Stepwise Construction of Polysubstituted Phenanthroline-Based Glutamate Pockets for Lanthanide Complexation

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Abstract: A multi-functionalized ligand, based on a glutamic acid skeleton, bearing phenanthroline carboxylic units as chromophores and chelating arms has been designed. A base-assisted bis N-alkylation of dimethyl glutamate hydrochloride with the pivotal 2-carbomethoxy-4-methoxy-9-bromomethyl-1,10-phenanthroline building block, followed by a saponification step, provided the target ligand as its tetrahydrochloride salt. The spectroscopic properties of the ligand and its lanthanide(III) complexes were investigated in aqueous 0.01 M TRIS/HCl buffer at pH 7.0. The europium(III) complex was highly luminescent, exhibiting a quantum yield of 6% despite the presence of ca. one molecule of water in the first coordination sphere, whereas the terbium(III) complex was only weakly luminescent.

Key words: phenanthroline, carboxylate, lanthanides, luminescence

Molecular architectures composed of chelating items (cryptands, branched macrocycles, podands) for lanthanide (Ln) binding underpin the development of many luminescent labels for biomedical analysis.[1,2] Such europium(III) and terbium(III) complexes are luminescent in aqueous solution if a few conditions are fulfilled: (i) the ligand should include a highly absorbing chromophore (since f→f transitions of naked lanthanides have very low absorption coefficients), (ii) energy transfer from the ligand-centered excited states to the Ln center should be fast and efficient, (iii) water should be largely excluded from the first coordination sphere, since the detrimental, non-radiative deactivation pathways for the excited Ln atoms are promoted by the vibrational modes of the surrounding solvent molecules.[3,4] The objective of creating an antenna effect, which arises when the first two requirements are met, makes the ligand design crucial for the photophysical properties of the resulting complexes.[5] If luminescent lanthanide chelates are to be used as labels in advanced analytical technologies such as homogeneous fluoroimmuno assays, fluorescence imaging, immunohistochemistry, or in situ hybridization techniques, additional severe requirements are needed such as (iv) high thermodynamic and kinetic stability, (v) good hydrophilicity, (vi) very efficient cation emission and high absorption at a suitable wavelength, (vii) a chemical structure allowing proper covalent linkage of the label to the target biomolecule, (viii) the affinity and non specific binding properties of the labeled biomolecules have to be retained and the biological target should have minimum influence on the photophysical properties of the tag. As a consequence of all these strict requirements, only a few viable labels have so far been tested, developed and made commercially available.

We recently required an efficient method by which to substitute a glutamic acid skeleton with two bipyridine carboxylate arms (**A**), allowing the possibility of complexing one glutamate carboxylate to the lanthanide whilst activating the second carboxylate as its hydroxysuccinimide ester (Figure 1).[6] This challenging approach provided complexes with exceptional reactivity towards proteins and very interesting optical properties in aqueous medium. These examples offer a wide range of possibilities where the precise molecular architecture can lead to the development of new molecules in which the lanthanide cation is well protected against solvent molecules with suitable chemical functionalities. The proper combination of light absorbers and anionic function, such as carboxylates, provides a wealth of opportunities with which to finely tune the physical properties of the lanthanide complexes.

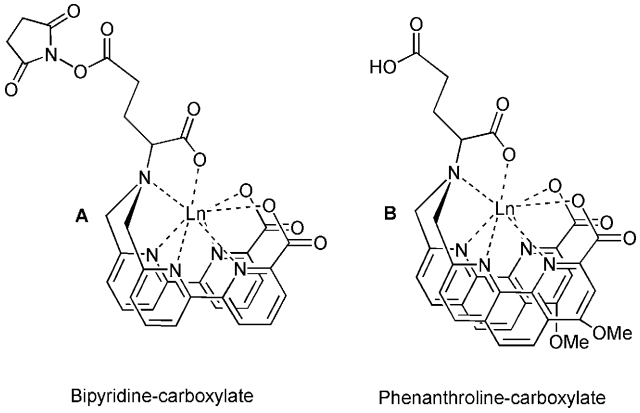

Figure 1

However, one clear disadvantage of using bipyridine-carboxylate moieties in **A** is its absorption in the near-UV (around 308 nm). Other chromophores (phenanthroline, terpyridine, hydroxyquinoline, isoquinoline) possess the necessary properties to absorb above 350 nm. Up until now, the synthetic methodologies available for the con-

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struction of such building blocks remain limited, and the provision of sufficient amounts of these substances may represent a bottleneck for further studies and development in biomedical analysis.

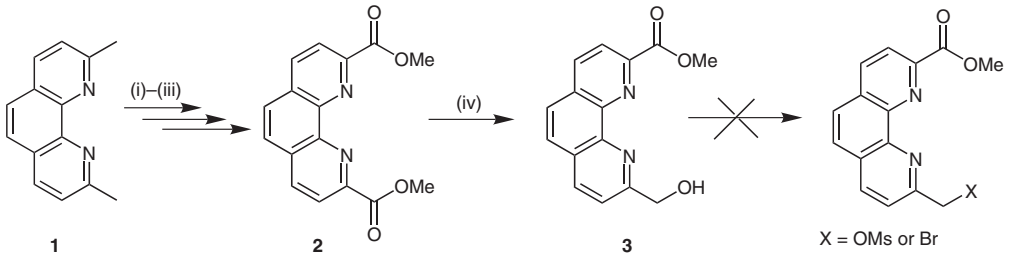
From a general point of view, 1,10-phenanthroline derivatives have enjoyed long-time popularity in the design of ionophores for alkali metal ions,[7] enantioselective catalytic systems,[8] efficient antenna chromophores for luminescent lanthanides ions[9,10] and as templates in the construction of esthetic macrocyclic loops.[11] Recent applications involve the use of lanthanide complexes in the functionalization of hybrid sol-gel glasses[12] and as efficient electroluminescent materials in light-emitting diodes.[13] Also well recognized is the nucleolytic activity of copper-phenanthroline complexes and some derivatives considered to be efficient chemical nucleases.[14,15] The cytotoxicity of some phenanthroline derivatives is also well recognized and these ligands have been used to selectively incorporate metallic cations in monoclonal antibodies.[16]

