CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200701024

Preparation and Study of New Poly-8-Hydroxyquinoline Chelators for an anti-Alzheimer Strategy

Céline Deraeve,[a] Christophe Boldron,[a] Alexandrine Maraval,[a] Honoré Mazarguil,[b] Heinz Gornitzka,[c] Laure Vendier,[a] Marguerite Pitié,*[a] and Bernard Meunier[a, d]

Abstract: Fourteen different ligands have been synthesized with two covalently linked 8-hydroxyquinoline motifs that favor metal complexation. These bis-chelators include different bridges at the C2 positions and different substituents to modulate their physicochemical properties. They can form metal complexes in a ratio of one ligand per metal ion with Cu^{II} and Zn^{II}, two metal ions involved in the formation of amyloid aggregates of the toxic Aβ-peptides in the Alzheimer disease. The apparent affinity of all bis-8-hydroxyquinoline ligands for Cu^{II} and Zn^{II} are similar with $\log K_{Cu^{II}} \approx 16$ and $\log K_{Zn^{II}} \approx 13$ and are 10000 times more efficient than for the corresponding 8-hydroxyquinoline monomers. Their strong chelating capacities allow them to inhibit more efficiently than the corresponding monomers the precipitation of Aβ-peptides induced by Cu^{II} and Zn^{II} and also to inhibit the toxic formation of H_2O_2 due to copper complexes of Aβ. The best results were obtained with a one-atom linker between the two quinoline units. X-ray analyses of single-crystals of Cu^{II}, Zn^{II} or Ni^{II} complexes of 2,2′-(2,2-propanediyl)-bis(8-hydroxyquinoline), including a one-atom linker, showed that all heteroatoms of the bis-8-hydroxyquinoline ligand chelate the same metal ion in a distorted square-planar geometry. The Cu^{II} and Zn^{II} complexes include a fifth axial ligand and are pentacoordinated.

Keywords: amyloids · chelates · copper · peptides · zinc

Introduction

The Alzheimer disease is associated with the aggregation of β-type amyloid peptides (Aβ) in the brain, leading to the formation of amyloid plaques.[1] These 39- to 43-mer peptides result from the cleavage of the amyloid precursor protein (APP). $A\beta_{1-40}$ and $A\beta_{1-42}$ are the two major pathology-related peptides. They are toxic to neurons and their capacity to strongly chelate metal ions, particularly Cu^{II} and Zn^{II} (and also iron in a lesser extent), has been associated to this toxicity. It has also been suggested that Cu–Aβ complexes can generate H_2O_2.[1,2] The accumulation of redox active metal ions in these amyloid plaques is probably involved in the oxidative stress inducing neuronal lesions leading to irreversible brain damage. Both Cu^{II} and Zn^{II} also affect interactions between Aβ peptides and lipid membranes.

Among the different Aβ peptides, $A\beta_{1-42}$ has the highest affinity to metal ions and is fostered by Alzheimer disease-promoting mutations and risks factors. $A\beta_{1-42}$ is probably the most toxic amyloid peptide, but also the most difficult to study due to its capacity to generate self-organized aggregates.

Different metal-chelating agents are able to dissolve Aβ-deposits by removing metal ions from the amyloid aggregates.[3] The use of a hydrophobic metal chelator like clioquinol showed some clinical improvements in a Phase II trial, indicating that chelators are not only useful to study the disease but might also have some therapeutic potential.[4]

We have recently shown that ligand **1**, with a covalent linkage between two 8-hydroxyquinoline units was particu-

[a] Dr. C. Deraeve, Dr. C. Boldron, Dr. A. Maraval, Dr. L. Vendier, Dr. M. Pitié, Dr. B. Meunier
Laboratoire de Chimie de Coordination du CNRS
205 route de Narbonne, 31077 Toulouse cedex 4 (France)
Fax: (+33) 561-55-30-03
E-mail: pitie@lcc-toulouse.fr

[b] Dr. H. Mazarguil
Institut de Pharmacologie et Biologie Structurale du CNRS
205 route de Narbonne, 31077 Toulouse cedex 4 (France)

[c] Pr. H. Gornitzka
Laboratoire d'Hétérochimie Fondamentale et Appliquée (UMR 5069-CNRS), Université Paul Sabatier
118 route de Narbonne, 31062 Toulouse cedex 9 (France)

[d] Dr. B. Meunier
Current adress:
PALUMED, BP 28262, 31682 Labège cedex (France)

larly efficient to inhibit the Cu^{II}- or Zn^{II}-induced aggregation of the β-amyloid peptide $A\beta_{1-42}$ when compared with clioquinol which is a monomeric 8-hydroxyquinoline derivative (Figure 1).[5] Our idea was to favor metal complexation

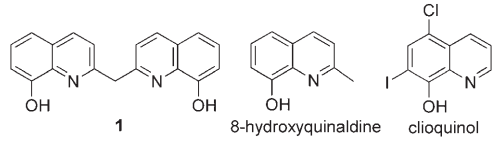


Figure 1. Structures of compound **1**, 8-hydroxyquinaldine and clioquinol.

by having two 8-hydroxyquinoline motifs around the metal ion to obtain in vivo a classical metal/ligand ratio for Cu^{II} and Zn^{II} for such type of ligand, having in mind that this ratio has a very poor probability to be observed in vivo when the two chelating entities are not covalently linked. Ligand **1** can also inhibit the production of H_2O_2 induced by the copper–$A\beta_{1-42}$ complexes and involved in the toxicity of the peptide. These results suggest that this ligand is a good candidate to study the role of metal ions in Alzheimer disease and might be considered as a prototype of a drug candidate.

We report here the preparation of different bis-8-hydroquinoline in order to study the influence of the linker length or of the nature of substituents with respect to their behavior with amyloid plaques. These parameters can modulate their physicochemical properties and, therefore, can influence their bioavailability, their stability and their metabolisation in vivo. All ligands are without asymmetric centers and are symmetric for synthetic purposes. Their activity was compared with clioquinol but also to 8-hydroxyquinaldine (Figure 1) that can be both considered as parent monomers of this bis-8-hydroxyquinoline series. They showed a good anti-amyloid activity to dissolve metal-aggregated $A\beta_{1-42}$ and to inhibit H_2O_2 production that correlates with their high ability to chelate the metal ions involved in the Alzheimer disease. Since the experiments were performed on the most amyloidogenic $A\beta_{1-42}$ peptide, it is reasonable to propose that these ligands can be active against all the other Aβ-peptides encountered in amyloid aggregates.

Results and Discussion

Ligands design and synthesis: Different substitutions on the CH_2 linker of **1** were performed (Scheme 1). Methylation yielded **3** as previously described by Yamamoto et al.[6] During the synthesis, single-crystals of [2,2-isopropylidenedi-8-quinolinolato]nickel(II) complex **2** suitable for X-ray analysis were obtained (Figure 2 and Table 1). A symmetric and monomeric Ni^{II} complex was observed. The geometry is square planar around the metal center with the two deprotonated 8-hydroxyquinoline chelating entities in *cis*-position, as expected from the design of this ligand series.[5]

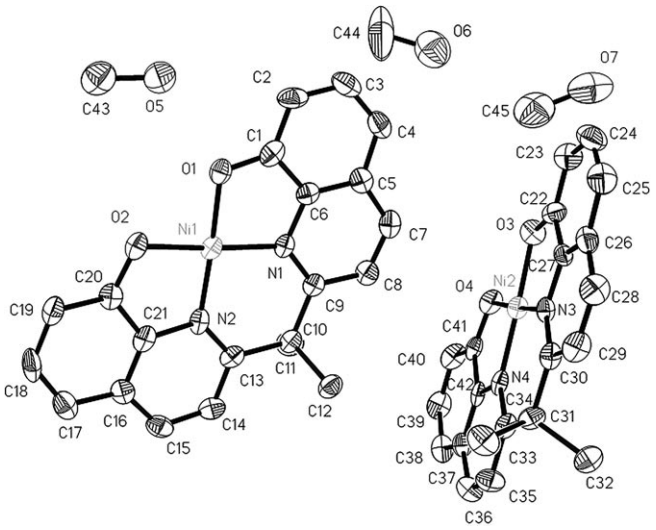


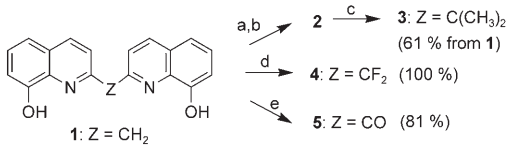
Figure 2. ORTEP drawing of complex **2**.

Table 1. Selected bonds lengths [Å] and angles [°] for **2**, **27**, **28** and **29**.

	2[a]	**27**	**28**	**29**
O1−C1	1.327(6)	1.330(3)	1.301(7)	1.342(3)
O2−C20	1.326(6)	1.333(3)	1.322(7)	1.334(3)
M1−O1	1.860(3)	1.9511(18)	1.942(5)	2.0982(19)
M1−O2	1.854(3)	1.9643(19)	1.941(4)	1.9706(19)
M1−N1	1.859(4)	1.969(2)	1.982(5)	1.983(2)
M1−N2	1.840(4)	1.956(2)	1.970(5)	1.960(2)
M1−O2(1		2.2641(18)		
M1−O3			2.283(5)	
M1−Cl1				2.3267(9)
O1-M1-N1	88.04(17)	85.50(9)	84.5(2)	80.88(8)
O1-M1-N2	177.28(17)	160.10(8)	162.0(2)	135.99(9)
O1-M1-O2	88.92(16)	95.03(8)	94.93(18)	90.75(8)
N1-M1-N2	94.34(18)	91.70(10)	90.7(2)	88.46(9)
O2-M1-N1	176.93(17)	171.96(9)	165.7(2)	157.65(9)
O2-M1-N2	88.69(17)	85.07(9)	85.38(19)	83.24(9)
O1-M1-O2(1		94.12(7)		
O2-M1-O2(1		86.23(8)		
N1-M1-O2(1		101.74(8)		
N2-M1-O2(1		105.73(8)		
O1-M1-O3			98.30(19)	
O2-M1-O3			92.09(19)	
N1-M1-O3			102.2(2)	
N2-M1-O3			99.72(19)	
O1-M1-Cl1				94.09(6)
O2-M1-Cl1				100.70(7)
N1-M1-Cl1				100.55(7)
N2-M1-Cl1				129.89(7)
C9-C10-C13	118.5(4)	113.0(2)	116.0(5)	111.1(2)
C11-C10-C12	110.0(4)	107.8(2)	106.7(6)	106.8(2)

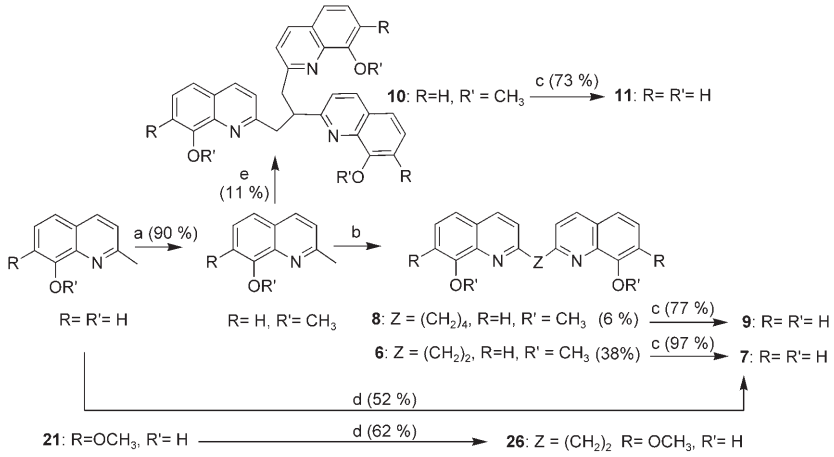
[a] Two molecules of complex appeared in the asymmetric crystal unit cell. Both showed similar values. Therefore, for clarity, values were only given here for one of both complexes.

Fluorinated ligand **4** was obtained by electrophilic fluorination of the linker of **1** with Selectfluor.[7] When compared with methylation, fluorination does not induce steric constraints because H and F substituents are isosteric. Ligand **1** was also quantitatively oxidized in **5** in aqueous alkaline conditions.



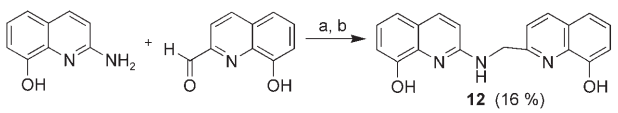
Scheme 1. Modifications of the CH_2 linker of compound **1**. a) $Ni(CH_3CO_2)_2$, CH_3OH, 60 °C; b) CH_3I, NaOH, CH_3OH, reflux; c) HCl, ethanol or EDTA, H_2O; d) Selectfluor, CH_3CN, RT; e) $NaHCO_3$, H_2O, THF, RT.

The length of the linker between the 8-hydroxyquinoline entities was also increased, from one to two and four atoms. Compound **7**, with an ethylene linker, was previously prepared by Albrecht et al.[8] and by Kitamura et al.[9] but with very poor yields: 5.6 and 13 % from 8-hydroxyquinaldine, respectively. We optimized the synthesis (Scheme 2). A first improvement consisted in performing a homo-coupling between two 8-methyloxyquinaldine in the presence of LDA (lithium diisopropylamine) and 1,2-dibromoethane at −95 °C to produce **6** (with the ethylene linker) in 38 % yield. The product was contaminated by compound **8** (with a butylene linker) that was easily removed by recrystallization. Then acid hydrolysis to remove the 8-methyloxy protective group yielded **7** (or **9** with the butylene linker). In fact the protection of the 8-hydroxyl function was found later as unnecessary and **7** was directly obtained in a better yield (52 %) by the same homo-coupling protocol from 8-hydroxyquinaldine. Interestingly, modifications of the reaction conditions (replacement of 1,2-dibromoethane by $CuCl_2$ as oxidizing agent and increase of the reaction temperature to 0 °C) gave an additional product from 8-methyloxyquinaldine: trimer **11** (Scheme 2). Compound **11** allowed us to document the effect of a higher number of 8-hydroxyquinoline residues in poly-8-hydroxyquinoline against metal complexes of Aβ.



