DOI: 10.1002/ejoc.200600317

Regioselectivity in the Multi-Component Synthesis of Indolizinoquinoline-5,12-dione Derivatives

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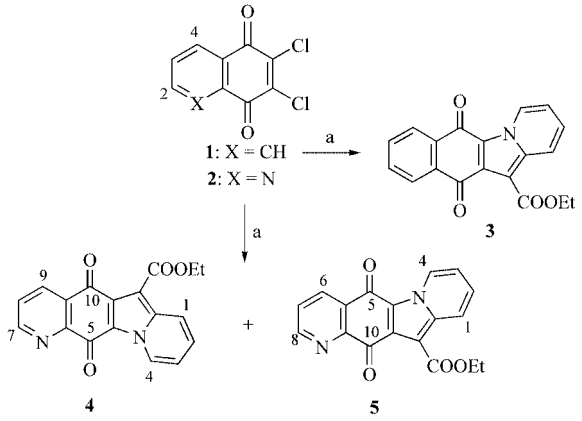
Keywords: Cyclisation / Nitrogen heterocycles / N,O ligands / Regioselectivity / Solvent effects

The one-pot cyclisation to form the indolizinoquinoline-5,12-dione ring system has been investigated starting from 6,7-dichloroquinoline-5,8-dione by reaction with pyridine and ethyl acetoacetate. Both the *N*,*N*-*syn* and the *N*,*N*-*anti* products have been fully characterised by mass spectrometry and NMR analysis, and the regioselectivity of their formation is discussed in terms of solvent polarity and/or the nature of the metal ion. We demonstrate here that, among a wide series of polar and apolar solvents, *tert*-butyl alcohol is the best choice to enhance the yield of the *N*,*N*-*syn* regioisomer, while the *N*,*N*-*anti* regioisomer can be obtained with a very high selectivity when the reaction is carried out with scandium triflate. In the presence of metal chelation, semi-empirical ZINDO calculations offer an appropriate tool to explain the regioselectivity observed when different metal ions are used. The study of the regioselectivity is also supported by the data for the corresponding two-step reactions with reversed addition of the nucleophilic reagents, and it has been extended to the cases involving 4-methylpyridine and 6,7-dichloroisoquinoline-5,8-dione as reagents.

Introduction

One interesting group of chemotherapeutic agents used in recent cancer therapy comprises molecules that interact with DNA. In particular, polycyclic aromatic compounds have been successfully studied as DNA-intercalating topoisomerase inhibitors. In accordance with Moore and Pindur's theory,[1] the structural requirements for active agents are that the molecules contain both a planar tricyclic or tetracyclic ring system and a conjugated *p*-quinone moiety for the generation of reactive oxygen species, which are ultimately considered to be the DNA fragmentation effectors. The presence of one or more nitrogen atoms in this skeleton is therefore vital for the formation of hydrogen bonds with DNA.

In our recent study on the design and synthesis of new compounds and on the evaluation of their bioactivity as antitumour agents,[2] we have focused our attention on suitable derivatives bearing a naphthindolizinedione ring system. The preparation of the central skeleton is based on a single-step, multi-component reaction between 2,3-dichloro-1,4-naphthoquinone (**1**), pyridine and an active methylene compound.[3] Aza analogues bearing the quinoline-5,8-dione system can be obtained by using 6,7-dichloroquinoline-5,8-dione (**2**),[4] which gives the possibility of producing two regioisomers due to the presence of the asymmetric chlorine atoms at the C-6 and C-7 positions in **2** (Scheme 1). This reactivity finds examples in the nucleophilic attack both of amines[5] and active methylene reagents.[6] Surprisingly, the single-step reaction of **2** with active methylene compounds and pyridine derivatives has been reported to give indolizino[2,3-*g*]quinoline-5,12-dione as the only product,[7] which roused our interest in investigating the key parameters that control the selectivity of the production of the two regioisomers. We chose the effect of solvent and the coordination of metal ions as variables for both the one-pot and two-step reactions, and the outcome of this study is here reported.



Scheme 1. Sequence for the cyclisation. Reagents: a) pyridine, ethyl acetoacetate under different conditions.

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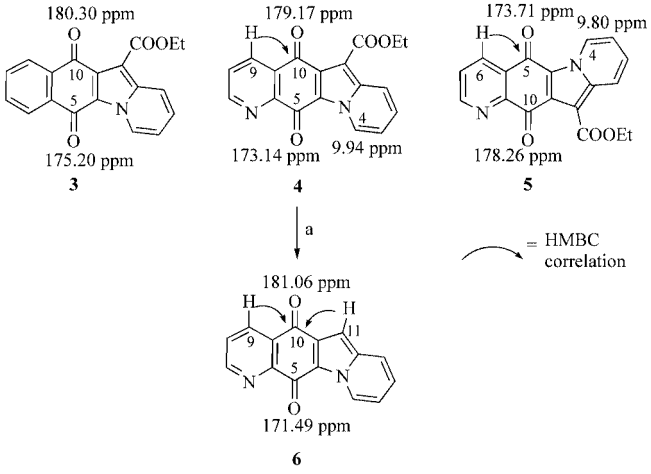
Results and Discussion

The one-pot cyclisation giving product **3**[8] has been extended here to the preparation of the aza analogues. Refluxing 6,7-dichloroquinoline-5,8-dione (**2**) with ethyl acetoacetate and an excess of pyridine in EtOH for 4 h gave a deep-reddish mixture consisting of the *N,N-syn* (**4**) and *N,N-anti* (**5**) regioisomers in a 64:36 ratio. The relative amounts of the two products were deduced by ^{1}H NMR analysis of the reaction mixture. Each isomer could be isolated in pure form by chromatography and fully characterised by mass spectrometry and NMR analysis.

The same molecular composition $C_{18}H_{12}N_2O_4$ was deduced for both regioisomers by HR-EIMS and further structural indications came from tandem fragmentation experiments in ESI(+)-MS analysis (see Experimental Section). NMR spectroscopic data, including NOESY and HMBC experiments, indicated that : (i) in the regioisomer **4**, the resonance at δ = 179.17 ppm is attributable to C-10 by long-range hetero-correlation with 9-H at δ = 8.56 ppm; as a consequence, the resonance at δ = 173.14 ppm must be assigned to C-5; (ii) in the regioisomer **5**, the resonance at δ = 173.71 ppm is attributable to C-5 by long-range hetero-correlation with 6-H at δ = 8.57 ppm, hence the resonance at δ = 178.26 ppm must be assigned to C-10 (Scheme 2). In the ester **3**, the ^{13}C NMR signals at δ = 180.30 and 175.20 ppm can easily be assigned to the carbon atoms C-5 and C-10, respectively,[2] and this assignment is also in good agreement with empirically calculated chemical shift values from shielding effects (see Experimental Section). It must be emphasised that the NMR spectroscopic data for regioisomers **4** and **5** do not allow us to establish which isomer is which. Empirical calculations, used for ester **3**, are not helpful here since they lead to the same values for both regioisomers. A decisive hint for the unambiguous regiochemical assignment, based on ^{1}H-^{13}C long-range couplings, requires the availability of a compound with protons located at the right and left side of the quinone ring which could correlate with one or both of the carbonyl groups. We reasoned that the product obtainable by decarboxylation of **4** or **5** could be a good choice. Therefore, the pure regioisomer showing the 4-H signal at δ = 9.94 ppm was subjected to decarboxylation[9] to give a compound for which both the doublet for 9-H and the singlet for 11-H were found to be hetero-correlated to the same signal (δ = 181.06 ppm; Scheme 2). This is clear-cut evidence for the *N,N-syn* structure of the regioisomer **6**; more importantly, the starting regioisomer must be **4**, with the same *N,N* geometry.

Among all the active methylene compounds previously used in the cyclisation involving substrate **1**,[9] ethyl acetoacetate was selected here in order to introduce a carboxyethyl unit for further functionalisation towards molecules as potential antitumour agents.[2] This choice was also dictated by its better yield when compared with other nucleophiles such as diethyl malonate[8] and ethyl cyanoacetate.[9] In the one-pot cyclisation, pyridine performs the double role of nucleophile and base, leading to the acetoacetate anion, as



Scheme 2. Structural assignments of the *N,N-syn* and *N,N-anti* regioisomers.

verified by carrying out the reaction in two steps. In fact, a stoichiometric amount of sodium acetate was required when ethyl acetoacetate was added as the first reagent[10] (Table 1, Entry 2). In this case, a slightly better yield could be obtained than in the one-pot reaction, although significant amounts of the dipolar by-products **7** and **8** (Figure 1) were generated. On the other hand, when pyridine was added as the first reagent to the substrate **2**, a lower yield was obtained due to a major formation of the same by-products (Table 1, Entry 3). These were characterised by NMR spectroscopy and ESI-MS analysis and the structures were established in analogy with that of by-product **9**, already reported from the reaction of 2,3-dichloronaphthoquinone (**1**),[8,11] by the assumption that the *N,N-anti* product **7** would be the major isomer (see Experimental Section).