Scheme 2. Synthesis of compounds **7**, **9**, **11** and **26**. a) CH_3I, K_2CO_3, acetone, RT; b) 1 equiv LDA, THF, −95 °C then $Br(CH_2)_2Br$; c) 48 % HBr, reflux; d) 2 equiv LDA, THF, −95 °C then $Br(CH_2)_2Br$; e) 3.7 equiv LDA, THF, 0 °C then $CuCl_2$.

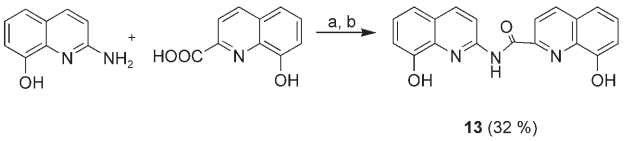
Bis-8-hydroxyquinoline ligands with other two-atom linkers were also prepared. A reductive amination of 8-hydroxyquinoline-2-carboxaldehyde by 2-amino-8-hydroxyquinoline produced **12** (with a CH_2NH linker, Scheme 3). A peptidic coupling reaction between 2-amino-8-hydroxyquinoline and 8-hydroxyquinoline-2-carboxylic acid allowed to obtain **13** (with a C(O)NH linker, Scheme 4).



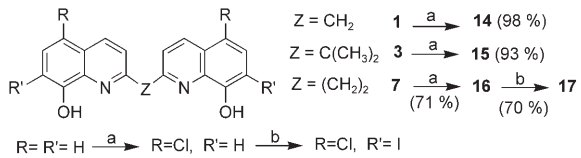
Scheme 3. Synthesis of compound **12**. a) $Cl(CH_2)_2Cl$, RT; b) $NaBH(CH_3CO_2)_3$, RT.

Different substituents were also introduced in the bis-8-hydroxyquinoline ligands. In the presence of *N*-chlorosuccinimide in acidic conditions, a selective chlorination on C5 carbons of **1**, **3** and **7** allowed to obtain **14**, **15** and **16**, respectively, in high yields (Scheme 5).[10] Then, a successive reaction with *N*-iodosuccinimide in the same conditions led to **17** (a dimeric analogue of clioquinol) from **16**.

Ligands **25** and **26** with methyloxy substituents on C7 carbon, in the *ortho*-position of the phenolic function, were also prepared. This type of substituents provide ligands with a par-

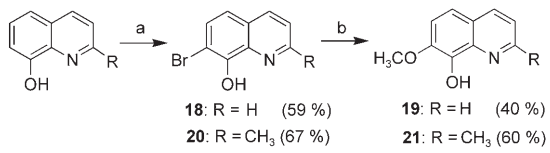


Scheme 4. Synthesis of compound **13**. a) BOP, HOBT, Et_3N, CH_2Cl_2, RT; b) H_2O, RT.



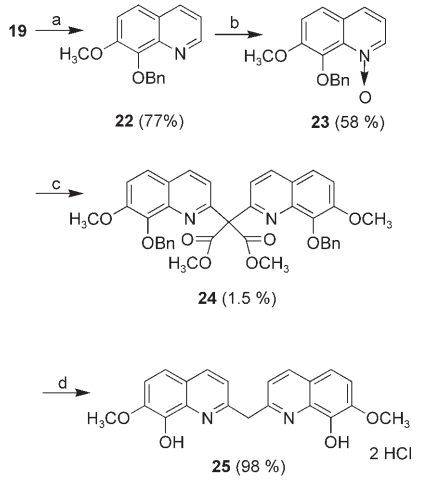
Scheme 5. Halogenation of bis-8-hydroxyquinoline ligands. a) *N*-chlorosuccinimide, H_2SO_4, RT; b) *N*-iodosuccinimide, H_2SO_4, CH_3OH, RT.

tial homology of structure with curcumin, which is a dimeric molecule active in dissolving Aβ-deposits.[11] Selective bromination, in alkaline conditions, on the C7 position of 8-hydroxyquinoline monomer generated the expected bromine-containing compounds (Scheme 6).[12] Then, a nucleophilic



Scheme 6. Synthesis of 7-methyloxy-8-hydroxyquinoline derivatives. a) *N*-bromosuccinimide, *t*BuNH$_2$, toluene; b) NaOCH$_3$, CuCl$_2$, DMF, reflux.

substitution of the bromine atom by CH$_3$ONa led to the 7-methyloxy-8-hydroxyquinoline precursors **19** and **21**.[13] They were used to prepare **25** (with a methylene linker, Scheme 7) and **26** (including an ethylene linker, Scheme 2)



Scheme 7. Synthesis of compound **25**. a) Benzylchloride, K$_2$CO$_3$, CH$_3$CN, reflux; b) *m*-CPBA, CH$_2$Cl$_2$, RT; c) CH$_2$(CO$_2$CH$_3$)$_2$, acetic anhydride, RT; d) 37% HCl, reflux.

with methods used for the syntheses of **1**[6] and **7**, respectively. Interestingly, X-ray analysis of single-crystals of intermediate **24** confirmed the position of the substituents on the ligand (Figure 3).

Copper(II) and zinc(II) chelation: The stoichiometry of the complexes with these different ligands and Cu^{II} or Zn^{II}—the two major metal ions (M) involved in the formation of amyloid plaques—was determined by metal titration. Experiments were performed in buffered saline conditions (at pH 7.4) in order to mimic the medium used during experiments involving the amyloid-β peptide.

For the majority of the bis-8-hydroxyquinoline ligands (L) studied here for a M/L ratio increased from 0 to 1, the π→π* transition centered near 248–262 nm for the free ligand

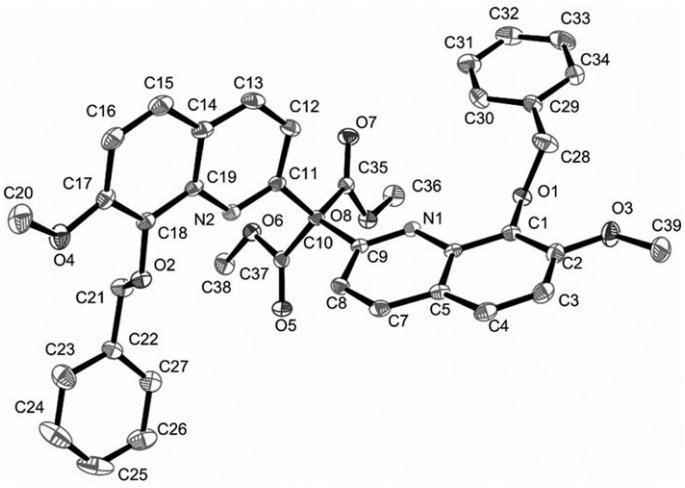


Figure 3. ORTEP drawing of compound **24**.

shifted to lower energy with concomitant appearance of an absorption band in the visible region (λ_{max} near 374–420 nm) probably due to a MLCT transition. At higher M/L ratios, no further change in UV-visible absorption was observed. The observation of isobestic points was in accordance with the formation of only one type of metal complex. These results correlated with the ability of the bis-8-hydroxyquinoline ligands to form only one type of Cu^{II} or Zn^{II} complex with a 1:1 metal/ligand ratio. This M/L ratio was confirmed by mass spectrometry. A typical example, obtained for the CuCl$_2$ titration with **3**, is given on Figure 4.

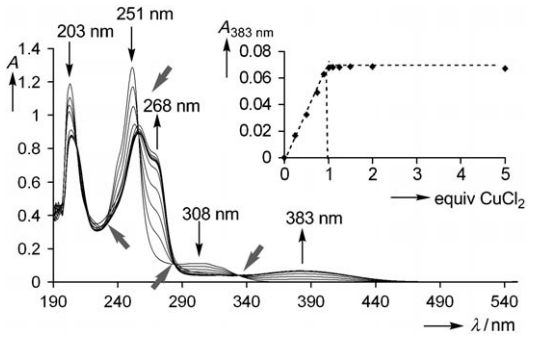


Figure 4. UV-visible spectra of ligand **3** (L, 15μM) in CH$_3$OH/Tris saline buffer pH 7.4 1:1, in the presence of different ratios of CuCl$_2$. Black arrows show the variation of absorbance when the Cu/L ratio increases. Grey arrows show isobestic points. The variation of absorbance at $\lambda =$ 383 nm show the stabilization of the complexation when Cu/L = 1.

Interestingly, major differences were observed in the case of **13** (including an amide linker) that formed multiple complexes with CuCl$_2$. This ligand also required more than one equivalent of Zn^{II} per ligand to obtain the stabilization of the titration although only one species was formed. The **13**-Zn^{II} complex, with a M/L ratio of one, was observed by mass spectrometry. One can propose that the affinity constant of **13** for Zn^{II} was weak in the conditions used. There-

fore an excess of Zn^{II} was required for 100% of complexation.

No reliable data were obtained during UV/Vis analyses involving Cu^{II} or Zn^{II} ions and the ligands **1**, **14** and **25** containing a CH_2 linker. The spectra changed with time showing a poor stability of these complexes under the conditions used. However, the ability of **1** and **14** to form a 1:1 metal/ligand complexes was confirmed by mass spectrometry.

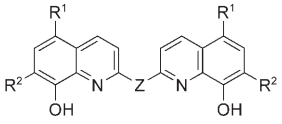
The affinity of bis-8-hydroxyquinoline ligands (excepted **1**, **13**, **14** and **25**) for Cu^{II} or Zn^{II} was estimated, in the buffered saline conditions used for UV titrations, via competitive chelation experiments with EDTA. This well-known ligand was selected after several trials with different chelators covering a large scale of affinity constants for Cu^{II} or Zn^{II}. EDTA appeared to be the most convenient competitor for all ligands. The logarithm of the apparent affinity constants of EDTA are 15.9 for Cu^{II} and 13.7 for Zn^{II} in the used conditions at pH 7.4.[14,15] The percentage of bis-8-hydroxyquinoline complex obtained in the presence of one equivalent of EDTA and one equivalent of metal ion was deduced from UV/Vis spectra and this value was used to estimate the apparent affinity constants (Table 2), as described in the Experimental Section.

All studied bis-8-hydroxyquinoline ligands have similar apparent affinity with $\log K_{Cu^{II}} \approx 16$ and $\log K_{Zn^{II}} \approx 13$ in the experimental conditions used. Interestingly, a comparison with the apparent affinity constants of 8-hydroxyquinaldine ($\log K_{LCu^{II}} = 11.0$, $\log K_{L_2Cu^{II}} = 7.7$ and $\log K_{appLCu^{II}} = 9.3$ at pH 7.4; $\log K_{LZn^{II}} = 9.1$, $\log K_{L_2Zn^{II}} = 8.8$ and $\log K_{appLZn^{II}} = 6.4$ at pH 7.4),[14b] the parent compound for the bis-8-hydroxyquinoline series, indicates that the bis-8-hydroxyquinoline strategy is particularly efficient to obtain ligands with high affinity constants for Cu^{II} and Zn^{II}.

Crystal structures of copper(II) complexes of ligand 3: Ligand **3** was metalated with one equivalent of different cupric salts [$Cu(OAc)_2$, $CuSO_4$ or $CuCl_2$]. Different single-crystals were obtained and were analyzed by X-ray diffraction. All complexes are five-coordinated, neutral, with a more or less distorted square-pyramidal configuration and a coordination sphere with all the heteroatoms of the ligand. Oxygen and nitrogen atoms of the ligand occupy the equatorial plane. However, major differences are observed due to the recrystallization conditions or to the copper salt used for metalation. They provide information concerning the structures that can adopt such cupric complexes of bis-8-hydroxyquinoline bridged on the C2 position of the quinoline entity. To our knowledge, they have never been previously described from analysis of single-crystals.

When ligand **3** was metalated in the presence of $Cu(OAc)_2$, precipitated and then recrystallized in CH_3OH, the dimeric complex **27** was obtained (Figure 5 and Table 1) with both copper centers showing the same distorted square-pyramidal geometry. One of the two oxygens of each ligand is μ-coordinated, with a participation to the equatorial plane of one copper center and a second engagement in

Table 2. Affinity constants of bis-8-hydroxyquinoline ligands for Cu^{II} and Zn^{II} ($\log K_{aff}$), partition coefficients ($\log D_{7.4}$) and inhibition of the precipitation of Aβ and of the production of H_2O_2.



Compound	Size of Z	Z	R^1	R^2	$\log K_{aff}$ for LM^{II} Cu^{II}	$\log K_{aff}$ for LM^{II} Zn^{II}	$\log D_{7.4}$	Soluble Aβ (%)[a] No metal	Cu^{II}	Zn^{II}	nmol H_2O_2 (% recovered H_2O_2)[b] No Aβ	Cu/Aβ = 2	Cu/Aβ = 0.75
no ligand	–	–	–	–	–	–	–	55 ± 4	18 ± 2	29 ± 3	2.93 ± 0.18	2.77 ± 0.16	1.08
1	1	CH_2	H	H	nd	nd	3.3 ± 0.1	63 ± 4	60 ± 5	63	0.54	0.75 ± 0.11	0.76
14	1	CH_2	Cl	H	nd	nd	3.5 ± 0.1	66	45 ± 5	57	0.56	0.80 ± 0.23	0.61
25	1	CH_2	H	CH_3O	nd	nd	nd	53	48 ± 3	50	nd	0.70	nd
3	1	$C(CH_3)_2$	H	H	15.9	13.0	4.4 ± 0.1	56	47 ± 3	59	0.84 (100)	0.71 ± 0.02 (100)	0.55
15	1	$C(CH_3)_2$	Cl	H	15.5	12.5	4.9 ± 0.1	55	30	49	0.12	0.37 ± 0.09 (97)	0.35
4	1	CF_2	H	H	15.7	13.9	3.8 ± 0.1	60	51 ± 1	50	0.39	0.55 ± 0.08 (97)	nd
5	1	C=O	H	H	16.6	14.4	3.9 ± 0.1	56	58 ± 3	54	0.82	0.86 ± 0.11 (92)	nd
7	2	$(CH_2)_2$	H	H	16.1	13.9	3.5 ± 0.1	59	35	60	0.75	0.89 ± 0.07	0.68
16	2	$(CH_2)_2$	Cl	H	15.5	13.0	4.2 ± 0.1	59	36	58	0.66	0.89 ± 0.09	0.64
17	2	$(CH_2)_2$	Cl	I	16.2	14.2	5.6 ± 0.5	36	19	47	0.66	0.82 ± 0.08	0.82
26	2	$(CH_2)_2$	H	CH_3O	16.0	13.3	3.5 ± 0.1	63 ± 3	38 ± 4	42 ± 4	nd	0.69	nd
12	2	CH_2NH	H	H	15.7	12.6	nd	45	43	nd	nd	nd	nd
13	2	C(O)NH	H	H	nd	nd	3.0 ± 0.1	66	46 ± 3	62 ± 5	0.47	0.47	0.45
9	4	$(CH_2)_4$	H	H	15.7	13.5	3.8 ± 0.1	52	42	43	nd	nd	nd
11(trimer)	–	–	–	–	nd	nd	3.7 ± 0.1	48	48	45	nd	nd	nd
8-hydroxyquinaldine		–	–	–	11[c]	9.1[c]	2.4 ± 0.1	69	52	62	0.72	0.77	0.79
clioquinol		–	–	–	–	–	3.8 ± 0.1	63	55 ± 3	37	0.54	0.78 ± 0.08	0.57

[a] $A\beta_{1-42}$ (5 μM) was incubated for 1 h at 37 °C in 20 mM Tris·HCl buffer (pH 7.4), 150 mM NaCl with or without 20 μM metal salt ($CuCl_2$ or $ZnCl_2$) then for another 1 h in the presence of 200 μM ligand followed by centrifugation. Quantities of soluble and precipitated $A\beta_{1-42}$ were determined in the supernatant and the pellet, respectively, with MicroBCA. [b] Experiments were performed in the presence of $A\beta_{1-42}$ (0.2 or 0.53 μM), 0.4 μM $CuCl_2$, 0.4 μM ligand (0.8 μM in the case of 8-hydroxyquinaldine and clioquinol), 10 μM ascorbate and air. Generated H_2O_2 (in nanomoles) was quantified with AmplexRed Assay. The recovered percentage of 1.5 nmol of H_2O_2 added in the medium was given into brackets. [c] From reference [14b]; nd: not determined.