Table 1. Synthesis of the regioisomers **4** and **5** from 6,7-dichloroquinoline-5,8-dione (**2**) upon changing the solvent.

Entry	Solvent	Conditions[a]	Ratio[b] **4/5**
1	EtOH	A	64:36
2	EtOH	B	75:25
3	EtOH	C	12:88
4	EtOH	D	64:36
5	*n*PrOH	A[c]	63:37
6	*n*BuOH	A[c]	61:39
7	*i*PrOH	A	76:24
8	*t*BuOH	A	85:15
9	*t*BuOH	B	93:7
10	2-methyl-2-butanol	A[c]	83:17
11	benzene	A	80:20
12	toluene	A[c]	75:25
13	C_6H_5Cl	A[c]	78:22
14	THF	A	80:20
15	DME	A	78:22
16	dioxane	A[c]	74:26
17	DMSO	A[c]	78:22
18	DMF	A[c]	80:20

[a] Reaction conditions: see Experimental Section. [b] Ratio evaluated by integration of ^{1}H NMR signals for 4-H of products **4** and **5**. [c] Reaction temperature fixed at 80 °C, for comparison.

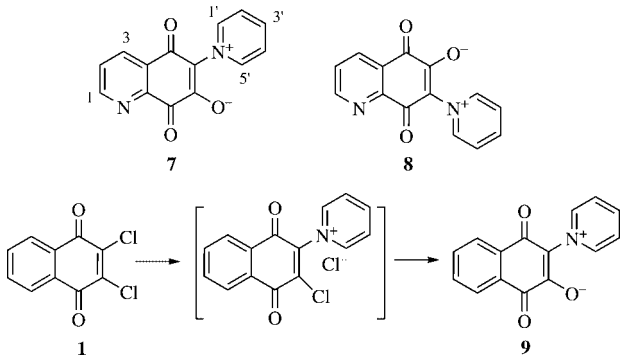


Figure 1. Structures of by-products **7**–**9**. Adopted numbering is for convenience.

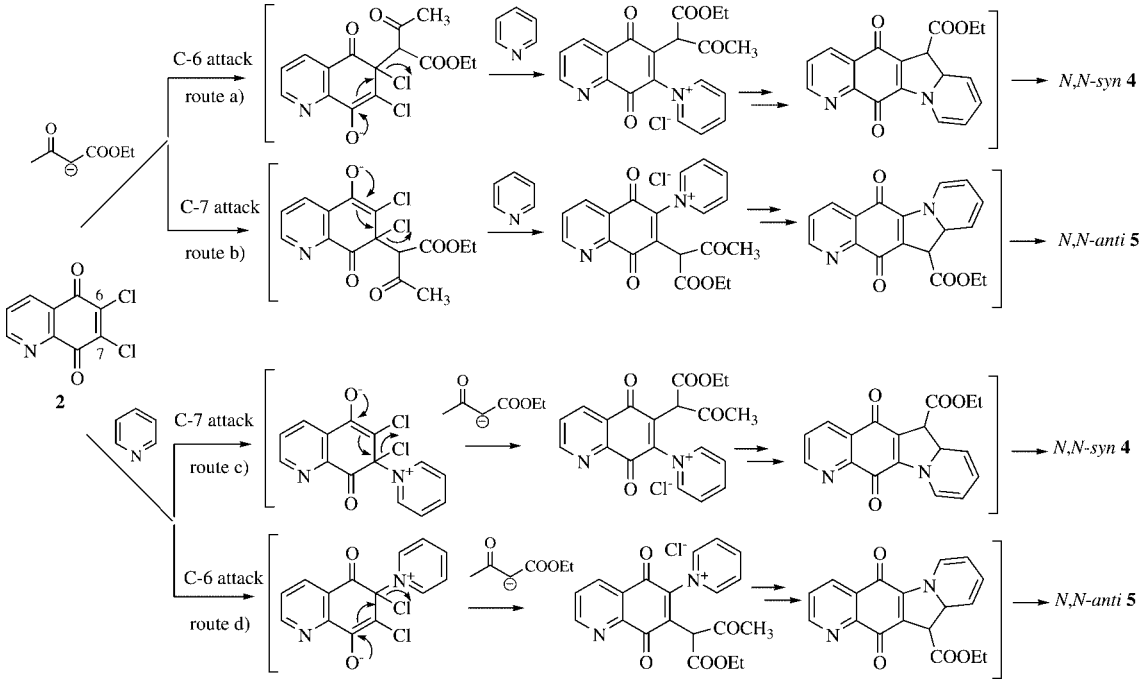
The regioselectivity, as observed in the one-pot sequence (**4**/**5** = 64:36), derives from a combination of the contributions of the nucleophilic attacks at the C-6 and C-7 positions of the substrate **2**. As shown in Scheme 3, the *N*,*N-syn* isomer **4** can be obtained either by route (a), where the first attack is by ethyl acetoacetate ion at the C-6 position, or by route (c), where the first attack is by pyridine at the C-7 position. Likewise, the *N*,*N-anti* isomer **5** is obtained by routes (b) and (d), which suggests that the major role in the selectivity is played by the nucleophilicity of the first attacking reagent.

When such a reaction is carried out in two steps, the regioselective ratio of the products changes depending upon the addition sequence of the reagents. In particular, we obtained a 75:25 ratio when acetoacetate ion was added as the first nucleophile and a 12:88 ratio when pyridine was the first one (Table 1, Entries 2 and 3). Following arguments similar to those previously suggested to explain the formation of product **3**,[8,11] we propose here a comprehensive mechanism for the formation of **4** and **5** (Scheme 3).

After a first nucleophilic attack (possibly by a Michael addition in the case of a C nucleophile) and displacement of one chlorine atom, the second nucleophilic attack induces the displacement of the second chlorine atom, followed by cleavage of the acetyl group to give ring closure and final aromatisation to the products.[8,9] In such a mechanism the inversion in the regioselectivity ratios obtained by changing the order of the reagents might be explained by a preferential attack at C-6 of both the nucleophiles. Our results are in fair agreement with the data reported for nucleophilic attacks on compound **2** by ethyl acetoacetate ion,[12] whereas amines have been reported to give substitution at C-6,[5a] at both C-6 and C-7[13] or only at C-7.[5b] The slight enhancement of regioselectivity (12:88 vs. 25:75) observed when pyridine is the first reagent is explained by a major role of route (d) with respect to route (b), possibly due to a mass-law effect of pyridine, which is present in excess.

Surprisingly, Yanni[7] has reported that the same reaction gives a single product, to which the *N*,*N-syn* structure of compound **4** was ascribed mainly on mechanistic grounds. However, the reported structural details cannot unambiguously establish the structure of such a regioisomer and, more importantly, the reaction carried out under the same experimental conditions as reported by Yanni[7] affords two regioisomers in the expected ratio (Table 1, Entry 4).

Changing from EtOH to other alcohols as solvent gave practically the same selectivity as was obtained with primary alcohols, irrespective of their chain length, as observed with EtOH, 1-propanol and 1-butanol (Table 1, Entries 1, 5 and 6, respectively). However, a slightly increased



Scheme 3. Possible routes to products **4** and **5**.

N,*N*-*syn*/*N*,*N*-*anti* ratio was obtained for the reaction conducted in 2-propanol (*i*PrOH) and *tert*-butyl alcohol (*t*BuOH) or 2-methyl-2-butanol (Table 1, Entries 7–10). These results indicate that the selectivity can be modulated by solvents with similar polarities. Moreover, they are in agreement with the lower solvent acidity of the tertiary alcohol such that when the latter is used as solvent, an increased availability of the free ethyl acetoacetate anion occurs. This explanation finds support in the higher regioselectivity (93:7) observed in *t*BuOH for the two-step reaction by route (a) (Table 1, Entry 9).

In the presence of aprotic solvents, such as benzene, toluene, chlorobenzene, THF, DME, 1,4-dioxane, DMSO and DMF, similar high regioselectivity was found (Table 1, Entries 11–18). This behaviour has been interpreted as deriving from an increased C-6 attack of ethyl acetoacetate anion, which is a better nucleophile in aprotic media than in EtOH and other primary alcohols as solvents.