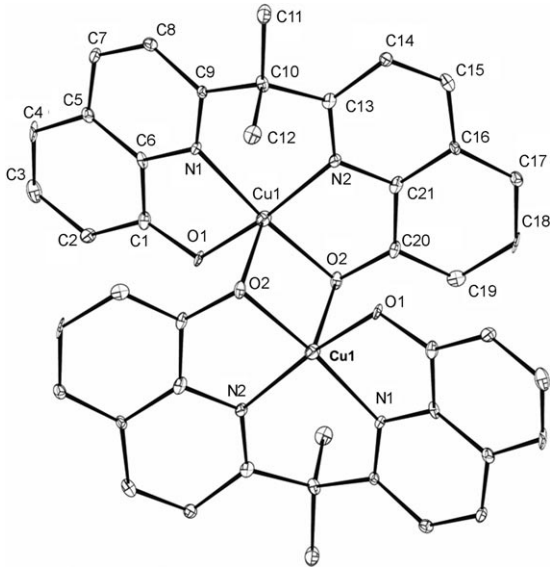


Figure 5. ORTEP drawing of complex **27**.

the axial position of the second copper center. Since the complex is neutral the phenolic substituents of **3** are deprotonated to generate **27**.

When ligand **3** was dissolved in DMF to be metalated with an aqueous solution of $CuSO_4$, single-crystals of monomeric complex **28** were obtained (Figure 6 and Table 1).

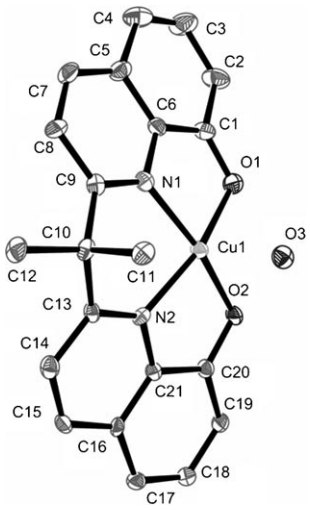


Figure 6. ORTEP drawing of complex **28**.

These experimental conditions are similar to those used by Di Vaira et al. to obtain single-crystals of (clioquinol)$_2$Cu^{II}.[17] Oxygen of a molecule of water crystallization solvent (O3) occupies the axial position of the copper complex.

When ligand **3** was dissolved in CH_3OH and then metalated with an aqueous solution of $CuCl_2$ (the same salt used for the determination of affinity constants), mono-crystals of monomeric complex **29** were obtained (Figure 7 and Table 1). The geometry of the copper center of **29** can be described as a highly distorted square-planar pyramid with a

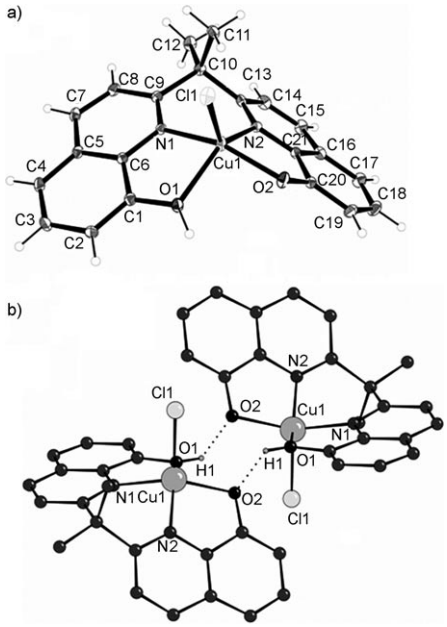


Figure 7. a) ORTEP drawing of complex **29**. b) Stabilization of two complexes by hydrogen bonds.

chloride ion as axial ligand. Only one of the two phenolic positions of **3** is deprotonated to form **29**, it was named O2 on the structure. On the other quinoline unit of the ligand, a hydrogen atom was located on O1 by Fourier differences map, with an O1–H bond of 0.93 Å. However, the angles in the crystal structure deviate from ideal values for a square planar pyramid and the geometry can be also described as a highly distorted trigonal bipyramidal arrangement around the metal ion. In this last proposition, the equatorial positions are occupied by the chloride ion, the phenolic atom of one of the both quinoline units of the ligand and by the nitrogen of the other quinoline unit. The other nitrogen and oxygen of the ligand are in axial position and this last oxygen is deprotonated in a phenolate form. Importantly, two copper complexes are stabilized by two intermolecular hydrogen bonds between the phenol hydrogen of one complex and the phenolate of the other one at 1.69 Å. The O···O separation was 2.439(3) Å and the angle O-H···O was 135.7°.

Interestingly, the two pK values of 8-hydroxyquinoline and 2-methyl-8-hydroxyquinoline monomer have been reported to be around 9.8 (close to the 9.6 value of phenol) and 11.7 for pK_1 and 5.0 (close to the 5.2 value of pyridine) and 4.6 for pK_2, respectively.[14b] It is reasonable to assume that bis-8-hydroxyquinoline ligands have similar values. Therefore, their cupric complexes could be deprotonated on both phenolic moieties of the ligand during experiments in the buffered medium (at pH 7.4) that was used in the other

experiments described in this manuscript. It can be noted that crystal structures of cupric complexes of 8-hydroxyquinoline[18] or clioquinol[17] have been obtained with two quinoline entities, chelating, as phenolate, a copper ion in a more or less distorted square-planar geometry. Copper was four-coordinated. Therefore, such square-planar geometry could exist in buffered medium for cupric complexes of bis-8-hydroxyquinoline. This proposition is reasonable because: i) all the heteroatoms of the ligand are more or less in an equatorial position in the copper complexes **27**, **28** and **29**; ii) the phenolic part of these complexes appeared easily deprotonable since they are partially or totally deprotonated in the complexes although no special treatment (like an addition of base) was performed to favor this deprotonation; and iii) this type of square planar geometry was also observed in the case of **2**, the nickel(II) complex of ligand **3**.

However, in such organization of bis-8-hydroxyquinoline copper complexes involving two phenolate residues, five-or six-coordinated copper complexes with one or two solvent molecule (such as water) could be also present in the buffered solution since this type of structure has been also previously observed for Cu^{II} complexes involving two 8-hydroxyquinolines[19] or two 8-hydroxyquinaldines.[20]

Crystal structure of the (ligand 3)–zinc(II) complex: For comparison, we tried to grow single-crystals of the Zn^{II} complexes of ligand **3**. Unfortunately they crystallized as particularly thin needles and it was difficult to obtain single-crystals suitable for X-ray analysis. We obtained, however, single-crystals of complex **30** (Figure 8) when ligand **3** was

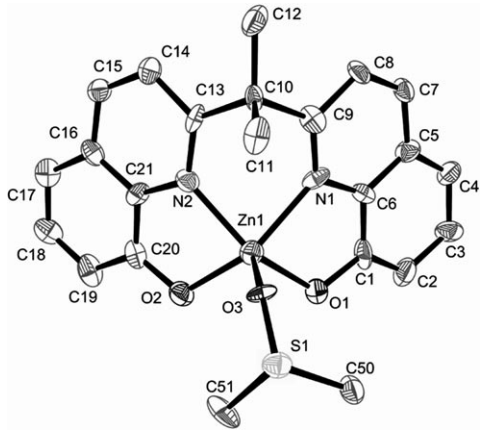


Figure 8. ORTEP drawing of complex **30**.

metalated with one equivalent of $ZnSO_4$ then recrystallized from a H_2O/DMSO mixture. The crystal was very weakly diffracting, so that a large proportion of essentially "unobserved" reflections were used in the refinement, with an elevated R_{int} value (>20%). Nevertheless, our model is good enough for the discussion of the main features of this complex. Zn^{II} complex **30** shows a structure similar to the ones observed for Cu^{II} complexes of the same ligand. Complex **30** is five-coordinated, distorted square pyramidal and shows a structure where all the heteroatoms of a same ligand molecule coordinate the same metal ion. Oxygen and nitrogen atoms of the ligand **3** occupy the equatorial plane. The axial position is occupied by the oxygen of a molecule of DMSO used as solvent of crystallization.

Analysis of the hydrophobicity of the ligands: The partition of the ligands between 1-octanol and a physiological aqueous phase was also measured (Table 2). 8-Hydroxyquinaldine appeared to be poorly hydrophobic ($\log D_{7.4}=2.4$ suggesting that this molecule is 250 times more soluble in 1-octanol than in the buffer at physiological pH 7.4 value). The halogenation increased highly the solubility in 1-octanol since clioquinol had a log $D_{7.4}$ value of 3.8 (the hydrophobicity is known to favor the passage of a molecule through the blood-brain barrier).[21]

The $\log D_{7.4}=3.3$ value for **1** was higher than that of 8-hydroxyquinaldine, its monomeric analogue. This can be considered as an advantage for molecules that should go to the brain. The $\log D_{7.4}$ increased also with the addition of fluoro, keto or methyl substituents on the linker of **1** ($\log D_{7.4}=3.3$, 3.8, 3.9 and 4.4 for **1**, **4**, **5** and **3**, respectively). This $\log D_{7.4}$ value also increased with the length of the aliphatic linker ($\log D_{7.4}=3.3$, 3.5 and 3.8 for **1**, **7** and **9**, respectively). Chlorination of the aromatic heterocycles allowed also to increase this parameter ($\log D_{7.4}=3.3$ and 3.5, 4.4 and 4.9, 3.5 and 4.2 when **1**, **3** and **7** are compared to their 5-dichloro derivatives **14**, **15** and **16**, respectively). Iodination had the same effect (compound **17**). Other substituents such as methyloxy on the C7 carbons did not change the $\log D_{7.4}$ value (that was the same for **7** and **26**).

Therefore the modifications of bis-8-hydroxyquinoline could modulate the biodistribution of the molecules. Importantly, excepted for **17**, the modifications allowed to obtain molecules with molecular weight <500, which is another parameter known to be favorable to a good bioavailability.[21]

Inhibition of the precipitation of $A\beta_{1-42}$ induced by Cu^{II} or Zn^{II}: The ability of poly-8-hydroxyquinoline ligands to inhibit the precipitation of $A\beta_{1-42}$ induced by Cu^{II} or Zn^{II} was compared in the conditions previously used to analyze ligand **1** (Table 2).[5] The peptide was incubated for one hour in the presence of four equivalents of $CuCl_2$ or $ZnCl_2$ followed by the addition of 40 equivalents of the ligand. After another hour of incubation, the quantity of soluble $A\beta_{1-42}$ after a centrifugation step was determined by microBCA assay.

Without addition of metal ion or ligand, only 55% of $A\beta_{1-42}$ was soluble. The addition of $CuCl_2$ strongly reduced the solubility of Aβ-peptide (only 18% of $A\beta_{1-42}$ remained soluble), more than with $ZnCl_2$ (29% of soluble $A\beta_{1-42}$).

Compound **1** appeared as the most efficient ligand to inhibit the peptide precipitation but other 8-hydroxyquinoline derivatives were active. Important remarks can be deduced from a comparison of the results obtained.

The incorporation of a substituent on the quinoline residues induced a decrease of the inhibition of the precipitation of $A\beta_{1-42}$ (compare **1** with **14** and **25**; **3** with **15**; **7** with **26**; **16** with **17**, respectively). Compound **17** with chloride and iodide substituents was rather inefficient.

Compounds including a one-atom linker (**1**, **14**, **25**, **3**, **15**, **4** and **5**) are generally more active than ligands including larger linkers (**7**, **16**, **17**, **26**, **12**, **13** and **9**). This result was particularly clear in the inhibition of the precipitation of $A\beta_{1-42}$ induced by $CuCl_2$ (47–60% of solubility of $A\beta_{1-42}$ for experiments involving one-atom linker ligand to compare to only 35–46% for experiments involving two-atom linkers in the case of $R^1=R^2=H$).

The nature of the linker modulates also the activity. In the one-atom linker series, the best results were obtained with a CH_2 linker but bis-8-hydroxyquinoline ligands including CF_2 (**4**) or CO (**5**) gave good results since they were also able to restore the solubility of $A\beta_{1-42}$ observed in the absence of added metal ion.

The incorporation of another 8-hydroxyquinoline residue did not increase the activity of poly-8-hydroxyquinoline for the activity studied here (compare dimers **1** or **7** with the trimer **11**).