The effect of some metal ions was also investigated for the chelation of compounds like 5,8-quinolinequinone[14] and 6,7-dichloroquinoline-5,8-dione (**2**),[15] where, in particular, the regioselectivity of amination[5a,16] is affected by the formation of a metal chelate complex. The complex where the N-1 atom and the carbonyl oxygen atom at C-8 in **2** are coordinated to the metal ion was proposed to be an activated intermediate that increases the electrophilicity at C-6 (Figure 2). We reasoned that such a metal chelation effect would be of great help in the investigation of the regioselectivity and we focused our attention only on salts soluble in a given solvent.

Complex **10**, prepared by treating a solution of **2** with an equimolar amount of silver perchlorate in CD_3OD, confirmed the metal–ligand binding by showing downfield shits in the NMR spectra (Figure 2). In particular, the stronger electrophilic nature of the C-6 centre in the chelate was indicated by a major deshielding effect observed for this carbon atom ($\Delta\delta$ = 1.50 ppm) than for C-7 ($\Delta\delta$ = 0.02 ppm). Similar effects were also detected with other metal chelates, including the Sc^{III} complex, as discussed below. The molecular composition of the complex was determined from the ESI-MS data recorded in positive-ion mode, where the most intense peak at *m*/*z* = 336 is attributable to the pseudomolecular ion $[\mathbf{2}\cdot Ag]^+$; the isotopic distribution of the latter was in perfect agreement with the simulated spectrum for a molecular composition $C_9H_3AgCl_2NO_2$ (Figure 3).

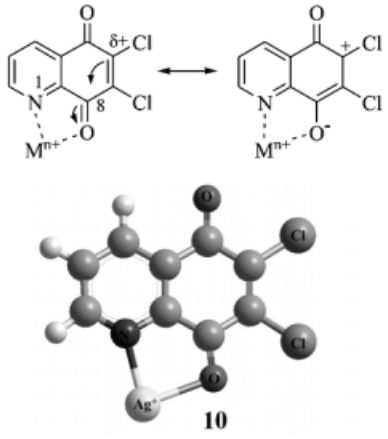


Figure 2. Metal ion coordination to 6,7-dichloroquinoline-5,8-dione (**2**) and the minimised structure of silver complex **10**.

In the one-pot cyclisation of **2**, when assisted by metal chelation, a preferential C-6 attack carried out at the same time by ethyl acetoacetate anion and pyridine was involved. Thus, in the presence of 0.3 mol-equiv. of scandium triflate in EtOH, a 21:79 ratio of the regioisomers **4** and **5** was obtained due to preferential attack at C-6 by pyridine through enhancement of route (d) with respect to route (a) in Scheme 3 (Table 2, Entry 1).

The regioselectivity was also affected by varying the amount of the metal salt; in particular, the effect was more marked in the case of scandium triflate than silver perchlorate (Table 2, Entries 1, 4, 5 and 9 and 10, respectively). Again, this evidence suggests that a competitive formation

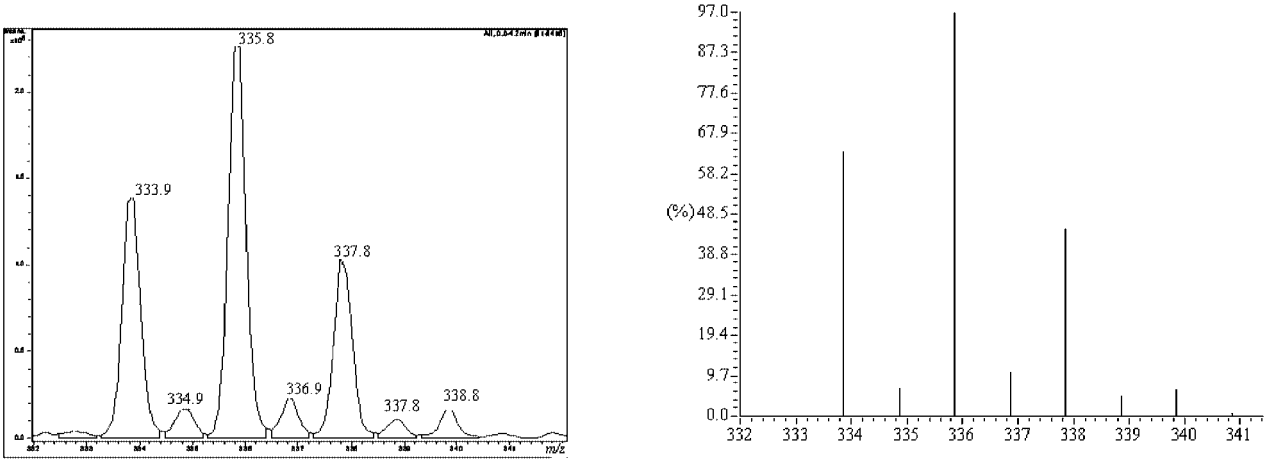


Figure 3. Experimental (left) and simulated (right) ESI(+) mass spectra for the silver complex **10**, showing the cluster with the most intense signal at *m*/*z* = 336 $[C_9H_3AgCl_2NO_2^+]$.

Table 2. Synthesis of regioisomers **4** and **5** from 6,7-dichloroquinoline-5,8-dione (**2**) in the presence of a metal ion.

Entry	Metal salt (equiv.)	Solvent	Conditions[a]	Ratio[b] **4/5**
1	$Sc(OTf)_3$ (0.3)	EtOH	A	21:79
2	$Sc(OTf)_3$ (0.3)	EtOH	B	60:40
3	$Sc(OTf)_3$ (0.3)	EtOH	C	0:100
4	$Sc(OTf)_3$ (1.0)	EtOH	A	0:100
5	$Sc(OTf)_3$ (0.1)	EtOH	A	40:60
6	$Sc(OTf)_3$ (0.3)	*t*BuOH	A	58:42
7	$Sc(OTf)_3$ (0.3)	benzene	A	54:46
8	$Sc(OTf)_3$ (0.3)	DMSO	A	80:20
9	$AgClO_4 \cdot H_2O$ (1.0)	EtOH	A	42:58
10	$AgClO_4$ H_2O (0.3)	EtOH	A	45:55
11	$FeCl_3$ (0.3)	EtOH	A	43:57
12	$NiCl_2 \cdot 6H_2O$ (0.3)	EtOH	A	22:78
13	$Cu(OAc)_2 \cdot H_2O$ (0.3)	EtOH	A	52:48
14	$CoCl_3 \cdot 6H_2O$ (0.3)	EtOH	A	68:32
15	$ZnBr_2$ (0.3)	EtOH	A	32:68
16	$AlCl_3 \cdot 6H_2O$ (0.3)	EtOH	A	50:50
17	CdI_2 (0.3)	EtOH	A	42:58
18	$Pd(OAc)_2$ (0.3)	EtOH	A	66:34
19	$CeCl_3 \cdot 7H_2O$ (0.3)	EtOH	A	22:78
20	$CeCl_3 \cdot 7H_2O$ (0.3)	*i*PrOH	A	52:48
21	$Sm(OTf)_3$ (0.3)	EtOH	A	15:85

[a] In one step (A) and two steps (B and C, with initial addition of ethyl acetoacetate and pyridine, respectively), under the conditions reported in Table 1; 40–75% yields of the purified regioisomers after chromatography; lower yields were obtained with conditions C. [b] Ratio evaluated by integration of ^{1}H NMR signals for 4-H in products **4** and **5**.

of stable metal chelates with ethyl acetoacetate[17] or pyridine could play a role. The formation of a stable chelate with pyridine was also confirmed by the isolation of a ruthenium complex when $RuCl_3$ (0.3 mol-equiv. with respect to **2**) was used in the one-pot reaction. In this case a 63:37 ratio of the regioisomers **4** and **5** was found, a value practically identical to the regioselectivity obtained in the absence of the metal salt (Table 1, Entry 1). The complex was established to be $[RuCl_3(py)_4]$ from its ^{1}H NMR spectrum and by ESI(+)-MS analysis. In particular, the mass spectrum shows a cluster centred at m/z = 488, in full agreement with the simulated spectrum, and tandem mass fragmentation experiments indicated the loss of pyridine molecules (see Experimental Section). The same 63:37 ratio was also obtained for the reaction in the presence of $RhCl_3 \cdot 3H_2O$, thus indicating that metal coordination to substrate **2** does not occur. This outcome is not unexpected since it has been already reported that Rh^{III} gives stable complexes with pyridine.[18]

The reaction in the presence of metal ions was also observed to be affected by the solvent. Changing from EtOH to aprotic solvents gave a lower regioselectivity in benzene due to the two opposite effects caused by the solvent and the scandium coordination (cf. Table 2, Entry 7 with Entry 1 in the same table and Entry 11 in Table 1). A similar effect was observed upon changing from EtOH to *t*BuOH or *i*PrOH (Table 2, Entries 6 and 20, respectively). In DMSO, the reaction gave the same regioisomeric ratio as without scandium triflate (cf. Table 2, Entry 7 with Entry 17 in Table 1); these results can be explained by the formation of a competitive complex between scandium and DMSO, as reported for hexacoordinate Sc^{III} complexes with donor monodentate molecules such as DMSO.[19]

When working at constant metal concentration, the one-pot reaction in EtOH gave moderate to good regioselectivity depending on the nature of the metal itself (Table 2, Entries 10–21); the best regioselectivities were obtained upon chelation with samarium, scandium, cerium or nickel ions.

Semi-empirical ZINDO calculations are useful for the study of complexes of the first two rows of transition metals and they provide a useful addition to experimental studies.[20] When this methodology was applied to metal chelates of substrate **2** (except cerium and samarium complexes due to a lack of parametrisation), the electron density at C-6 and C-7 could be reliably estimated (Table 3). This allowed us to establish a relationship between the regioselectivity and the different electron densities at C-6 and C-7 in a given chelate. In particular, the data indicate that a given increase of the difference of the calculated charge at such positions is linearly correlated to the relative amount of *N*,*N*-*anti* isomer **5** (Figure 4).

The ZINDO calculations proved to be a good tool for the screening of expected regioselectivity when different metal ions were used in the reaction of **2** with different nucleophiles, and we used such an approach to choose the "best metal" in the case of substituted pyridines (see below).

Table 3. ZINDO calculated charges, q, at C-6 and C-7 for the complex of **2** with metal ions.

Metal ion	Charge at C-6	Charge at C-7	Δq [C(6) – C(7)]	Yield of *N*,*N*-*anti* **5** (%±3%)
None (0)	0.141	0.148	–0.007	36
Ag^+	0.189	0.121	0.068	55
Cu^{2+}	0.245	0.198	0.047	48
Cd^{2+}	0.201	0.114	0.087	58
Fe^{3+}	0.278	0.202	0.076	57
Al^{3+}	0.167	0.136	0.031	50
Sc^{3+}	0.293	0.119	0.174	79
Co^{2+}	0.158	0.212	–0.054	32
Pd^{2+}	0.150	0.185	–0.035	34
Ni^{2+}	0.272	0.110	0.162	78
Zn^{2+}	0.239	0.106	0.133	68

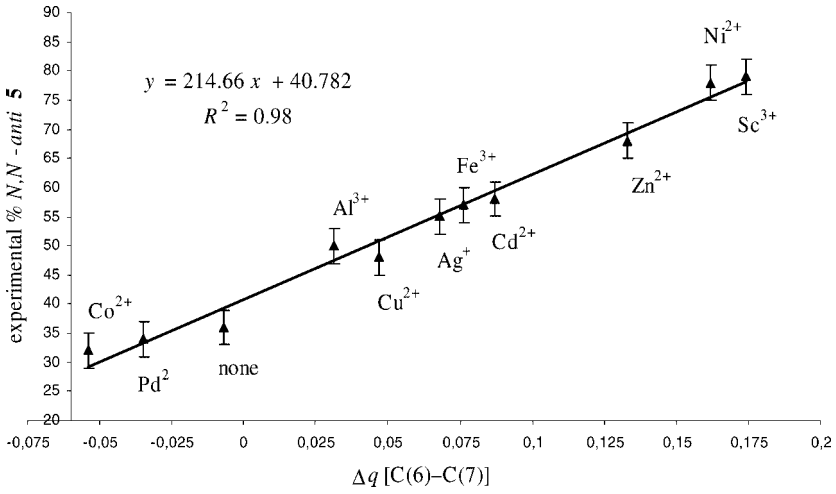
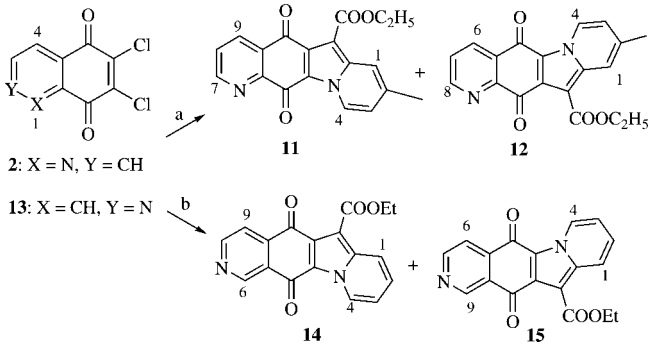


Figure 4. Linear correlation of the experimental amount of *N,N-anti* **5** as a function of the difference between the calculated charges at C-6 and C-7 for the regioselective one-pot synthesis in the presence of metal ions.

The double cyclisation studied here was also applied to the preparation of substituted analogues (Scheme 4). The one-pot reaction of compound **2** with 4-methylpyridine and ethyl acetoacetate in EtOH gave the regioisomers **11** and **12** in a 72:28 ratio (Table 4).



Scheme 4. Synthesis of the methyl-substituted regioisomers **11/12** and of isoquinoline-like regioisomers **14/15**. Reagents: a) 4-methylpyridine, ethyl acetoacetate under different conditions; b) pyridine, ethyl acetoacetate under different conditions.

The same molecular composition ($C_{19}H_{14}N_2O_4$) was deduced by HR-EIMS analysis (see Experimental Section) and the structural assignments for both the isomers were achieved from the NMR spectroscopic data according to considerations similar to those described above for products **4** and **5**. In particular, 9-H with signal at δ = 8.52 ppm is long-range heterocorrelated to the most deshielded carbon atom (δ_C = 179.32 ppm, attributable to C-10), so the remaining downfield singlet at δ = 172.72 ppm could be assigned to C(5)=O in the *N,N-syn* compound **11**; similarly, 6-H with signal at δ = 8.50 ppm in the *N,N-anti* product **12** is long-range hetero-correlated to C-5 (δ = 173.34 ppm), so the most downfield singlet at δ = 178.42 ppm could be assigned to C=O at the C-10 position.

The better regioselectivity in favour of the *N,N-syn* isomer than the corresponding reaction with pyridine (Table 1, Entry 1) can be explained by the higher nucleophilic character of 4-methylpyridine[21] than pyridine, which induces a higher reactivity both for the preferential attack at C-6 and at C-7 of substrate **2**.[5b,13] The same reason could account for the partially reversed selectivity in the two-step sequence, where the first addition of 4-methylpyridine gave a minor selectivity (Table 4, Entries 2 and 3).

Replacing EtOH with *t*BuOH gave a higher ratio in favour of the *N,N-syn* product (90:10), and this increased even more when the cyclisation was realized in two steps with initial addition of acetoacetate anion (93:7).

When the reaction was carried out in EtOH in the presence of a metal ion, an inversion of the regioisomeric ratio for products **11** and **12** was obtained (12:88) with 0.3 molequiv. of $Sc(OTf)_3$, whereas practically pure *N,N-anti* product **12** was obtained in the presence of a stoichiometric amount of metal ion (Table 4, Entries 6 and 7). This behaviour was somehow expected in light of the higher selectivity obtained from the one-pot reaction giving **4** and **5** when Sc^{III} ions were chosen for metal chelation (Table 2, Entry 1). When compared to the corresponding reaction with pyridine, which affords **4** and **5** in an 8:92 ratio, the better nucleophilicity of 4-methylpyridine might be responsible for its exclusive attack at the C-6 position of the chelated substrate **2**.

The versatility of this double cyclisation was also tested with 6,7-dichloroisoquinoline-5,8-dione (**13**), which gives the new tetracyclic structures *N,N-syn* (**14**) and *N,N-anti* (**15**), respectively. Their structural assignments were based on one- and two-dimensional experiments, including NOESY and HSQC, where the ^{13}C NMR chemical shifts and long-range hetero-correlations are quite diagnostic. The relevant HMBC correlations involve 6-H/C-5 and 9-H/C-10 (δ = 9.47 ppm with δ = 174.56 ppm and δ = 8.00 ppm with δ = 179.35 ppm for **14**, and δ = 8.02 ppm with δ =

Table 4. One-pot synthesis of regioisomers **11** and **12** and of isoquinoline-like regioisomers **14** and **15**.