A clear interest of the use of **1** compared with clioquinol was previously observed when $A\beta_{1-42}$ was precipitated with 2.5 equiv of $CuCl_2$.[5] This ratio of Cu^{II} per peptide is the minimal quantity of Cu^{II} inducing the maximum of $A\beta_{1-42}$ precipitation. One equivalent of **1** per Cu^{II} was sufficient to obtain the same peptide solubility observed without metal ion. Clioquinol was less efficient, although two equivalents of clioquinol per Cu^{II} were used to respect the metal/ligand ratio of two 8-hydroxyquinoline residues per metal ion in the case of **1** and clioquinol.

The same experiment was performed for the most active ligands of the amyloid dissolution experiments: **1**, **3**, **4** and **5** (with one-atom linker) and **13** (the best compound in the two-atom linker series) (Figure 9). Importantly, all bis-8-hydroxyquinoline gave statistically the same results for $A\beta_{1-42}$ solubility when compared to results obtained in conditions involving an excess of metal ion and ligand. For these ligands, a 1:1 ratio for ligand/Cu was sufficient whereas mono-8-hydroxyquinoline derivatives (8-hydroxyquinaldine or clioquinol) failed to induce a large inhibition of $A\beta_{1-42}$ precipitation.

Therefore, the use of bis-8-hydroxyquinoline ligands having a one-atom linker is favorable to maintain the solubility of Aβ-peptides.

Inhibition of the synthesis of H_2O_2 induced by copper complexes of $A\beta_{1-42}$: Aβ-Cu, in the presence of a reductant, exerts a toxicity correlated with the generation of H_2O_2.[2] The ability of the bis-8-hydroxyquinoline chelators to inhibit the formation of H_2O_2 by $CuCl_2$ in the absence of $A\beta_{1-42}$ or for a $Cu/A\beta_{1-42}$ ratio = 2 or 0.75 was compared, see Table 2. In the conditions used, $A\beta_{1-42}$, $CuCl_2$ or copper complexes of $A\beta_{1-42}$ did not generate a significant quantity of H_2O_2 in the absence of ascorbate but copper complexes ($A\beta{\cdot}Cu_n$ or $CuCl_2$) in the presence of ascorbate were catalytically able to produce H_2O_2.

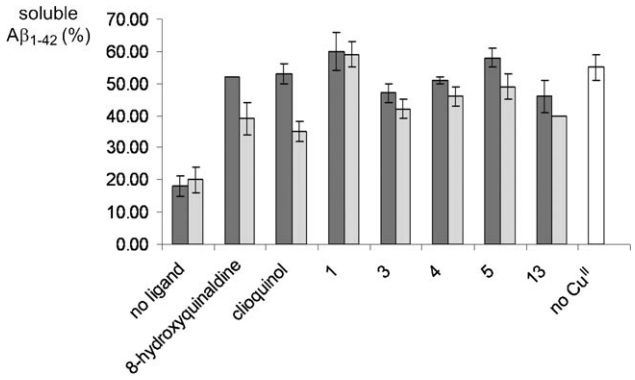


Figure 9. Variation of the percentage of soluble $A\beta_{1-42}$ peptide in the presence of different stoichiometries of $CuCl_2$ and 8-hydroxyquinoline derivatives. Dark grey: $A\beta_{1-42}$ (5 μM) was incubated in the presence of 4 equiv of $CuCl_2$ then 40 equiv of 8-hydroxyquinoline derivative then centrifuged. Clear grey: $A\beta_{1-42}$ (5 μM) was incubated in the presence of 2.5 equiv of $CuCl_2$ then 2.5 equiv of bis-8-hydroxyquinoline derivative (5 equiv in the case of 8-hydroxyquinaldine and clioquinol) then centrifuged. Percentage of peptide in the supernatant (soluble Aβ) and the precipitate (aggregated Aβ) were quantified with MicroBCA Assay.

In the absence of Aβ, all ligands inhibited the H_2O_2 formation mediated by $CuCl_2$ in the presence of ascorbate (2.93 nmol H_2O_2).

All chelators were also able to inhibit H_2O_2 formation mediated by $A\beta_{1-42}{\cdot}Cu_n$ complexes in the presence of ascorbate. For a $Cu/A\beta_{1-42}$ ratio = 2, the ligands (in the proportion of one equivalent per copper ion) were able to reduce the production of H_2O_2 to 15–30% of the quantity produced by $A\beta_{1-42}{\cdot}Cu_2$ in the absence of chelator (only 0.37–0.89 nmol H_2O_2 were produced compare with 2.77 nmol without ligand added). For a $Cu/A\beta_{1-42}$ ratio = 0.75, the copper complex of amyloid peptide produced less H_2O_2 (1.08 nmol) and again one equivalent of chelator per copper reduced significantly the formation of H_2O_2.

The addition of a known quantity of H_2O_2 in the reaction medium containing **3**, **15** or **4** (three one-atom linker ligands that showed interesting performances in the experiments just above) allowed us to verify that their copper complexes did not degrade H_2O_2 (97–100% of the added H_2O_2 was still present at the end of the analysis). We have previously shown that Aβ-Cu did not degrade H_2O_2 in these conditions.[22] This control allowed us to confirm that the observed decrease of H_2O_2 formation was in fact due to product inhibition (and not to its putative degradation) by the addition of **3**, **15** or **4**. These ligands probably removed copper from Aβ to generate redox-inactive copper complexes (**3**·Cu, **15**·Cu or **4**·Cu) in the presently used experimental conditions. Importantly, this proposition is not valuable for all the different ligands evaluated here since for experiments performed in the presence of **5**, a weak but real degradation of H_2O_2 was observed (only 92% of added H_2O_2 was recovered).

Conclusion

Fourteen symmetric bis-8-hydroxyquinoline ligands were prepared and they are efficient chelators of metal ions such as Cu^{II} and Zn^{II}. These metal chelators are able to inhibit the precipitation of a 42-mer Aβ-peptide enhanced by Cu^{II} and Zn^{II} ions and also to inhibit the H_2O_2 production associated to an oxidative stress induced by Aβ. The hydrophobicity of bis-8-hydroxyquinoline ligands seems to be adapted for the passage of the blood-brain barrier to reach the toxic amyloid aggregates involved in Alzheimer disease. Variations of substituents on these bis-8-hydroxyquinolines have limited effects on the metal affinity or on the ability to inhibit H_2O_2 production but significant structure/activity relationship can be deduced from ligand-induced dissolution of metal-aggregated $A\beta_{1-42}$. The best results were obtained for a one-atom linker between both 8-hydroxyquinoline units. Compound **1** (with a CH_2 linker) was particularly active but its chelation to Cu^{II} or Zn^{II} was difficult to analyze in physiological conditions. Substitutions of the methylene hydrogens of **1** by two methyl groups (ligand **3**), fluoro atoms (ligand **4**) or a keto function (ligand **5**) induced only a limited decrease of their activity and these chelators are less susceptible to an oxidative metabolism in vivo. Introduction of substituents such as chloride, iodide or methyloxy on 8-hydroxyquinoline heterocycle had a negative effect in experiments involving Aβ-peptide solubility.

Better in vitro results were obtained against the amyloid peptide with these bis-quinoline ligands compared with the monomeric derivatives, especially when a 1:1 Cu/ligand ratio was utilized.

Experimental Section

General: 2,2′-Methanediyl-bis(8-hydroxyquinolinium) dichloride dihydrate (**1**),[6] 8-methyloxyquinaldine[9] and 7-bromo-8-hydroxyquinoline (**18**)[12] were synthesized as previously described. DMF and CH_3CN were dried over 4 Å molecular sieves. CH_2Cl_2 was dried over basic alumina. THF was distilled over benzophenone and sodium. Other commercially available reagents and solvents were purchased from standard chemical suppliers and used without further purification. NMR spectra were recorded on Bruker spectrometers at 200, 250, 300 or 500 MHz. UV/Vis spectra were recorded on a Hewlett-Packard 8452 A diode array spectrophotometer or a Perkin-Elmer Lambda 35 spectrophotometer. Chromatographic purifications were performed on silica gel.

X-ray analysis: Data collection were collected at low temperature (180 or 193 K) on a Bruker-CCD (for **2**), an Xcalibur Oxford Diffraction diffractometer (for **24**, **28** and **29**) or an IPDS STOE diffractometer (for **27** and **30**) using a graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) and equipped with an Oxford Cryosystems (Cryostream Cooler Device). The structures have been solved by direct methods using SHELXS-97[23] (for **2**) or SIR92[24] (for **24**, **27**–**30**) and refined by means of least-squares procedures on F^2 with the aid of the program SHELXL97[25] include in the software package WinGX version 1.63.[26] The Atomic Scattering Factors were taken from International tables for X-Ray Crystallography.[27] Most of the hydrogens atoms were geometrically placed and refined by using a riding model, few of them were located by Fourier differences map and refined by using a riding model. All non-hydrogens atoms were anisotropically refined, and in the last cycles of refinement a weighting Scheme was used, where weights are calculated from the following formula: $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ where $P = (F_o^2 + 2F_c^2)/3$. Drawings of molecule are performed with the program ORTEP32 with 30% probability displacement ellipsoids for non-hydrogen atoms (excepted for **2** that was drawn with 50% probability).[28] The crystal data for the determined structures are given in Table 3.

CCDC 652781 (**29**), 652782 (**24**), 652783 (**28**), 652784 (**27**), 652785 (**30**), and 652786 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Table 3. Details of crystallographic measurements and refinements.

	2	**24**	**27**	**28**	**29**
formula	$C_{22.5}H_{22}N_2NiO_{3.5}$	$C_{39}H_{34}N_2O_8$	$C_{42}H_{32}Cu_2N_4O_4\,4(CH_4O)$	$C_{21}H_{18}CuN_2O_3$	$C_{21}H_{17}ClCuN_2O_2$
M_w	435.13	658.68	911.98	409.91	428.36
T [K]	193 (2)	180	110	180	180
crystal system	triclinic	monoclinic	triclinic	orthorhombic	triclinic
space group	$P\bar{1}$	$P12_11$	$P\bar{1}$	*Pbca*	$P\bar{1}$
a [Å]	12.456(4)	10.2593(9)	8.4208(12)	15.9572(15)	9.4456(10)
b [Å]	12.650(4)	10.8311(10)	10.5632(16)	9.1716(8)	9.9688(12)
c [Å]	13.984(5)	15.2192(14)	11.4970(17)	23.850(2)	11.4489(8)
α [°]	112.260(5)	–	89.769(12)	–	64.633(9)
β [°]	105.956(5)	97.622(7)	87.858(12)	90	82.430(7)
γ [°]	90.115(6)	–	81.443(12)	–	63.962(11)
V [Å^3]	1946.7(11)	1676.2(3)	1010.6(3)	3490.5 (5)	873.12(19)
Z	4	2	1	8	2
ρ_{calcd} [g cm^{-3}]	1.485	1.305	1.499	1.560	1.629
μ [mm^{-1}]	1.026	0.092	1.114	1.276	1.423
$F(000)$	908	692	474	1688	438
crystal size [mm]	0.1× 0.4×0.8	0.49×0.17×0.15	0.15×0.12×0.05	0.125×0.1×0.05	0.2×0.1×0.02
θ range [°]	5.10–23.26	2.70–32.12	2.89–32.04	2.13–25.99	2.46–32.16
collected/independent reflns	12267/5521	17105/5799	10909/6440	25850/3402	9591/5588
reflns with $[F^2 > 4\sigma(F^2)]$	5521	3358	6440	3402	5588
R_{int}	0.0614	0.0593	0.0869	0.1251	0.0386
parameters	533	446	277	246	246
GOF on F^2	0.977	0.892	0.661	0.966	0.959
$R_1[F^2 > 4\sigma(F^2)]/wR2$	0.0532/0.1081	0.0478/0.0980	0.0466/0.0522	0.0625/0.1612	0.0367/0.0880
$\Delta\rho_{max}/\Delta\rho_{min}$ [e Å^3]	0.821/−0.466	0.366/−0.426	0.771/−0.587	0.650/−1.294	0.589/−0.689

[2,2′-(2,2-Propanediyl)-bis[8-quinolinolato]nickel(II) (2): The synthesis was performed according to ref. [6]. More precise ^{1}H NMR data than those previously published are given, other unpublished characterizations are added. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.15 (d, J = 9.0 Hz, 2H), 7.45 (br d, J = 8.5 Hz, 2H), 7.33 (br t, J = 8.0 Hz, 2H), 6.91 (br d, J = 7.5 Hz, 2H), 6.81 (br d, J = 6.5 Hz, 2H), 1.93 ppm (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$): δ = 166.4, 159.1, 144.3, 138.7, 130.7, 127.7, 119.3, 113.6, 110.4, 50.7, 32.4 ppm; MS (DCI, NH_3): m/z: 387 $[M+H]^+$, 404 $[M+NH_4]^+$; HRMS: m/z: calcd for $C_{21}H_{17}N_2O_2Ni$: 387.0644, found: 387.0646. Recrystallization from CH_3OH provided green single-crystals suitable for analysis by X-ray diffraction.

2,2′-(2,2-Propanediyl)-bis(8-hydroxyquinoline) (3): Complex **2** (3.32 g, 8.60 mmol) was suspended in ethanol (80 mL) and 37% HCl (16 mL) was added to give a green solution. Hot H_2O (320 mL) was added portion-wise resulting in yellow crystals. After cooling, the crystals were collected, washed with aqueous 1 M HCl, air-dried overnight at room temperature and dissolved in hot ethanol (32 mL). A hot aqueous solution of $AcONa\cdot 3H_2O$ (1.60 g in 64 mL) was then added portion-wise. After cooling, the precipitate was collected by centrifugation, washed with H_2O and extracted with CH_2Cl_2 to give **3** as a white product after evaporation of the solvent (1.81 g). The supernatant of the previous steps contained remaining nickel complex. After removal of the solvent, it was demetalled with EDTA (2×10 g in 500 mL H_2O) under stirring for 1 h in CH_2Cl_2 (1 L). The organic layer was collected, washed with saturated aqueous $NaHCO_3$ and concentrated under vacuum. The mixture was purified by chromatography (CH_2Cl_2, 0–1% CH_3OH) to give 828 mg of **3** (total mass = 2.64 g, 93%). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.30 (br s, 2H), 8.02 (d, J = 9.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 2H), 7.30 (dd, J = 1.2, 7.5 Hz, 2H), 7.22 (d, J = 9.0 Hz, 2H), 7.21 (dd, J = 1.5, 7.5 Hz, 2H), 2.0 ppm (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$): δ = 164.7, 152.1, 136.8, 136.5, 127.5, 126.8, 121.2, 117.5, 110.3, 49.3, 28.0 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 203 (77700), 251 (84600), 308 nm (6900 $M^{-1}cm^{-1}$); MS (DCI, NH_3): m/z: 331 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{18}N_2O_2$: C 76.34, H 5.49, N 8.48; found: C 75.80, H 5.30, N 8.38.