Entry	Metal salt (equiv.)	Solvent	Conditions[a]	Product ratio (*syn*/*anti*)[b]
		Compounds **11** and **12**		
1	–	EtOH	A	72:28
2	–	EtOH	B	80:20
3		EtOH	C	40:60
4	–	*t*BuOH	A	90:10
5	–	*t*BuOH	B	93:7
6	$Sc(OTf)_3$ (0.3)	EtOH	A	12:88
7	$Sc(OTf)_3$ (1.0)	EtOH	A	0:100
		Compounds **14** and **15**		
8	–	EtOH	A	75:25
9	–	EtOH	B	40:60
10	–	EtOH	C	90:10
11	–	*t*BuOH	A	78:22
12	–	benzene	A	75:25
13	$Sc(OTf)_3$ (0.3)	EtOH	A	70:30
14	$Sc(OTf)_3$ (1.0)	EtOH	A	65:35

[a] In one step (A) and two steps (B and C, with initial addition of ethyl acetoacetate and pyridine, respectively) under the conditions reported in Table 1; 55–65% yields of the purified regioisomers after chromatography; lower yields were obtained under conditions C condition. [b] Ratio evaluated by integration of ^{1}H NMR signals for 4-H in **11** and **12**, or 6-H in **14** and 9-H in **15**.

173.95 ppm and δ = 9.44 ppm with δ = 179.77 ppm for **15**). These correlations allowed us to assign the most downfield value of the two carbonyl signals to the C=O group lying on the same side as the pyrrole nitrogen atom, in analogy to the assignments for compounds **3**, **4** and **11**.

When the reaction was carried out under the two-step conditions (Table 4, Entries 9 and 10), the data can be interpreted by implying a resonance structure of **13** with a positive charge at C-7 (Figure 5).[4]

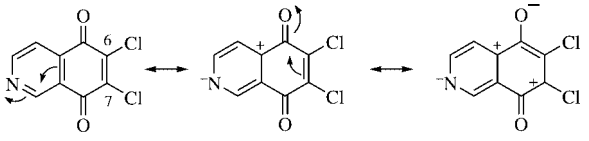


Figure 5. Resonance forms for 6,7-dichloroisoquinoline-5,8-dione (**13**).

Products from C-7 attack of reagent **13** have been observed upon reaction with substituted anilines.[22] In agreement with these reports, the reaction of **13** conducted with initial addition of pyridine furnished the *N*,*N*-*syn* product **14** with high selectivity (90:10), while the *N*,*N*-*anti* product **15** was obtained as the major regioisomer upon reverse addition of the two nucleophiles, although with lower selectivity.

Concerning the regioselectivity in the one-pot reaction in different solvents, a 75:25 ratio of products **14** and **15** was achieved both in EtOH and *t*BuOH, as well as in benzene (Table 4, Entries 8, 11 and 12, respectively). Compared with the results for substrate **2** (Table 1, Entry 1), the better regioselectivity with substrate **13** is due to the preferential attack of pyridine at the more electrophilic C-7 position. The absence of a solvent effect on the regioselectivity is additional evidence for the prevalent role played by the C-7 electrophilicity in comparison with the change of the nucleophilic nature of the reagents due to solvent effects.

Finally, the unchanged or moderately changed regioselectivity when using $Sc(OTf)_3$ in catalytic or stoichiometric amounts (Table 4, Entries 13 and 14), suggests a decisive role of the electrophilic character of the C-7 centre and a marginal role of metal coordination. The latter is due to the peculiar position of the nitrogen atom in compound **13**, which is responsible for the inability to form a stable bidentate chelate, in contrast to **2**.

Conclusions

We have studied the regioselectivity of the multi-component cyclisation to the indolizinoquinoline-5,12-dione ring system starting from 6,7-dichloroquinoline-5,8-dione (**2**). The *N*,*N*-*syn* (**4**) and *N*,*N*-*anti* (**5**) regioisomers were obtained and their structures established by NMR analysis and comparison with the NMR spectroscopic data of a suitable compound derived from decarboxylation of the pure *N*,*N*-*syn* isomer. The ratio of products **4** and **5** was found to be dependent on solvent and metal ion. For the 15 solvents under investigation, the best regioselectivity in the one-pot reaction was obtained in *t*BuOH as protic solvent (85:15) and in benzene, THF or DMF as aprotic solvent (80:20). The greater formation of the *N*,*N*-*syn* product **4** can be rationalised as being due to the preferential attack of ethyl acetoacetate anion at the C-6 position in **2**, as supported by the higher ratio (93:7) obtained for the two-step sequence with initial addition of ethyl acetoacetate ion in *t*BuOH.

On the contrary, the metal-ion chelation of substrate **2** furnishes C-6 as the better electrophilic centre and gives a fully reversed regioselectivity of the products. Among the twelve soluble metal salts examined in different solvents and molar amounts, the best results were obtained with 0.3 mol-equiv. of $Sm(OTf)_3$, $Sc(OTf)_3$, $NiCl_2$ and $CeCl_3$ (from 15:85 to 22:78 for the **4**/**5** ratio) in EtOH or, even

better, with complete selectivity for the *N*,*N*-*anti* product in the two-step sequence with initial addition of pyridine or with a stoichiometric amount of $Sc(OTf)_3$ in the case of the one-pot reaction.

In addition, semi-empirical ZINDO calculations have been performed for a series of metal chelates of **2**, thereby furnishing a relationship in full agreement with the experimental regioisomeric ratios. This reaction provides an efficient tool for the selective access to both regioisomers by a suitable choice of solvent and/or metal ion. The same approach has also been successfully applied to obtain analogous tetracyclic molecules starting from 4-methylpyridine or 6,7-dichloroisoquinoline-5,8-dione; thus, the system investigated here represents an effective and versatile access to practically pure regioisomers that could be used as precursors in the synthesis of potential antitumour agents.

Experimental Section

General Methods: All evaporations were carried out at room temp. at reduced pressure. Anhydrous benzene and pyridine were obtained by distillation from calcium chloride and KOH, respectively. The other reagents and solvents were used without purification. Caution must be taken when using benzene, CdI_2, $NiCl_2$ and $CoCl_2$ due to their carcinogenicity. TLC was performed on Merck Kieselgel 60 PF_{254} and Merck RP-18 F_{254}. Preparative TLC was carried out on 20×20 cm Merck Kieselgel 60 F_{254} 0.5 mm plates. Melting points were recorded with a Kofler hot-stage microscope. FT-IR spectra were recorded with an Equinox 55 Bruker apparatus, using a film obtained by evaporation of a solution of compounds in CH_2Cl_2. UV spectra were recorded with a Perkin–Elmer Lambda-3 spectrophotometer. NMR spectra were recorded with an Avance 400 Bruker spectrometer; ^1H at 400 MHz and ^{13}C at 100 MHz in $CDCl_3$ (previously treated with basic alumina to avoid acidic traces) or CD_3OD; δ values in ppm are given relative to the solvent residual signals in either $CDCl_3$ (δ_H = 7.25 ppm and δ_C = 77.00 ppm) or CD_3OD (δ_H = 3.31 ppm and δ_C = 49.00 ppm). Multiplicities are taken from APT experiments. Structural assignments are from ^1H,^1H-COSY, HSQC, HMBC and 2D NOESY experiments. Calculated ^{13}C NMR spectroscopic data for **3** were obtained with CS Chem NMR Pro Version 6.0. EI mass spectra and HR-EI data were recorded with a Kratos-MS80 mass spectrometer with home-built computerized acquisition software. ESI-MS data and tandem fragmentation spectra (MS^n), were recorded with a Bruker Esquire-LC™ spectrometer equipped with an electrospray ionisation ion source used, positive- or negative-ion mode, by injection of the sample into the source from a methanol solution.

Semi-Empirical Calculations: Semi-empirical ZINDO/1 calculations were carried out with the HyperChem 7.5 (demo version) software package assuming vacuum conditions and setting the charge of the minimised structure of the complex on the metal ion. The final criterion of RMS gradient was 0.1 kcal mol^{-1} Å^{-1} and the overlap weighting factor for σ-σ and π-π was set at 1.00.