2,2′-(Difluromethanediyl)-bis(8-hydroxyquinoline) (4): Compound **1** was suspended in CH_2Cl_2 and washed with sodium acetate buffer (0.1 M, pH 7.0) to obtain its neutral form **1′** (quantitative yield). Then, to an orange suspension of **1′** (110 mg, 0.36 mmol) in dry CH_3CN (30 mL) was added, under N_2, 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo(2.2.2)octane bis-(tetrafluoroborate) (F-TEDA-BF_4, Selectfluor, 258 mg, 0.73 mmol) in small portions, resulting in the subsequent products dissolution to a yellow solution. The mixture was stirred for 1.5 h at room temperature. After solvent removal, the product was dissolved in CH_2Cl_2 and washed two times with H_2O. The organic layer was concentrated and dried under vacuum to give **4** as a yellow powder (123 mg, quantitative yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.34 (d, J = 8.5 Hz, 2H), 8.00 (d, J = 8.5 Hz, 2H), 7.78 (s, 2H), 7.52 (t, J = 8.0 Hz, 2H), 7.39 (dd, J = 1.0, 8.0 Hz, 2H), 7.19 ppm (dd, J = 1.0, 7.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 150.3 (t, ^2J(C,F) = 30.1 Hz, Cq), 152.3, 137.6, 137.2, 129.3, 128.5, 118.7 (t, ^3J(C,F) = 3.3 Hz, CH), 117.9, 117.0 (t, ^1J(C,F) = 245.6 Hz, Cq), 111.0 ppm; ^{19}F NMR (188 MHz, $CDCl_3$, ref: CF_3CO_2H): δ = −22.2 ppm (s); UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 241 (50000), 251 (55600), 314 nm (4700 $M^{-1}cm^{-1}$); MS (DCI, NH_3): m/z: 339 $[M+H]^+$, 356 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{19}H_{12}F_2N_2O_2\cdot 0.3H_2O$: C 66.39, H 3.69, N 8.15; found: C 66.25, H 3.62, N 8.27.

Bis(8-hydroxy-2-quinolinyl)methanone (5): Compound **1** (60 mg, 0.16 mmol) was stirred in THF/saturated aqueous $NaHCO_3$ 1:1 (8 mL total) for 48 h. CH_2Cl_2 (10 mL) and H_2O (10 mL) were added. The organic phase was collected and the aqueous phase extracted further with CH_2Cl_2 (2×10 mL). The organic phase was washed with H_2O (10 mL) and the solvent was evaporated under vacuum. The product was purified by filtration over silica gel with CH_2Cl_2/CH_3OH 97:3 to give **5** as an orange powder (42 mg, 81%). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.36 (d, J = 8.5 Hz, 2H), 8.19 (d, J = 8.5 Hz, 2H), 8.04 (br s, 2H), 7.59 (m, 2H), 7.43 (dd, J = 1.0, 8.5 Hz, 2H), 7.22 ppm (dd, J = 1.0, 7.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.2, 153.1, 151.4, 137.1, 137.0, 130.4, 129.5, 121.8, 117.9, 111.2 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 254 (43300), 273 (26100), 310 (9600, sh), 375 nm (2300 $M^{-1}cm^{-1}$); MS (DCI, NH_3): m/z: 317 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{12}N_2O_3\cdot 0.5H_2O$: C 70.15, H 4.03, N 8.61; found: C 70.11, H 3.83, N 8.94.

2,2′-(1,2-Ethanediyl)-bis(8-methyloxyquinoline) (6): A solution of 8-methyloxyquinaldine (5.32 g, 30.75 mmol) in dry THF (47 mL) under N_2 was cooled to −95 °C in a CH_3OH/liquid N_2 bath. Lithium diisopropylamine·THF (20.5 mL, 30.7 mmol, 1.5 M in cyclohexane) in dry THF (63 mL) was then added over 2 h. The solution was stirred 2 h more at −95 °C before the addition of 1,2-dibromoethane (5.30 mL, 61.5 mmol). The mixture was allowed to warm to room temperature and stirred overnight. Water (32 mL) was then added, resulting in the formation of a white precipitate removed by filtration. The filtrate was concentrated under vacuum and purified by chromatography ($CHCl_3$, 0–90% AcOEt). The resulting product and the precipitate were recrystallized from hot CH_3OH to give **6** as a white solid (2.04 g, 38%). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.04 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.35 (dd, J = 1.5, 8.0 Hz, 2H), 7.06 (dd, J = 1.5, 7.5 Hz, 2H), 4.10 (s, 6H), 3.61 ppm (s, 4H); ^{13}C NMR (63 MHz, $CDCl_3$): δ = 160.8, 155.0, 139.8, 136.3, 128.0, 125.9, 122.1, 119.5, 107.8, 56.1, 39.2 ppm; MS (DCI, NH_3): m/z: 345 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{20}N_2O_2$: C 76.72, H 5.85, N 8.13; found: C 76.58, H 6.04, N 7.99.

2,2′-(1,2-Ethanediyl)-bis(8-hydroxyquinoline) (7): *Method A*: To a solution of 8-hydroxyquinaldine (1.00 g, 6.29 mmol) in THF (10 mL), under argon and at −95 °C, lithium diisopropylamine·THF (8.4 mL, 12.6 mmol, 1.5 M in cyclohexane) in dry THF (12.5 mL) was added over 45 min. The solution was stirred for 1.5 h then 1,2-dibromoethane (1.08 mL, 12.58 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 15 h. The product was precipitated with H_2O (12 mL), collected by filtration and washed with H_2O. Then it was dissolved in CH_2Cl_2 (150 mL) where a few drops of AcOH were added to dissolve the product that was washed with saturated aqueous $NaHCO_3$ (2×100 mL) then with H_2O to give **7** (0.51 g, 1.62 mmol, 52%) after drying.

Method B: A solution of **6** (2.83 g, 8.23 mmol) in 48% HBr (150 mL) was heated under reflux for 24 h. After cooling to room temperature, the mixture was neutralized with aqueous 3 M NaOH, resulting in the formation of a pale green precipitate. The product was extracted with CH_2Cl_2 and washed with H_2O and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated under vacuum to afford **7** as a pale green powder (2.53 g, 97%).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.35 (br s, 2H), 8.20 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.5 Hz, 2H), 7.37 (t, J = 6.5 Hz, 2H), 7.32 (dd, J = 2.0, 6.5 Hz, 2H), 7.05 (dd, J = 2.0, 6.5 Hz, 2H), 3.57 ppm (s, 4H); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 160.2, 153.0, 138.1, 136.6, 127.6, 127.0, 122.8, 118.0, 111.4, 37.2 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 204 (71800), 248 (74200), 305 nm (5200 $M^{-1}cm^{-1}$); MS (DCI, NH_3): m/z: 317 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{16}N_2O_2\cdot 0.1NaBr$: C 73.53, H 4.93, N 8.58; found: C 73.81, H 4.73, N 8.50.

2,2′-(1,4-Butanediyl)-bis(8-methyloxyquinoline) (8): It was obtained during the synthesis of **6** as major by-product in the supernatant of the CH_3OH recrystallization. A second crystallization in CH_3OH allowed to obtain a pure methanolic solution of the desired product which was evaporated and dried under vacuum to give **8** as a white powder (0.68 g, 6%). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.01 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.35 (dd, J = 1.5, 7.5 Hz, 2H), 7.06 (dd, J = 1.5, 7.5 Hz, 2H), 4.01 (s, 6H), 3.10 (m, 4H), 1.95 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.8, 155.0, 139.7, 136.2, 127.9, 125.7, 121.8, 119.4, 107.7, 56.1, 39.4, 30.0 ppm; MS (DCI, NH_3): m/z: 373 $[M+H]^+$; elemental analysis calcd (%) for $C_{24}H_{24}N_2O_2\cdot 0.5H_2O$: C 75.57, H 6.61, N 7.34; found: C 75.41, H 6.62, N 7.16.

2,2′-(1,4-Butanediyl)-bis(8-hydroxyquinoline) (9): Compound **8** (50 mg, 0.13 mmol) was heated under reflux in 48% HBr (2.7 mL) for 24 h. After cooling down to 0 °C, the reaction mixture was alkalinized to pH 8 with aqueous 3 M NaOH (5 mL, 15 mmol) and saturated aqueous $NaHCO_3$ (2 mL). After adding H_2O until the volume reached 15 mL, the aqueous

phase was extracted with CH_2Cl_2 (3×10 mL). The organic phase was washed with H_2O (10 mL) and the solvent was evaporated under vacuum. The product was purified by flash chromatography (CH_2Cl_2/CH_3OH/AcOH 96:3:1). The product was then dissolved in CH_2Cl_2 (10 mL) and washed with sodium acetate buffer (10 mL, 0.1 M, pH 7). Evaporation of the solvent gave **9** as a white powder (34 mg, 77%). ^{1}H NMR (250 MHz, $CDCl_3$): δ=8.04 (d, J=8.5 Hz, 2H), 7.38 (t, J=7.5 Hz, 2H), 7.31–7.25 (m, 4H), 7.15 (d, J=7.5 Hz, 2H), 3.02 (m, 4H), 1.93 ppm (m, 4H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=160.8, 153.2, 138.3, 136.7, 127.6, 126.9, 122.5, 117.9, 111.4, 38.3, 29.4 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε)=202 (81300), 244 (90800), 303 nm (6300 M^{-1} cm^{-1}); MS (DCI, NH_3): m/z: 345 [M+H]$^+$; elemental analysis calcd (%) for $C_{22}H_{20}N_2O_2$·0.25 H_2O: C 75.73, H 5.92, N 8.35; found: C 75.39, H 5.69, N 8.63.

2,2′,2″-(1,2,3-Propanetriyl)-tris(8-methyloxyquinoline) (10): To a solution of 8-methyloxyquinaldine (1.41 g, 8.14 mmol) in dry THF (20 mL), under argon and cooled on an ice-bath, was added lithium diisopropylamine·THF (20 mL, 30.52 mmol, 1.5 M) over 1 min. The solution was stirred for 1 h at 4°C before the addition of dry $CuCl_2$ (1.32 g, 9.81 mmol) then the mixture was stirred for 36 h at room temperature under argon. Water (100 mL) was then added, and the crude product was extracted with $CHCl_3$, washed with brine and the solvent was evaporated before a purification step by chromatography ($CHCl_3$, 20–100% AcOEt). The resulting product was dissolved in CH_2Cl_2 and washed with an aqueous solution of EDTA to remove copper traces and purified by chromatography again in the previous conditions to give **10** as a white powder (150 mg, 11%). ^{1}H NMR (250 MHz, $CDCl_3$): δ=7.81 (d, J=8.5 Hz, 1H), 7.75 (d, J=8.5 Hz, 2H), 7.37–7.18 (m, 9H), 7.00 (dd, J=1.3, 7.5 Hz, 1H), 6.95 (dd, J=1.3, 7.5 Hz, 1H), 4.49 (dd, J=8.0, 7.0 Hz, 1H), 4.05 (s, 3H), 4.01 (s, 6H), 3.78 (dd, J=14.0, 8.0 Hz, 2H), 3.62 ppm (dd, J=14.0, 7.0 Hz, 2H); ^{13}C NMR (63 MHz, $CDCl_3$): δ=162.7, 159.8, 155.4, 155.1, 140.0, 139.7, 135.8, 135.6, 128.1, 127.8, 125.7, 125.6, 122.8, 122.0, 119.5, 119.3, 108.2, 107.6, 56.4, 56.0, 48.4, 44.3 ppm; MS (DCI, NH_3): m/z: 516 [M+H]$^+$; HRMS: m/z: calcd for $C_{33}H_{30}N_3O_3$: 516.2287, found: 516.2300.

2,2′,2″-(1,2,3-Propanetriyl)-tris(8-hydroxyquinoline) (11): Compound **10** (30 mg, 0.06 mmol) was refluxed in 48% HBr (1.2 mL) for 36 h. The acid was evaporated under vacuum and the residue was dissolved in CH_2Cl_2 (10 mL) and washed with sodium acetate buffer (10 mL, 0.1 M, pH 7.0). The organic phase was collected and the aqueous phase extracted further with CH_2Cl_2 (2×10 mL). The pooled organic phases were washed with H_2O (10 mL) and the solvent was evaporated under vacuum to give **11** as an orange powder (20 mg, 73%). ^{1}H NMR (250 MHz, $CDCl_3$): δ=7.94 (d, J=8.0 Hz, 1H), 7.91 (d, J=8.0 Hz, 2H), 7.42–7.10 (m, 12H), 4.47 (dd, J=8.0, 6.5 Hz, 1H), 3.71 (dd, J=14.5, 8.0 Hz, 2H), 3.57 ppm (dd, J=14.5, 6.5 Hz, 2H); ^{13}C NMR (63 MHz, [D$_6$]DMSO): δ=162.8, 159.3, 152.9, 152.8, 138.03, 138.01, 136.3, 136.0, 127.5, 127.4, 127.0, 123.6, 123.3, 118.0, 117.9, 111.2, 111.0, 45.9, 43.6 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε)=204 (106000), 248 (113000), 302 nm (9200 M^{-1} cm^{-1}); MS (CDI, NH_3): m/z: 474 [M+H]$^+$; elemental analysis calcd (%) for $C_{30}H_{23}N_3O_3$·0.5 H_2O: C 74.67, H 5.01, N 8.71; found: C 74.54, H 5.09, N 9.19.

2-{[8-Hydroxy-2-quinolinyl)amino]methyl}-8-quinolinol (12): A solution of 2-amino-8-hydroxyquinoline (100 mg, 0.62 mmol) and 8-hydroxyquinoline-2-carboxaldehyde (130 mg, 0.75 mmol) in 1,2-dichloroethane (8 mL) was stirred during 1 h at room temperature then AcO_3BHNa (291 mg, 1.29 mmol) was added. After 1.5 h, CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added and the product was extracted with CH_2Cl_2 (2×120 mL), dried (Na_2SO_4), then the solvent was evaporated. The product was dissolved in CH_2Cl_2 (75 mL) and one volume of hexane was added. After filtration and reduction of the solvent volume, the product was purified by chromatography (CH_2Cl_2, 0.5–1% CH_3OH) to give **12** as a clear brown powder (32 mg, 16%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ=8.29 (d, J=8.5 Hz, 1H), 8.01 (t, J=4.5 Hz, 1H), 7.93 (d, J=9.0 Hz, 1H), 7.57 (d, J=8.5 Hz, 1H), 7.39 (m, 2H), 7.08 (m, 4H), 6.91 (dd, J=1.5, 8.0 Hz, 1H), 5.08 ppm (d, J=4.5 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=157.3, 156.0, 153.2, 150.8, 137.5, 137.5, 137.0, 137.0, 128.0, 127.4, 123.4, 122.2, 121.1, 118.04, 118.02, 114.0, 111.7, 111.0, 46.8 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε)=253 (52100), 267 (42900), 345 nm (3600 M^{-1} cm^{-1}, sh); MS (DCI, NH_3): m/z: 318 [M+H]$^+$; HRMS: m/z: calcd for $C_{19}H_{16}N_3O_2$: 318.1243, found: 318.1238.

8-Hydroxy-*N*-(8-hydroxy-2-quinolinyl)-2-quinolinecarboxamide (13): To a suspension of 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol) in CH_2Cl_2 (5 mL) were added 1-hydroxybenzotriazole monohydrate (71 mg, 0.53 mmol), (benzotriazole-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (177 mg, 0.40 mmol) and Et_3N (0.037 mL, 0.26 mmol). After stirring for 0.5 h at room temperature, 2-amino-8-hydroxyquinoline (85 mg, 0.53 mmol) and Et_3N (0.037 mL, 0.26 mmol then, after 5 min, 0.117 mL, 0.794 mmol) were added. After stirring for 18 h, H_2O (10 mL) and CH_2Cl_2 (5 mL) were added and the organic phase was collected. The product was purified by chromatography (CH_2Cl_2/CH_3OH 99.5:0.5). The product was then dissolved in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$. The solvent was evaporated to give **13** as a white powder (28 mg, 32%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ=11.89 (s, 1H), 10.89 (s, 1H), 9.55 (s, 1H), 8.60 (d, J=8.5 Hz, 1H), 8.41 (s, 2H), 8.32 (d, J=8.5 Hz, 1H), 7.63 (dd, J=7.0 and 8.0 Hz, 1H), 7.53 (dd, J=1.0, 8.0 Hz, 1H), 7.42 (dd, J=2.0, 8.0 Hz, 1H), 7.37 (dd, J=7.0, 8.0 Hz, 1H), 7.23 (dd, J=1.0, 7.0 Hz, 1H), 7.13 ppm (dd, J=2.0, 7.0 Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ= 163.8, 154.7, 152.7, 150.0, 147.3, 138.6, 138.6, 137.6, 137.2, 130.50, 130.46, 127.4, 126.5, 119.6, 118.3, 118.0, 116.6, 112.8, 112.5 ppm; UV/Vis [DMSO/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 8:2]: λ_{max} (ε)=259 (49100), 332 nm (11200 M^{-1} cm^{-1}, sh); MS (DCI, NH_3): m/z: 332 [M+H]$^+$; HRMS: m/z: calcd for $C_{19}H_{14}N_3O_3$: 332.1035, found: 332.1011.

General protocol for chlorination of C5 of bis-8-hydroxyquinoline derivatives: To a solution 0.1 M of ligand in 97% H_2SO_4 at 0°C was added *N*-chlorosuccinimide (2 equiv) in small portions. The mixture was stirred for 15 min at 0°C and 4 h at room temperature. It was then poured onto ice to give a suspension that was neutralized with aqueous 6 M NaOH. The mixture was centrifuged, the supernatant was removed and the precipitate was suspended in H_2O and extracted with CH_2Cl_2. The organic layer was washed with H_2O and the solvent was evaporated under vacuum to give the product.

2,2′-Methanediyl-bis(5-chloro-8-hydroxyquinoline) (14): Pale orange powder (440 mg, 98%). ^{1}H NMR (250 MHz, $CDCl_3$): δ=8.45 (d, J=8.5 Hz, 2H), 8.12 (br s, 2H), 7.56 (d, J=8.5 Hz, 2H), 7.48 (d, J=8.0 Hz, 2H), 7.10 (d, J=8.0 Hz, 2H), 4.74 ppm (s, 2H); ^{13}C NMR (63 MHz, $CDCl_3$): δ=157.4, 151.0, 138.1, 134.2, 127.2, 125.0, 123.3, 120.4, 110.3, 47.5 ppm; UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε)=258 (59900), 320 (7000), 460 (2000), 489 (2900), 518 nm (2100 M^{-1} cm^{-1}); MS (DCI, NH_3): m/z: 371 [M+H]$^+$; elemental analysis calcd (%) for $C_{19}H_{12}Cl_2N_2O_2$·0.1 Na_2SO_4: C 59.21, H 3.14, N 7.27; found: C 59.27, H 2.58, N 7.05.

2,2′-(2,2-Propanediyl)-bis(5-chloro-8-hydroxyquinoline) (15): White powder (55 mg, 93%). ^{1}H NMR (250 MHz, $CDCl_3$): δ=8.37 (d, J=9.0 Hz, 2H), 8.19 (br s, 2H), 7.50 (d, J=8.0 Hz, 2H), 7.32 (d, J=9.0 Hz, 2H), 7.13 (d, J=8.0 Hz, 2H), 2.0 ppm (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$): δ=165.2, 151.1, 137.3, 134.0, 127.2, 124.7, 121.9, 120.5, 110.2, 49.4, 27.9 ppm; UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε)=258 (72500), 315 nm (8300 M^{-1} cm^{-1}); MS (DCI, NH_3): m/z: 399 [M+H]$^+$; elemental analysis calcd (%) for $C_{21}H_{16}Cl_2N_2O_2$: C 63.17, H 4.04, N 7.02; found: C 63.02, H 3.76, N 6.87.

2,2′-(1,2-Ethanediyl)-bis(5-chloro-8-hydroxyquinoline) (16): Pale green solid (258 mg, 71%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ=9.73 (br s, 2H), 8.37 (d, J=8.5 Hz, 2H), 7.70 (d, J=8.5 Hz, 2H), 7.52 (d, J=8.0 Hz, 2H), 7.06 (d, J=8.0 Hz, 2H), 3.64 ppm (s, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=161.5, 153.0, 139.2, 133.5, 127.4, 125.4, 124.4, 119.6, 112.1, 37.1 ppm. ^{1}H/^{13}C NMR correlations spots between C5 and H4 allowed to attribute the halogen position; UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε)=254 (75000), 313 nm (7200 M^{-1} cm^{-1}); MS (DCI, NH_3): m/z: 385 [M+H]$^+$; elemental analysis calcd (%) for $C_{20}H_{14}Cl_2N_2O_2$·0.1 Na_2SO_4: C 60.14, H 3.53, N 7.01; found: C 59.92, H 3.07, N 6.65.

2,2′-(1,2-Ethanediyl)-bis(5-chloro-7-iodo-8-hydroxyquinoline) (17): To a solution of **16** (300 mg, 0.78 mmol) in CH_3OH (12 mL) under N_2 was added 97% H_2SO_4 (250 μL) to give a yellow suspension cooled on an ice

bath. *N*-Iodosuccinimide (418 mg, 1.86 mmol) was then slowly added. The mixture was stirred for 15 min at 0 °C and at room temperature for 5 d. It was then poured onto ice and decolorized by addition of $Na_2S_2O_5$ (450 mg). The suspension was neutralized with aqueous ammonia and centrifuged. The supernatant was removed and the precipitate was dissolved in CH_2Cl_2 and washed with Tris·HCl buffer (0.1 M, pH 7). The organic layer was evaporated under vacuum to afford **17** as a yellow powder (350 mg, 70 %). ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 8.36$ (d, $J =$ 8.5 Hz, 2H), 7.90 (s, 2H), 7.70 (d, $J = 8.5$ Hz, 2H), 3.68 ppm (s, 4H); ^{13}C NMR (63 MHz, [D$_6$]DMSO): $\delta = 162.1$, 153.1, 137.3, 134.4, 133.5, 124.6, 124.5, 120.2, 78.4, 37.1 ppm; UV/Vis [DMSO/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 8:2]: λ_{max} (ε) = 262 (69500), 319 nm (7900 M^{-1} cm^{-1}); MS (DCI, NH_3): m/z: 637 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{12}Cl_2I_2N_2O_2$: C 37.71, H 1.90, N 4.40; found: C 38.15, H 2.04, N 4.15.

7-Bromo-2-methyl-8-hydroxyquinoline (20): *Tert*-butylamine (3.23 mL) was stirred under argon in toluene (100 mL) during 2 h at room temperature with 4 Å molecular sieves (10 g). After cooling to −70 °C, *N*-bromosuccinimide (5.48 g, 30.78 mmol) and 8-hydroxyquinaldine (5.00 g, 30.78 mmol) were added and the temperature was slowly increased to 20 °C (4 h). After filtration and washing with diethyl ether, the filtrate was washed with H_2O (3 × 50 mL), dried (Na_2SO_4) then the solvent was evaporated. The obtained solid was refluxed in hexane (100 mL) then filtrated and dried to give **20** as a white powder (4.85 g, 67 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.00$ (d, $J = 8.5$ Hz, 1H), 7.52 (d, $J = 9.0$ Hz, 1H), 7.31 (d, $J = 8.5$ Hz, 1H),7.16 (d, $J = 9.0$ Hz, 1H), 2.72 ppm (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 157.9$, 149.2, 137.7, 136.2, 130.1, 125.4, 122.9, 118.2, 103.9, 24.8 ppm; MS (DCI, NH_3): m/z: 238 $[M+H]^+$; elemental analysis calcd (%) for $C_{10}H_8NOBr$: C 50.45, H 3.39, N 5.88, found: C 50.02, H 3.37, N 6.12.

General method for synthesis of 7-methyloxy-8-hydroxyquinoline derivatives: To a solution of 7-bromo-8-hydroxyquinoline derivative (70 mM in DMF) was added 10 equiv of CH_3ONa (30 % wt in CH_3OH) and the mixture was stirred for 10 min under argon. $CuCl_2.2H_2O$ (0.3 equiv) was added and the mixture was heated under reflux for 20 h. After cooling to room temperature, H_2O and EDTA (0.3 equiv) were added and the stirring was carried on for 1 h. The solution was slightly acidified with AcOH and then carefully basified with saturated $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by chromatography using a gradient from CH_2Cl_2/CH_3OH/AcOH 94:4:2 to CH_2Cl_2/CH_3OH 90:10. The fractions containing the product were combined and washed with saturated aqueous $NaHCO_3$ (3 × 100 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure affording desired product.

7-Methyloxy-8-hydroxyquinoline (19): Grey powder (0.65 g, 40 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.77$ (dd, $J = 1.5$, 4.0 Hz, 1H), 8.11 (dd, $J = 1.5$, 8.5 Hz, 1H), 7.36 (m, 2H), 7.31 (dd, $J = 4.0$, 8.5 Hz, 1H), 4.06 ppm (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 148.6$, 144.0, 139.7, 138.6, 136.0, 123.5, 119.7, 117.7, 116.4, 57.3 ppm; MS (DCI, NH_3): m/z: 176 $[M+H]^+$; elemental analysis calcd (%) for $C_{10}H_9NO_2 \cdot 0.1\,H_2O$: C 67.86, H 5.24, N 7.91; found: C 67.82, H 5.11, N 7.95.

7-Methyloxy-2-methyl-8-hydroxyquinoline (21): White powder (480 mg, 60 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.95$ (d, $J = 8.5$ Hz, 1H), 7.26 (s, 2H), 7.15 (d, $J = 8.5$ Hz, 1H), 4.03 (s, 3H), 2.69 ppm (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 157.6$, 143.9, 139.3, 138.0, 136.0, 121.7, 120.6, 117.3, 115.2, 57.2, 25.1 ppm; MS (DCI, NH_3): m/z: 190 $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{11}NO_2$: C 69.83, H 5.86, N 7.40, found: C 69.40, H 5.85, N 7.47.

8-(Benzyloxy)-7-methyloxyquinoline (22): Compound **19** (1.10 g, 6.29 mmol) and K_2CO_3 (1.30 g, 9.43 mmol) in dry CH_3CN (30 mL) were stirred for 10 min under argon. Benzyl chloride (0.87 mL, 7.54 mmol) was added and the mixture was heated under reflux overnight. After cooling to room temperature, the precipitate was filtered, washed with CH_2Cl_2 and the filtrate was dried under reduced pressure. The product was dissolved in CH_2Cl_2 (50 mL) and washed with aqueous 2 M NaOH (3 × 50 mL) then H_2O (50 mL). The organic layer was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure affording **22** as a brown oil (1.28 g, 77 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.94$ (dd, $J = 1.5$, 4.2 Hz, 1H), 8.06 (dd, $J = 1.5$, 8.5 Hz, 1H), 7.59–7.51 (m, 3H), 7.36–7.24 (m, 5H), 5.40 (s, 2H), 3.92 ppm (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta =$ 151.3, 149.5, 142.9, 141.2, 137.4, 135.3, 127.9, 127.4, 127.1, 123.6, 122.9, 118.5, 114.8, 75.2, 56.1 ppm; MS (DCI, NH_3): m/z: 266 $[M+H]^+$; elemental analysis calcd (%) for $C_{17}H_{15}NO_2 \cdot 0.05\,CHCl_3$: C 75.49, H 5.59, N 5.16; found: C 75.74, H 5.40, N 5.33.