Preparation of Compounds 2 and 13: 6,7-Dichloroquinoline-5,8-dione (**2**) and 6,7-dichloroisoquinoline-5,8-dione (**13**) were prepared according to Shaikh et al.,[4] by treating 8-hydroxyquinoline in concentrated HCl solution and 5-hydroxyisoquinoline in concd. HNO_3, respectively, with sodium chlorate. **2:** M.p. 221–222 °C. ^1H NMR (400 MHz, CD_3OD, 25 °C): δ = 9.00 (dd, $J_{2,3}$ = 4.8, $J_{2,4}$ = 1.7 Hz, 1 H, 2-H), 8.58 (dd, $J_{4,3}$ = 7.9, $J_{4,2}$ = 1.7 Hz, 1 H, 4-H), 7.90 (dd, $J_{3,2}$ = 4.8, $J_{3,4}$ = 7.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 175.57 (s, C-5), 174.25 (s, C-8), 155.35 (d, C-2), 146.70 (s, C-8a), 144.32 (s, C-7), 143.10 (s, C-6), 135.60 (d, C-4), 128.28 (s, C-4a), 128.22 (d, C-3) ppm. ESI(+)-MS: *m*/*z* = 250 $[M + Na]^+$, 228 $[M + H]^+$ for $C_9H_3{}^{35}Cl_2NO_2$. **13:** M.p. 178–180 °C. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.47 (br. s, 1 H, 1-H), 9.15 (d, $J_{4,3}$ = 4.8 Hz, 1 H, 3-H), 8.00 (d, $J_{3,4}$ = 4.8 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 175.57 (s, C-5), 174.25 (s, C-8), 155.35 (d, C-2), 146.70 (s, C-8a), 144.32 (s, C-7), 143.10 (s, C-6), 135.60 (d, C-4), 128.28 (s, C-4a), 128.22 (d, C-3) ppm. ESI(+)-MS: *m*/*z* = 228 $[M + H]^+$ for $C_9H_3{}^{35}Cl_2NO_2$.

One-Pot Synthesis of 4/5 (Method A): Ethyl acetoacetate (7.5 μL, 0.059 mmol) and pyridine (22 μL, 0.27 mmol) were added to a solution of **2** (10 mg, 0.044 mmol) in absolute EtOH (1 mL), to give an intense blue colour. The solution was refluxed whilst stirring for 4 h, to give a reddish-brown suspension, which was then concentrated to give a residue that was subsequently subjected to preparative TLC with CH_2Cl_2/MeOH (96:4) as eluent. Extensive extraction of the reddish band with CH_2Cl_2/EtOH (3:1) gave a mixture of **4** and **5** (7.6 mg, 54%). These products were separated by preparative TLC with EtOAc/Et_3N (95:5). After a reaction time of 10 h, the same **4/5** ratio and yield were obtained. Repeating the reaction under the experimental conditions reported by Yanni (method D),[7] namely **2** (1 equiv.), ethyl acetate (1 equiv.), pyridine (1.5 equiv.) and refluxing in absolute EtOH for 10 h gave a 63:37 mixture of products in 34% yield. When the reaction was performed in other solvents the same volume (1 mL) was used. For the reaction in the presence of metal salts, the salt was added to a suspension of **2** in the selected solvent and the mixture stirred at room temperature for 5 min before addition of ethyl acetoacetate and pyridine. Further details are reported in Table 1. The **4/5** ratio was evaluated by integration of NMR signals for the 4-H proton in the spectrum of the residue obtained by evaporation of the reaction mixture or the regioisomeric mixture separated by preparative TLC; the same ratio was obtained in both cases.

Two-Step Synthesis of 4/5 (Method B): Ethyl acetoacetate (5.6 μL, 0.044 mmol) and anhydrous sodium acetate (3.6 mg, 0.044 mmol) were added to a solution of **2** (10 mg, 0.044 mmol) in absolute EtOH (1 mL) and the mixture refluxed for 2 h whilst monitoring the disappearance of **2** by TLC (*n*-hexane/EtOAc = 1:1). The suspension was then filtered, pyridine (22 μL, 0.27 mmol) was added to the filtrate and the mixture refluxed for 4 h. Workup was performed as above to give a mixture of **4** and **5** in a 75:25 ratio (11 mg, 78%). For the reaction in the presence of a metal salt, the salt was added to a suspension of **2** in the selected solvent and this mixture stirred at room temperature for 5 min before the addition of ethyl acetoacetate and solid sodium acetate.

Two-Step Synthesis of 4/5 (Method C): Pyridine (7.1 μL, 0.088 mmol) was added to a solution of **2** (10.0 mg, 0.044 mmol) in absolute EtOH (1 mL). The mixture was refluxed for 1 h, then ethyl acetoacetate (5.6 μL, 0.044 mmol) was added and the mixture refluxed for 4 h. Workup was performed as above to obtain **4** and **5** in a 12:88 ratio (1.4 mg, 10%). The low yield is due to the isolation of by-products **7** and **8** in higher amounts than with methods A and B. For the reaction in the presence of a metal salt, the salt was added to a suspension of **2** in the selected solvent and the mixture stirred at room temperature for 5 min before the addition of pyridine.

Ethyl 5,10-Dioxo-5,10-dihydro-4,6-diazabenzo[*b*]fluorene-11-carboxylate (4): R_f = 0.61 (silica gel; EtOAc/Et_3N, 95:5); m.p. 225–226 °C. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.94 (d, $J_{4,3}$ = 7.1 Hz, 1 H, 4-H), 9.03 (br. d, $J_{7,8}$ = 4.6 Hz, 1 H, 7-H), 8.56 (d, $J_{6,5}$ = 7.9 Hz,

1 H, 6-H), 8.37 (d, $J_{1,2}$ = 9.1 Hz, 1 H, 1-H), 7.66 (dd, $J_{7,6}$ = 7.9, $J_{7,8}$ = 4.6 Hz, 1 H, 8-H), 7.50 (ddd, $J_{2,1}$ = 9.1, $J_{2,3}$ = 7.0, $J_{2,4}$ = 1.1 Hz, 1 H, 2-H), 7.26 (td, $J_{3,4}$ = $J_{3,2}$ = 7.0, $J_{3,1}$ = 1.1 Hz, 1 H, 3-H), 4.50 (q, J = 6.7 Hz, 2 H, CH_3CH_2), 1.52 (t, J = 6.7 Hz, 3 H, CH_3CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 179.17 (s, C-10), 173.14 (s, C-5), 162.99 (*C*OOEt), 154.09 (d, C-7), 149.52 (s, C-5a), 139.95 (s, C-11a), 135.40 (d, C-9), 130.92 (s, C-9a), 128.11 (s, C-4b), 128.57 (d, C-4), 126.98 (d, C-8), 126.93 (d, C-2), 122.99 (s, C-10a), 121.11 (d, C-1), 117.98 (d, C-3), 106.36 (s, C-11), 61.17 (t, O*C*H_2), 14.32 (q, *C*H_3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 460 (1700), 350 (2220), 335 (3120), 320 (3340), 280 (5060), 250 (11220 $M^{-1}cm^{-1}$) nm. FT-IR (film) $\tilde{\nu}$ = 2928, 1723, 1682, 1645, 1587, 1504, 1387, 1317, 1235, 1187, 1121, 1027, 790, 754 cm^{-1}. ESI(+)-MS: *m*/*z* = 321 $[M + H]^+$, 293 $[M + H - C_2H_5]^+$, 275 $[M + H - C_2H_5OH]^+$. MS (70 eV, EI): *m*/*z* (%) = 320 (100) $[M]^+$, 275 (96) $[M - C_2H_5O]^+$, 248 (99), 220 (16), 191 (20), 164 (24). HR-EIMS: calcd. for $C_{18}H_{12}N_2O_4$ 320.0797; found 320.0791 ± 0.0030.

Ethyl 5,10-Dioxo-5,10-dihydro-4,9a-diazabenzo[*b*]fluorene-11-carboxylate (5): R_f = 0.69 (silica gel; EtOAc/Et_3N, 95:5); m.p. 219–220 °C. ^{1}H NMR (400 MHz, $CDCl_3$; 25 °C): δ = 9.80 (d, $J_{4,3}$ = 7.0 Hz, 1 H, 4-H), 9.01 (dd, $J_{8,7}$ = 4.6, $J_{8,6}$ = 1.2 Hz, 1 H, 8-H), 8.57 (dd, $J_{6,7}$ = 7.8, $J_{6,8}$ = 1.2 Hz, 1 H, 6-H), 8.40 (d, $J_{1,2}$ = 9.2 Hz, 1 H, 1-H), 7.66 (dd, $J_{6,7}$ = 7.8, $J_{6,8}$ = 4.6 Hz, 1 H, 7-H), 7.49 (ddd, $J_{2,1}$ = 9.2, $J_{2,3}$ = 7.3, $J_{2,4}$ = 1.2 Hz, 1 H, 2-H), 7.23 (dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 7.3 Hz, 1 H, 3-H), 4.50 (q, J = 6.7 Hz, 2 H, CH_3CH_2), 1.52 (t, J = 6.7 Hz, 3 H, CH_3CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 178.26 (s, C-10), 173.71 (s, C-5), 163.47 (*C*OOEt) 153.66 (d, C-8), 149.66 (s, C-9a), 140.23 (s, C-11a), 134.33 (d, C-6), 130.53 (s, C-5a), 128.56 (s, C-4b), 128.45, 128.44 (d, C-2 and C-4), 128.22 (d, C-7), 121.71 (s, C-10a), 121.11 (d, C-1), 117.93 (d, C-3), 106.88 (s, C-11), 61.28 (t, O*C*H_2), 14.15 (q, *C*H_3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 460 (2620), 350 (4000), 335 (5600), 320 (6270), 280 (9570), 250 (20000), 246 (20200 $M^{-1}cm^{-1}$) nm. FT-IR (film) $\tilde{\nu}$ 2958, 2918, 2850, 1722, 1691, 1640, 1610, 1501, 1389, 1371, 1354, 1319, 1272, 1223, 1181, 1160, 1115, 1076, 1026, 797, 757, 708 cm^{-1}. ESI(+)-MS: *m*/*z* = 321 $[M + H]^+$, 293 $[M + H - C_2H_5]^+$, 275 $[M + H - C_2H_5OH]^+$. MS (70 eV, EI): *m*/*z* (%) = 320 (100) $[M]^+$, 275 (96) $[M - C_2H_5O]^+$, 248 (99), 220 (14), 191 (21), 164 (24). HREI-MS calcd. for $C_{18}H_{12}N_2O_4$: 320.0797; found 320.0792 ± 0.0030.