8-(Benzyloxy)-7-methyloxyquinolin-*N*-oxide (23): To dry CH_2Cl_2 (11 mL) at 4 °C under argon were successively added **22** (0.30 g, 1.13 mmol) and *m*-chloroperbenzoic acid 77 % wt (0.38 g, 1.69 mmol). After stirring for 48 h at room temperature, CH_2Cl_2 (40 mL) was added. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and the solvent was evaporated. The product was purified by chromatography CH_2Cl_2/CH_3OH 95:5 affording **23** as a brown oil (0.18 g, 58 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.43$ (dd, $J = 1.0$, 6.0 Hz, 1H), 7.68–7.58 (m, 4H), 7.41–7.30 (m, 4H), 7.10 (dd, $J = 6.0$, 8.5 Hz, 1H), 5.23 (s, 2H), 3.96 ppm (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 138.4$, 128.9, 128.7, 128.2, 127.8, 127.6, 125.7, 124.9, 123.4, 119.5, 118.8, 116.8, 107.7, 75.2, 57.1 ppm; MS (DCI, NH_3): m/z: 282 $[M+H]^+$; elemental analysis calcd (%) for $C_{17}H_{15}NO_3$: C 72.58, H 5.37, N 4.98; found: C 72.74, H 4.91, N 4.87.

2,2′-(Dimethylmalonatediyl)-bis(8-(benzyloxy)-7-methyloxyquinoline) (24): To a solution of **23** (0.72 g, 2.56 mmol) in dry CH_2Cl_2 (7 mL) were added dimethylmalonate (0.307 mL, 2.69 mmol) and acetic anhydride (1.18 mL) and the mixture was stirred for 24 d protected from the light and the humidity ($CaCl_2$ trap). CH_3OH (20 mL) was added and, after 15 h at −20 °C, a precipitate was removed by centrifugation. The solvent of the supernatant was evaporated and the product was dissolved in CH_3OH (0.5 mL) and precipitated with H_2O (3 mL). Supernatant was removed and pellet was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (2 × 20 mL). After evaporation of the solvent, the product was purified by preparative thin layer chromatography eluted with CH_2Cl_2/CH_3OH 99:1. Residual impurities were then removed by crystallization from CH_3OH to give **24** as white needles (12.3 mg, 1.5 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.93$ (d, $J = 8.5$ Hz, 2H), 7.63 (d, $J = 8.5$ Hz, 2H), 7.59 (m, 4H), 7.47 (d, $J = 9.0$ Hz, 2H), 7.40–7.28 (m, 8H), 5.41 (s, 4H), 3.95 (s, 6H), 3.84 ppm (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 169.3$, 157.5, 152.2, 142.2, 142.1, 138.2, 135.9, 128.4, 128.2, 127.7, 123.3, 123.0, 121.1, 116.0, 75.8, 75.2, 57.2, 53.0 ppm; MS (DCI, NH_3): m/z: 659 $[M+H]^+$; HRMS: m/z: calcd for $C_{39}H_{35}N_2O_8$: 659.2393; found: 659.2399. Crystallization from CH_3OH provided single-crystals suitable for analysis by X-ray diffraction as white needles.

2,2′-Methanediyl-bis(7-methyloxy-8-hydroxyquinoline) dihydrochloride (25): Compound **24** (40 mg, 0.06 mmol) was refluxed in 37 % aqueous HCl (3 mL) for 1.5 h then the solvent was evaporated. The product was dissolved in CH_3OH then precipitated by pouring in diethyl ether (3 mL). The precipitate was centrifuged and washed with diethyl ether before to be dried under vacuum to give **24** as yellow crystals (28 mg, 98 %). ^{1}H NMR (250 MHz, CD_3OD): $\delta = 8.97$ (d, $J = 8.0$ Hz, 2H), 7.86 (s, 4H), 7.68 (d, $J = 8.0$ Hz, 2H), 4.16 ppm (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 152.5$, 150.4, 147.6, 135.1, 129.8, 123.9, 120.4, 119.8, 117.2, 56.4 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 203 (47800), 246 (34100, sh), 257 (49800), 334 nm (3500 M^{-1} cm^{-1}); MS (DCI, NH_3): m/z: 363 $[M+H]^+$.

2,2′-(1,2-Ethanediyl)-bis(7-methyloxy-8-quinolinol) (26): A solution under argon of **21** (0.50 g, 2.64 mmol) in THF (10 mL) was stirred 0.5 h with 4 Å molecular sieves (2.0 g) at room temperature. After cooling to −95 °C, lithium diisopropylamine·THF (3.70 mL, 5.57 mmol, 1.5 M in cyclohexane) was added over 10 min. The mixture was allowed to warm to −50 °C, then cooled again to −90 °C and 1,2-dibromoethane (0.50 mL, 5.82 mmol) was added and the mixture was allowed to warm to room temperature before adding H_2O (5 mL) and AcOH (0.50 mL). After 1 h of stirring, molecular sieves were removed by filtration. Saturated aqueous $NaHCO_3$ (10 mL), H_2O (10 mL) and diethyl ether (40 mL) were added and the organic phase was collected and dried (Na_2SO_4). Solvent was evaporated and the solid obtained was recrystallized from CH_3OH (10 mL) to give **26** as a white powder (306 mg, 62 %). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.00$ (d, $J = 8.5$ Hz, 2H), 7.28 (s, 4H), 7.24 (d, $J =$ 8.5 Hz, 2H), 4.04 (s, 6H), 3.57 ppm (s, 4H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 160.1$, 144.0, 139.3, 137.9, 136.3, 121.9, 120.4, 117.5, 115.5, 57.2,

37.1 ppm; UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 204 (83000), 208 (53200, sh), 252 (97200), 338 nm (6600 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 377 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{20}N_2O_4 \cdot 0.2\,H_2O$: C 69.53, H 5.41, N 7.37, found: C 69.40, H 5.30, N 7.47.

Single-crystals of copper(II) or zinc(II) complexes of ligand 3

Complex 27: To a solution of **3** (10.0 mg, 0.03 mmol) in ethanol (0.4 mL) was added $Cu(OAc)_2 \cdot H_2O$ (6.05 mg, 0.03 mmol) dissolved in H_2O (0.4 mL). The mixture was stirred for 0.5 h at 60 °C then precipitated at 4 °C. Supernatant was removed after a centrifugation step and the pellet was washed twice with H_2O before to be dried at 110 °C. Product was then recrystallized in CH_3OH to give **27** as green needles.

Complex 28: To a solution of **3** (11.7 mg, 0.035 mmol) in DMF (0.4 mL) was added $CuSO_4 \cdot 5\,H_2O$ (8.85 mg, 0.035 mmol) dissolved in H_2O (0.4 mL) to give a green solution. Slow evaporation of the solvent at room temperature gave **28** as green needles.

Complex 29: To a solution of **3** (10.6 mg, 0.032 mmol) in CH_3OH (0.4 mL) was added $CuCl_2 \cdot 2\,H_2O$ (5.53 mg, 0.032 mmol) dissolved in H_2O (0.4 mL). A green precipitate was obtained. It was dissolved by heating then CH_3OH (4 mL) was added and slow evaporation of the solvent gave **29** as green-yellow crystals.

Complex 30: To a solution of **3** (51.7 mg, 0.156 mmol) in THF (2.0 mL) was added $ZnSO_4 \cdot 7\,H_2O$ (45.08 mg, 0.156 mmol) dissolved in H_2O (2.0 mL). THF (2.0 mL) was added and the solution was slowly evaporated at room temperature until the formation of a biphasic system. Supernatant was removed and the volume of the lower phase was reduced. A minimum volume of DMSO was added to obtain a homogenous solution at 60–70 °C. The mixture was stand at room temperature until the formation of single-crystals of complex **30** as white needles. The overall quality of the data was poor due to the weak diffracting crystal, which leads to a high R_{int} value (11826/3679 collected/independents reflections, R_{int} 0.3374, $R_1[F^2 > 4\sigma(F^2)] = 0.0726$, $wR2 = 0.1582$). We have limited the discussion of the main features of the complex. Complex **30** crystallizes in the orthorhombic space group $Pca2_1$ with the cell parameters: a = 19.690(3), b = 10.036(2), c = 10.220(2) Å.

Determination of the metal to ligand stoichiometry: UV-visible absorption spectra and spectrophotometric titrations were performed in the presence of 20 mM Tris·HCl pH 7.4 buffer, 150 mM NaCl since it was the solvent used during Aβ dissolution assays. Organic solvent (CH_3OH, dioxane or DMSO) was added in order to have a good solubility of ligands and metal complexes at the concentrations used in these experiments. To a 15 μM solution of ligand were added aliquots of concentrated solutions of $CuCl_2$ or $ZnCl_2$ in order to induce negligible volume variations. Upon these additions changes in UV-visible absorption spectra were immediately observed and were stable between two additions as a result of a fast complexation process. The metal to ligand stoichiometries were also confirmed from elemental analysis of significant examples and are included here.

Preparation of metal complexes for mass spectrometry characterization: Solutions of ligands in CH_3OH or dioxane were metalated in the presence of 1 equiv of metal ion during 1 h at room temperature before to be evaporation of the solvent. $CuCl_2$ (for **1**, **4**, **5**, **9**, **12**, **13**, **25** and **26**), $Cu(OAc)_2$ (for **3**, **7**, **14**, **15**, **16** and **17**), $Zn(OAc)_2$ (for **1**, **3**, **7**, **14**, **15**, and **16**) or $ZnCl_2$ (for **4**, **5**, **9**, **12**, **13**, **17**, **25** and **26**) were used. Control UV/Vis spectrophotometric analyses of the different complexes are the same than those obtained during titration experiments.

1·Cu^{II}: UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 256 (41900), 272 (32200, sh), 304 (8800), 338 (5100), 376 nm (3100 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 364 $[LCu^{II}-H]^+$, 381 $[LCu^{II}NH_4-2H]^+$; elemental analysis calcd (%) for $C_{19}H_{12}N_2O_2Cu$: calcd C 52.73, H 2.33, N 6.47; found: C 52.52, H 1.64, N 6.48.

3·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 202 (70800), 254 (58900), 268 (42400, sh), 383 nm (4300 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 392 $[LCu^{II}-H]^+$, 409 $[LCu^{II}NH_4-2H]^+$; elemental analysis calcd (%) for $C_{21}H_{16}N_2O_2Cu \cdot 0.3\,C_2H_4O_2$: calcd C 63.33, H 4.16, N 6.84; found: C 63.30, H 3.50, N 6.82.

4·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 205 (64000), 255 (47600), 276 (34400), 415 nm (2900 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 400 $[LCu^{II}-H]^+$, 417 $[LCu^{II}NH_4-2H]^+$.

5·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 245 (41100), 306 (27900), 348 (12100, sh), 485 nm (2500 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 378 $[LCu^{II}-H]^+$, 395 $[LCu^{II}NH_4-2H]^+$.

7·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 202 (57700), 259 (68000), 268 (46200, sh), 392 nm (4900 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 378 $[LCu^{II}-H]^+$, 395 $[LCu^{II}NH_4-2H]^+$.

9·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 204 (56000), 260 (71800), 374 nm (4800 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 406 $[LCu^{II}-H]^+$, 423 $[LCu^{II}NH_4-2H]^+$.

12·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 267 (45600), 286 (26800, sh), 320 (4700, sh), 389 nm (2700 $M^{-1}cm^{-1}$); MS (ESMS): *m/z*: 379 $[LCu^{II}-H]^+$, 415 $[LCuCl]^+$.

14·Cu^{II}: UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 256 (38300), 278 (35900), 310 (10300), 342 (7300), 398 nm (4000 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 434 $[LCu^{II}-H]^+$, 451 $[LCu^{II}NH_4-2H]^+$.

15·Cu^{II}: UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 261 (59500), 274 (46900, sh), 347 (4700), 409 nm (5800 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 462 $[LCu^{II}-H]^+$, 479 $[LCu^{II}NH_4-2H]^+$; elemental analysis calcd (%) for $C_{21}H_{14}N_2O_2Cl_2Cu$: calcd C 54.74, H 3.06, N 6.08; found: C 54.50, H 2.84, N 5.92.

16·Cu^{II}: UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 264 (64700), 276 (46700), 413 nm (5300 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 448 $[LCu^{II}-H]^+$, 465 $[LCu^{II}NH_4-2H]^+$.

17·Cu^{II}: UV/Vis [DMSO/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 8:2]: λ_{max} (ε) = 278 (56000), 340 (55000), 392 (6200), 420 nm (5000 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 700 $[LCu^{II}-H]^+$, 717 $[LCu^{II}NH_4-2H]^+$.

26·Cu^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 212 (65300), 263 (65300), 275 (47300, sh), 319 (4500), 421 nm (5900 $M^{-1}cm^{-1}$); MS (ESMS): *m/z*: 438 $[LCu^{II}-H]^+$, 475 $[LCuCl]^+$.

1·Zn^{II}: MS (DCI, NH_3): *m/z*: 365 $[LZn^{II}-H]^+$, 382 $[LZn^{II}NH_4-2H]^+$.

3·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 202 (78700), 258 (74100), 268 (46700), 378 nm (4600 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 393 $[LZn^{II}-H]^+$, 410 $[LZn^{II}NH_4-2H]^+$.

4·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 205 (43300), 258 (50500), 274 (30900), 404 nm (3000 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 401 $[LZn^{II}-H]^+$.

5·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 238 (31100), 298 (26200), 346 (10500, sh), 473 nm (300 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 379 $[LZn^{II}-H]^+$, 396 $[LZn^{II}NH_4-2H]^+$.

7·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 202 (55500), 258 (63600), 268 (43400), 374 nm (4000 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 379 $[LZn^{II}-H]^+$, 396 $[LZn^{II}NH_4-2H]^+$.

9·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 202 (63600), 260 (65600), 368 nm (4400 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 407 $[LZn^{II}-H]^+$, 424 $[LZn^{II}NH_4-2H]^+$.

12·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 267 (41400), 287 (26900, sh), 320 (5600, sh), 372 nm (3100 $M^{-1}cm^{-1}$); MS (ESMS): *m/z*: 380 $[LZn^{II}-H]^+$, 415 $[LZnCl]^+$.

14·Zn^{II}: MS (DCI, NH_3): *m/z*: 435 $[LZn^{II}-H]^+$, 452 $[LZn^{II}NH_4-2H]^+$.