4,6-Diazabenzo[*b*]fluorene-5,10-dione (6): A 0.5 M solution of NaOH in EtOH (0.1 mL) was added to a solution of **4** (10.0 mg, 0.031 mmol) in EtOH (1 mL) and the mixture refluxed for 0.5 h. A solution containing an excess of NaOEt, previously prepared from sodium and EtOH, was then added and the mixture refluxed for 5 h. The reaction mixture was concentrated, added to H_2O (3 mL) and extracted three times with CH_2Cl_2. The combined organic phases were washed with H_2O, dried with Na_2SO_4 and concentrated to give a residue, which was subjected to preparative TLC with CH_2Cl_2/MeOH (96:4) to give pure **6** (6.3 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.77 (d, $J_{4,3}$ = 7.1 Hz, 1 H, 4-H), 9.00 (br. d, $J_{7,8}$ = 4.6 Hz, 1 H, 7-H), 8.51 (d, $J_{9,8}$ = 8.0 Hz, 1 H, 9-H), 7.70 (d, $J_{1,2}$ = 8.9 Hz, 1 H, 1-H), 7.59 (dd, $J_{7,6}$ = 8.0, $J_{7,8}$ = 4.6 Hz, 1 H, 7-H), 7.28 (dd, $J_{2,1}$ = 8.9, $J_{2,3}$ = 7.0 Hz, 1 H, 2-H), 7.12 (t, $J_{3,4}$ = $J_{3,2}$ = 7.0 Hz, 1 H, 3-H), 7.11 (s, 1 H, 11-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 181.06 (s, C-10), 171.04 (s, C-5), 154.06 (d, C-7), 151.17 (s, C-5a), 139.06 (s, C-11a), 135.11 (d, C-9), 131.16 (s, C-9a), 129.88 (d, C-4), 128.90 (s, C-4b), 126.36 (d, C-8), 126.17 (d, C-2), 122.07 (s, C-10a), 120.89 (d, C-1), 117.18 (d, C-3), 102.14 (s, C-11) ppm. MS (70 eV, EI): *m*/*z* (%) = 248 (54) $[M]^+$, 220 (13), 192 (11). HR-EIMS: calcd. for $C_{15}H_8N_2O_2$ 248.0586; found 248.0554 ± 0.0030.

By-Products 7 and 8: From the procedure adopted in method C in EtOH, a mixture of **7** and **8** (ca. 8:2; 8.8 mg, 80%) was also isolated from the more polar band in the preparative TLC purification. **7/8:** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C; the values for **8** are given in square brackets, when not superimposed): δ = 8.94 [9.02] (dd, $J_{1,2}$ = 4.7, $J_{1,3}$ = 1.5 Hz, 1 H, 1-H), 7.65 [7.58] (dd, $J_{2,1}$ = 4.7, $J_{2,3}$ = 7.6 Hz, 1 H, 2-H), 8.54 [8.43] (dd, $J_{3,2}$ = 7.6, $J_{3,1}$ = 1.5 Hz, 1 H, 3-H), 8.80 (d, $J_{1',2'}$ = $J_{5',4'}$ = 5.9 Hz, 2 H, 1′-H, 5′-H), 7.95 (t, $J_{2',1'}$ = $J_{2',3'}$ = $J_{4',3'}$ = $J_{4',5'}$ = 7.0 Hz, 2 H, 2′-H and 4′-H), 8.31 (t, $J_{3',2'}$ = $J_{3',4'}$ = 7.0 Hz, 1 H, 3′-H) ppm. ESI(+)-MS: *m*/*z* = 253 $[M + H]^+$, 275 $[M + Na]^+$, 527 $[2 M + Na]^+$. MS/MS(253): *m*/*z* = 225.

Silver Complex 10: This complex was prepared directly in an NMR tube by dissolving $AgClO_4 \cdot H_2O$ (5.0 mg, 0.022 mmol) in a solution of compound **2** (5.0 mg, 0.022 mmol) in CD_3OD (0.7 mL) to give a yellow solution. ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 9.06 (dd, $J_{2,3}$ = 4.8, $J_{2,4}$ = 1.7 Hz, 1 H, 2-H), 8.73 (dd, $J_{4,3}$ = 7.9, $J_{4,2}$ = 1.7 Hz, 1 H, 4-H), 8.07 (dd, $J_{3,2}$ = 4.8, $J_{3,4}$ = 7.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 176.18, 176.02 (s, C-5 and C-8), 156.95 (d, C-2), 146.09 (s, C-8a), 144.61, 144.30 (s, C-6 and C-7), 131.13 (d, C-3), 138.47 (d, C-4); 130.44 (s, C-4b) ppm. ESI(+)-MS (most intense signal of the cluster): *m*/*z* = 336 $[C_9H_3AgCl_2NO_2]^+$.

$[RuCl_3(py)_4]$: This complex was isolated as a less polar yellow portion by preparative TLC (CH_2Cl_2/MeOH, 94:6) of the crude mixture from the one-pot sequence according to method A that gives **4/5** in the presence of 0.3 equiv. of $RuCl_3$. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.61 (d, J = 5.3 Hz, 2 H), 7.64 (t, J = 7.8 Hz, 1 H), 7.11 (t, J = 7.0 Hz, 2 H) ppm. ESI(+)-MS (most intense signal of complex clusters): *m*/*z* = 488 $[Ru(Py)_4Cl_2]^+$, 409 $[Ru(Py)_3Cl_2]^+$, 330 $[Ru(Py)_2Cl_2]^+$, 251 $[Ru(Py)Cl_2]^+$.

Reactivity of Sc^{III} Complex of 2: An equimolar amount of solid $Sc(OTf)_3$ was added to an NMR tube containing a 0.014 M solution of **2** in CD_3OD to give a deeper yellow colour corresponding to the formation of **2**·Sc^{III}. Free **2** was observed in the ^{1}H NMR spectrum a few minutes after addition of an equimolar amount of solid NaOAc. Another sample of the same solution of **2**·Sc^{III} complex in CD_3OD was treated with ethyl acetoacetate − no change was observed − while the addition of NaOAc in stoichiometric amount again caused formation of free **2**. **2**·Sc^{III}**:** ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 9.03 (dd, $J_{2,3}$ = 4.8, $J_{2,4}$ = 1.6 Hz, 1 H, 2-H), 8.66 (dd, $J_{4,3}$ = 7.9, $J_{4,2}$ = 1.6 Hz, 1 H, 4-H), 7.96 (dd, $J_{3,2}$ = 4.8, $J_{3,4}$ = 7.9 Hz, 1 H, 3-H) ppm. ESI(+)-MS (most intense signal of the cluster): *m*/*z* = 570 $[2 \cdot Sc(OTf)_2]^+$.

Synthesis of the Regioisomers 11/12: Ethyl acetoacetate (7.5 μL, 0.059 mmol) and 4-methylpyridine (26 μL, 0.27 mmol) were added to a solution of **2** (10.0 mg, 0.044 mmol) in absolute EtOH (1 mL) to give an intense blue colour. After stirring for 4 h, a reddish-brown suspension had formed. The mixture was concentrated to give a residue, which was then subjected to preparative TLC with CH_2Cl_2/MeOH (96:4). Extensive extraction of the reddish band with hot EtOH gave a mixture of **11** and **12** in a 12:88 ratio (8.8 mg, 60%). When the reaction was performed in other solvents, the same volume (1 mL) was used. For the reaction in the presence of metal salts, or for the two-step sequence, the same procedure reported for **4/5** was adopted. Each pure product could be isolated by two successive elutions of the same preparative TLC plate with EtOAc/Et_3N (95:5). **11:** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.80 (d, $J_{4,3}$ = 7.0 Hz, 1 H, 4-H), 9.00 (dd, $J_{7,8}$ = 4.6, $J_{7,9}$ = 1.6 Hz, 1 H, 7-H), 8.52 (dd, $J_{9,8}$ = 7.8, $J_{9,7}$ = 1.6 Hz, 1 H, 9-H), 8.15 (br. s, 1 H, 1-H), 7.62 (dd, $J_{8,9}$ = 7.8, $J_{8,7}$ = 4.6 Hz, 1 H, 8-H), 7.07 (dd, $J_{3,4}$ = 7.0, $J_{3,1}$ = 1.1 Hz, 1 H, 3-H), 4.49 (q, J = 7.1 Hz, 2 H, CH_3CH_2), 2.54 (s, 3 H, ArCH_3) 1.54 (t, J = 7.1 Hz, 3 H, CH_3CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ = 179.32 (s, C-10), 172.72 (s, C-5), 163.72 (s, -*C*OOEt), 154.02 (d, C-7), 149.45 (s, C-5a), 140.52 (s,

C-2), 128.29 (s, C-4b), 140.76 (s, C-11a), 135.36 (d, C-9), 130.83 (s, C-9a), 127.99 (d, C-4), 126.86 (d, C-8), 120.65 (d, C-3), 119.61 (d, C-1), 104.60 (s, C-11), 60.96 (t, O*C*H$_2$), 21.85 (q, *C*H$_3$–Ar), 14.09 (q, *C*H$_3$CH$_2$O) ppm. MS (70 eV, EI): *m/z* (%) = 334 (15) [M]$^+$, 290 (22), 262 (47), 205 (14), 97 (28), 83 (35), 71(34), 55 (54), 43 (100). HREI-MS: calcd. for $C_{19}H_{14}N_2O_4$ 334.0954; found 334.0956 ± 0.0010. **12:** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.68 (d, $J_{4,3}$ = 7.2 Hz, 1 H, 4-H), 9.00 (dd, $J_{8,7}$ = 4.7, $J_{8,6}$ = 1.6 Hz, 1 H, 8-H), 8.50 (dd, $J_{6,7}$ = 7.8, $J_{6,8}$ = 1.6 Hz, 1 H, 6-H), 8.19 (s, 1 H, 1-H), 7.64 (dd, $J_{7,6}$ = 7.8, $J_{7,8}$ = 4.7 Hz, 1 H, 7-H), 7.05 (d, $J_{3,4}$ = 7.2 Hz, 1 H, 3-H), 4.49 (q, J = 7.1 Hz, 2 H, CH$_3$C*H*$_2$), 2.50 (br. s, 3 H, ArC*H*$_3$) 1.51 (t, J = 7.1 Hz, 3 H, C*H*$_3$CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ = 178.42 (s, C-10), 173.34 (s, C-5), 163.77 (*C*OOEt), 153.47 (d, C-8), 149.67 (s, C-9a), 130.56 (s, C-5a), 140.76 (s, C-11a), 140.63 (s, C-2), 134.25 (d, C-6), 128.84 (s, C-4b), 127.60 (d, C-7), 127.09 (d, C-4), 121.44 (s, C-10a), 120.54 (d, C-3), 119.63 (d, C-1), 108.66 (s, C-11), 61.18 (t, O*C*H$_2$), 21.80 (q, *C*H$_3$–Ar), 14.17 (q, OCH$_2$*C*H$_3$) ppm. MS (70 eV, EI): *m/z* (%) = 334 (15) [M]$^+$, 290 (22), 262 (47), 205 (14), 97 (28), 83 (35), 71(34), 55 (54), 43 (100). HREI-MS: calcd. for $C_{19}H_{14}N_2O_4$ 334.0954; found 334.0956 ± 0.0010.

Synthesis of the Regioisomers 14 and 15: Ethyl acetoacetate (7.5 μL, 0.059 mmol) and pyridine (22 μL, 0.27 mmol) were added to a solution of **13** (10 mg, 0.044 mmol) in absolute EtOH (1 mL) to give an intense blue colour. The mixture was refluxed whilst stirring for 4 h to give a reddish-brown suspension, which was concentrated to give a residue that was subjected to preparative TLC with CH_2Cl_2/MeOH (96:4). Extensive extraction of the reddish band with CH_2Cl_2/EtOH (3:1) gave an inseparable mixture of **14** and **15** (7.6 mg, 54%). When the reaction was performed in other solvents, the same volume (1 mL) was used. For the reaction in the presence of metal salts, or for the two-step sequence, the same procedure reported for **4** and **5** was adopted. Pure products **14** and **15** could not be obtained by the chromatographic techniques adopted to separate the regioisomers **4** from **5** and **11** from **12**. **14 (Mixture with 15):** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.85 (br. d, $J_{4,3}$ = 7.0 Hz, 1 H, 4-H), 9.47 (s, 1 H, 6-H), 9.03 (d, $J_{8,9}$ = 4.8 Hz, 1 H, 8-H), 8.36 (br. d, $J_{1,2}$ = 9.1 Hz, 1 H, 1-H), 8.00 (d, $J_{9,8}$ = 4.8 Hz, 1 H, 9-H), 7.51 (m, 1 H, 2-H), 7.23 (br. t, $J_{3,2}$ = $J_{3,4}$ = 7.0 Hz, 1 H, 3-H), 4.52 (q, J = 7.1 Hz, 2 H, CH$_3$C*H*$_2$), 1.50 (t, J = 7.1 Hz, 3 H, C*H*$_3$CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 179.35 (s, C-10), 174.56 (s, C-5), 163.00 (*C*OOEt), 154.77 (d, C-8), 148.22 (d, C-6), 139.79 (s, C-11a), 139.62(s, C-5a), 128.49 (d, C-2), 128.52 (d, C-4b), 127.80 (d, C-4), 126.79 (s, C-9a), 121.28 (d, C-1), 119.19 (d, C-9), 118.04 (d, C-3), 106.26 (s, C-11), 60.96 (t, OCH_2), 14.09 (q, *C*H$_3$) ppm. MS (70 eV, EI): *m/z* (%) = 320 (92) [M]$^+$, 275 (91), 248 (100), 219 (11), 191(19), 164(28). HREI-MS: calcd. for $C_{18}H_{12}N_2O_4$ 320.0797; found 320.0791 ± 0.0030. **15 (Mixture with 14):** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.83 (br. d, $J_{4,3}$ = 7.0 Hz, 1 H, 4-H), 9.44 (s, 1 H, 9-H), 9.02 (d, $J_{7,6}$ = 4.9 Hz, 1 H, 7-H), 8.36 (br. d, $J_{1,2}$ = 9.1 Hz, 1 H, 1-H), 8.02 (d, $J_{6,7}$ = 4.9 Hz, 1 H, 6-H), 7.49 (m, 1 H, 2-H), 7.24 (br. t, $J_{3,2}$ = $J_{3,4}$ = 7.0 Hz, 1 H, 3-H), 4.50 (q, J = 7.1 Hz, 2 H, CH$_3$C*H*$_2$), 1.49 (t, J = 7.1 Hz, 3 H, C*H*$_3$CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 179.77 (s, C-10), 173.95 (s, C-5), 163.20 (*C*OOEt), 154.97 (d, C-7), 148.41 (d, C-9), 140.30 (s, C-11a), 139.38 (s, C-9a), 126.78 (s, C-5a), 128.61 (d, C-2), 128.52 (d, C-4b), 128.17 (d, C-4), 126.78 (s, C-5a), 121.00 (d, C-1), 119.28 (d, C-6), 117.89 (d, C-3), 106.26 (s, C-11), 60.96 (t, O*C*H$_2$), 14.09 (q, *C*H$_3$) ppm. MS (70 eV, EI): *m/z* (%) = 320 (92) [M]$^+$, 275 (91), 248 (100), 219 (11), 191(19), 164 (28). HREI-MS: calcd. for $C_{18}H_{12}N_2O_4$ 320.0797; found 320.0791 ± 0.0020.

Acknowledgments

We are grateful to Mr. A. Sterni for his technical support with the mass spectrometer. This work was financially supported by the Italian project PRIN2003 (Rome).

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Received: April 10, 2006
Published Online: July 27, 2006