15·Zn^{II}: UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 265 (69200), 275 (45700), 346 (5800), 405 nm (6100 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 463 $[LZn^{II}-H]^+$, 482 $[LZn^{II}NH_4-2H]^+$.

16·Zn^{II}: UV/Vis [dioxane/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 264 (57800), 272 (43600, sh), 390 nm (5400 $M^{-1}cm^{-1}$); MS (DCI, NH_3): *m/z*: 449 $[LZn^{II}-H]^+$, 466 $[LZn^{II}NH_4-2H]^+$.

17·Zn^{II}: UV/Vis [DMSO/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 8:2]: λ_{max} (ε) = 276 (54200), 340 (5800), 352 (7700), 397 nm (5100 $M^{-1}cm^{-1}$); MS (ESMS): *m/z*: 735 $[LZnCl]^-$.

25·Zn^{II}: MS (ESMS): *m/z*: 425 $[LZn^{II}-H]^+$.

26·Zn^{II}: UV/Vis [CH_3OH/Tris·HCl 20 mM pH 7.4, NaCl 150 mM 1:1]: λ_{max} (ε) = 202 (66900), 264 (92500), 276 (47100, sh), 318 (5500), 398 nm (6700 M^{-1} cm^{-1}); MS (ESMS): m/z: 439 $[LZn^{II}-H]^+$.

Estimation of the metal to ligand affinity constants: Solutions (15 μM) of studied ligand (L_s), EDTA (competitor chelator L_c) and metal ion (M) in 1:1:1 ratio were analyzed spectrophotometrically at 20 °C to determine the concentration of L_s, ML_s, L_c or ML_c species at the equilibrium. Experiments were performed in 20 mM Tris·HCl pH 7.4, 150 mM NaCl buffer where an organic solvent (CH_3OH, dioxane or DMSO) was added in order to have a good solubility of ligands and metal complexes at the concentrations used. Controls showed that all ligands and complexes studied were in accordance with the Beer–Lambert law in the experimental conditions used. In these experimental conditions, and since all ligands studied L_s form ML_s species, complexation reactions can be summarized as: $L_s + M \overset{K_s}{\rightleftharpoons} ML_s$ with $K_s = [ML_s]/[L_s][M]$ and $L_c + M \overset{K_c}{\rightleftharpoons} ML_c$ with $K_c = [ML_c]/[L_c][M]$

K_c is the K_{app} value for the competitive chelator at pH 7.4 where the experiments were performed. It was determined as in reference 15. It can be deduced that at the equilibrium: $K_s = K_c[ML_s][L_c]/[ML_c][L_s]$.

The initial concentration was C. All the metal is chelated during the experiments and $[ML_s] + [ML_c] = C = [ML_s] + [L_s] = [ML_c] + [L_c]$. At the equilibrium $[ML_s] = x\,\%$ of C, then $[L_s] = (1-x)\,\%$ of C, $[ML_c] = (1-x)\,\%$ of C and $[L_c] = x\,\%$ of C. Therefore, the apparent affinity constant of L_s for the metal ion M, at pH 7.4, is: $K_s = (K_c x^2)/(1-x)^2$.

log$D_{7.4}$ determination: Ligand (2.0 mg) was dissolved in 1-octanol (2.00 mL) then Tris·HCl buffer (2.00 mL, 20 mM, pH 7.4), 150 mM NaCl was added. After a 3 min vortex at room temperature followed by centrifugation for 5 min at 5500 g, the concentration of ligand in each layer was determined by UV/Vis spectrophotometry. Samples from 1-octanol layer were repartitioned until consistent partition coefficient values were obtained. The measurement was carried out in triplicate.

Reagents for the experiments involving Aβ_{1-42} amyloid peptide: Before use, MilliQ H_2O (Millipore) and all buffers were treated with Chelex-100 resin (Biorad) (5.0 mg mL^{-1}) and filtered through a 0.2 μm filter (Whatman) to remove any metal ion or particulate matter. Aβ_{1-42} amyloid peptide was synthesized, purified (up to 95 % purity) and characterized by HPLC analysis and MALDI-TOF mass spectrometry by CNRS-IPBS (Toulouse, France). It was dissolved and quantified as previously described.[5,22] Ligands were used as chloride salts. Excepted for **1** and **25** that were synthesized as chloride salts, they were generated by addition of 1 equiv of HCl per nitrogenous functions of ligand dissolved in DMSO. After evaporation of the solvent, the chloride salts were dissolved at the desired concentration in DMSO and stored at −20 °C. All experiments involving the peptide were performed in duplicate when the discrepancy between experimental values was less than 5 %. In the other cases and for major results triplicates were done and standard deviations were calculated. Stirrings were performed with a Thermomixer.

Inhibition of Aβ precipitation: Final concentrations were given. Aβ_{1-42} (500 μL, 5 μM) was incubated in Tris·HCl buffer (20 mM, pH 7.4), 150 mM NaCl with $CuCl_2$ or $ZnCl_2$ (20 μM) for 1 h at 37 °C under stirring at 1400 rpm. Tested ligand (200 μM) dissolved in DMSO (50 μL) was added and the samples were incubated for one additional hour at 37 °C under stirring at 1400 rpm. Samples were then centrifuged for 20 min at 5500 g and 500 μL of supernatant was removed. The test tube containing residual supernatant and pellet received 450 μL of experimental buffer/DMSO 91:9. Then both supernatant and pellet were analyzed for protein concentration by MicroBCA Assay (Pierce). This double quantification allowed to monitor the initial quantity of peptide and to confirm the validity of the measurements. In the case of **1**, **5**, **12**, **13**, **14**, **25**, **26** and 8-hydroxyquinaldine that showed an absorption at 562 nm in the dosage conditions, blanks were performed in the presence of the tested ligand and the studied metal ion. Some experiments were performed with other ratios of Cu^{II} (2.5 equiv) and ligand (2.5 or 5 equiv) per Aβ_{1-42}.

Hydrogen peroxide assay: Final concentrations were given. Aβ_{1-42} (0.2 or 0.53 μM) and $CuCl_2$ (0.4 μM) were incubated for 1 h at 37 °C under stirring at 1400 rpm in 375 μL of sodium phosphate buffer (20 mM, pH 7.4). Then 2 μL of tested ligand in DMSO (0 to 0.8 μM) were added. After one more hour of incubation, 2 μL of sodium ascorbate (10 μM) were added and incubated 5 min under stirring. The amount of H_2O_2 produced was quantified using the AmplexRed H_2O_2 Assay Kit (Molecular Probes) from an analytical grade H_2O_2 standard curve. Some experiments received also known quantities of H_2O_2 before the addition of AmplexRed.

Acknowledgement

CNRS and PALUMED are gratefully acknowledged for their financial support. Dr F. Coslédan (PALUMED) is acknowledged for fruitful discussions.

[1] a) M. Mattson, *Nature* **2004**, *430*, 631–639; b) M. Citron, *Nat. Rev. Neurosci.* **2004**, *5*, 677–685; c) E. Gaggelli, H. Kozlowski, D. Valensin, G. Valensin, *Chem. Rev.* **2006**, *106*, 1995–2044.

[2] a) C. Opazo, X. Huang, R. Cherny, R. Moir, A. Roher, A. White, R. Cappai, C. Masters, R. Tanzi, N. Inestrosa, A. Bush, *J. Biol. Chem.* **2002**, *277*, 40302–40308; b) G. Ciccotosto, D. Tew, C. Curtain, D. Smith, D. Carrington, C. Masters, A. Bush, R. Cherny, R. Cappai, K. Barnham, *J. Biol. Chem.* **2004**, *279*, 42528–42534.

[3] a) R. Cherny, J. Legg, C. McLean, D. Fairlie, X. Huang, C. Atwood, K. Beyreuther, R. Tanzi, C. Masters, A. Bush, *J. Biol. Chem.* **1999**, *274*, 23223–23228; b) R. Cherny, K. Barnham, T. Lynch, I. Volitakis, Q. Li, C. McLean, G. Multhaup, K. Beyreuther, R. Tanzi, C. Masters, A. Bush, *J. Struct. Biol.* **2000**, *130*, 209–216; c) J. Lee, J. Friedman, I. Angel, A. Kozak, J. Koh, *Neurobiol. Aging* **2004**, *25*, 1315–1321; d) A. Dedeoglu, K. Cormier, S. Payton, K. Tseitlin, J. Kremsky, L. Lai, X. Li, R. Moir, R. Tanzi, A. Bush, N. Kowall, J. Rogers, X. Huang, *Exp. Gerontol.* **2004**, *39*, 1641–1649; e) Z. Cui, P. Lockman, C. Atwood, C. Hsu, A. Gupte, D. Allen, R. Mumper, *Eur. J. Pharm. Biopharm.* **2005**, *59*, 263–272; f) C. Boldron, I. van der Auwera, C. Deraeve, H. Gornitzka, S. Wera, M. Pitié, F. van Leuven, B. Meunier, *ChemBioChem* **2005**, *6*, 1976–1980.

[4] a) R. Cherny, C. Atwood, M. Xilinas, D. Gray, W. Jones, C. McLean, K. Barnham, I. Volitakis, F. Fraser, Y. Kim, X. Huang, L. Goldstein, R. Moir, J. Lim, K. Beyreuther, H. Zheng, R. Tanzi, C. Masters, A. Bush, *Neuron* **2001**, *30*, 665–676; b) C. Ritchie, A. Bush, A. Mackinnon, S. Macfarlane, M. Mastwyk, L. McGregor, L. Kiers, R. Cherny, Q. Li, A. Tammer, D. Carrington, C. Mavros, I. Volitakis, M. Xilinas, D. Ames, S. Davis, K. Beyreuther, R. Tanzi, C. Masters, *Arch. Neurol.* **2003**, *60*, 1685–1691.

[5] C. Deraeve, M. Pitié, B. Meunier, *New J. Chem.* **2007**, *31*, 193–195.

[6] Y. Yamamoto, A. Miura, A. Kawamata, M. Miura, S. Takei, *Bull. Chem. Soc. Jpn.* **1978**, *51*, 3489–3495.

[7] R. E. Banks, N. J. Lawrence, A. L. Popplewell, *J. Chem. Soc. Chem. Commun.* **1994**, 343–344.

[8] M. Albrecht, O. Blau, K. Witt, E. Wegelius, M. Nissinen, K. Rissanen, R. Fröhlich, *Synthesis* **1999**, *10*, 1819–1829.

[9] C. Kitamura, N. Maeda, N. Kamada, M. Ouchi, A. Yoneda, *J. Chem. Soc. Perkin Trans. 1* **2000**, 781–785.

[10] H. Gershon, M. W. Mc Neil, *J. Heterocycl. Chem.* **1972**, *9*, 659–667.

[11] a) F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy, G. M. Cole, *J. Biol. Chem.* **2005**, *280*, 5892–5901; b) Y. Porat, A. Abramowitz, E. Gazit, *Chem. Biol. Drug. Des.* **2006**, *67*, 27–37.

[12] a) D. E. Pearson, R. D. Wysong, C. V. Breder, *J. Org. Chem.* **1967**, *32*, 2358–2360; b) G. E. Collis, A. K. Burrell, K. D. John, P. G. Plieger, *Acta Crystallogr. Sect. C* **2003**, *59*, o443-o444.

[13] a) M. Numazawa, Y. Ogura, *J. Chem. Soc. Chem. Commun.* **1983**, 533–534; b) D. Nobel, *J. Chem. Soc. Chem. Commun.* **1993**, 419–420; c) D. Planchenault, R. Dhal, J. Robin, *Tetrahedron* **1995**, *51*, 1395–1404.

[14] a) The National Institute of Standards and Technology Standard Reference Data base 46″ NIST (Critically selected Stability Constants of Metal Complexes Database, version 4.0, US Departement of Commerce); b) L. G. Sillen, A. E. Martell, *Stability constants of*

metal-ion complexes, The Chemical Society London Publication, **1971**.
[15] a) A. Ringblom, *Complexation in Analytical Chemistry*, Interscience, New York, **1963**; b) G. Schwarznbach, H. Flaschka, *Complexometric Titrations*, Meuthuen, New York, **1969**.
[16] H. Gershon, M. W. McNeil, S. G. Schulman, J. W. Parkes, *Anal. Chim. Acta* **1972**, *62*, 43–47.
[17] M. Di Vaira, C. Bazzicalupi, P. Orili, L. Messori, B. Bruni, P. Zatta, *Inorg. Chem.* **2004**, *43*, 3795–3797.
[18] F. Kanamaru, K. Ogawa, I. Nitta, *Bull. Chem. Soc. Jpn.* **1963**, *36*, 422–427.
[19] N. Okabe, H. Saishu, *Acta Crystallogr. Sect. E Acta Cryst.* **2001**, *57*, m714–m716.
[20] a) M. Shoda, H. Gershon, D. Bray, D. D. Clarke, *Monatsh. Chem.* **1998**, *129*, 843–853; b) F. Jian, Y. Wang, L. Lu, X. Yang, X. Wang, S. Chantrapromma, H. K. Fun, I. Abdul Razak, *Acta Crystallogr. Sect. C* **2001**, *57*, 714–716.
[21] H. van de Waterbeemd, E. Gifford, *Nat. Rev. Drug Discovery* **2003**, *2*, 192–204.
[22] C. Deraeve, M. Pitié, B. Meunier, *J. Inorg. Biochem.* **2006**, *100*, 2117–2126.
[23] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467–473.
[24] SIR92—A program for crystal structure solution: A. Altomare, G. Cascarano, C. Giacovazzo and A. Guagliardi, *J. Appl. Crystallogr.* **1993**, *26*, 343–350.
[25] SHELX97 [Includes SHELXS97, SHELXL97, CIFTAB]—Programs for Crystal Structure Analysis (Release 97-2). G. M. Sheldrick, Institut für Anorganische Chemie der Universität, Tammannstrasse 4, 3400 Göttingen (Germany), **1998**.
[26] WINGX—1.63 Integrated System of Windows Programs for the Solution, Refinement and Analysis of Single Crystal X-Ray Diffraction Data: L. Farrugia, *J. Appl. Crystallogr.* **1999**, *32*, 837–838.
[27] International tables for X-ray crystallography, *Vol. IV*, Kynoch press, Birmingham (Great Britain), **1974**.
[28] ORTEP32 for Window: L. J. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.

Received: July 4, 2007
Published online: October 30, 2007