Here we present our synthetic approach to the engineering of phenanthroline-carboxylate based luminescent complexes **B** depicted in Figure 1. Since published procedures for the synthesis of asymmetrically substituted phenanthroline platforms are scant and preparative yields are usually very low,[17,18] we initially pursued the approach shown in Scheme 1. Starting from 2,9-dimethyl-1,10-phenanthroline, following the procedure developed by Chandler,[19] the dimethyl ester **2** could be obtained pure in three steps with an overall yield of 29%.

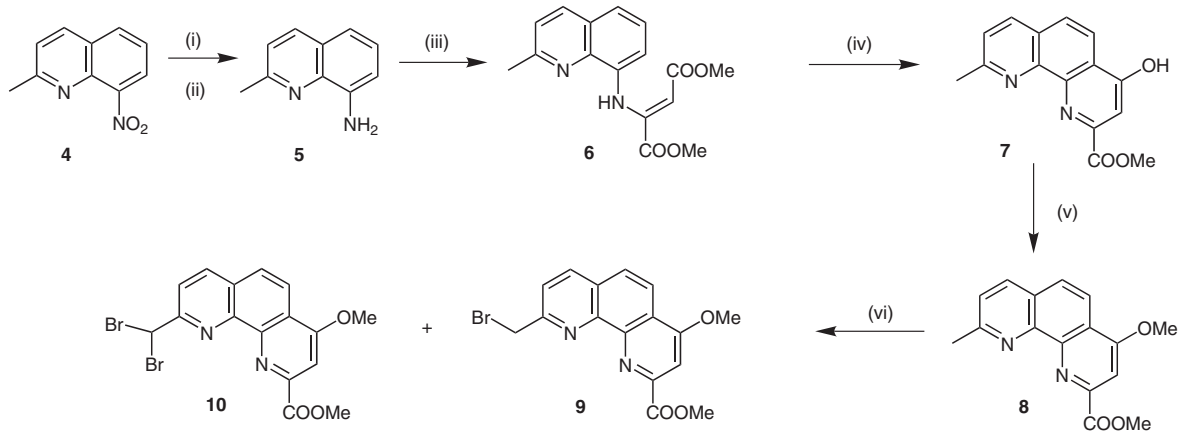
The selective reduction of a single ester function was inspired by related results obtained on pyridine derivatives with $NaBH_4$ in methanol (85% of the monoester/monobenzylalcoholpyridine derivative was isolated).[20] In our hands, attempts to adapt this protocol to the synthesis of phenanthroline was far less successful (12 to 22%). Furthermore, tentative conversion of the primary alcohol to the mesylate or bromo derivatives failed under various experimental conditions.

Adopting an alternative strategy inspired by analogous procedures,[21] we chose to investigate the preparation of the pivotal starting material **9** as described in Scheme 2. The reduction of the nitro derivative **4** to the corresponding amino compound was the first target and the best yield (95%) was obtained using concentrated HI under harsh conditions.[22] Various other conditions were tested and are summarized in Table 1. Amongst them, the use of molecular hydrogen and palladium supported catalysts provide compound **5** in variable yields, as did the use of various metals Fe, Sm, Sn with the exclusion of Zn.

With this viable protocol in hand, we turned our attention towards the addition and cyclization reaction of dimethyl acetylenedicarboxylate, leading to compound **7** after refluxing in diphenyl ether.[23] Alkylation of the hydroxypyridine ring provided derivative **8** in good yield. Classical



Scheme 1 *Reagents and conditions*: (i) SeO_2, dioxane–H_2O, reflux, 70%; (ii) 80% HNO_3, reflux, 61%; (iii) MeOH, HCl, reflux; aq $NaHCO_3$, 67%; (iv) $NaBH_4$, MeOH, 12–22%.



Scheme 2 *Reagents and conditions*: (i) 57% aq HI, 90 °C; (ii) aq $NaHCO_3$, 95%; (iii) MeO_2C-C≡C-CO_2Me, MeOH, r.t., 100%; (iv) Ph_2O, reflux, 83%; (v) MeI, K_2CO_3, MeCN, 80 °C, 95%; (vi) NBS, AIBN cat., benzene, reflux, 37% of **9**, 8% of **10**.

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Table 1 Experimental Conditions for the Reduction of 8-Nitroquinaldine **4** to 8-Aminoquinaldine **5**

Reagents and conditions	Isolated yields (%)
H_2 (5 bar), 5% Pd/C, EtOH–CH_2Cl_2, r.t.	42–62
Zn, aq NaOH, EtOH, reflux	–
$Na_2S_2O_4$, H_2O–THF, 70 °C	24
Fe, HCl, EtOH–H_2O, reflux, 17 h	30
Fe, HCl, EtOH–H_2O, reflux, 1 h	54
Sm, NH_4Cl, MeOH, sonication	45
$SnCl_2$, HCl, Et_2O	86
57% aq HI, 90 °C	95

bromination under radical conditions provided a mixture of the desired mono-bromo derivative **9** in 37% and the disubstituted compound **10** in 8% yield. Both compounds were relatively easy to separate from the unreacted starting material **8** (48% recovered yield) which could be recycled.

We were pleased to find that the key mono-bromo derivative **9** reacted with the hydrochloride salt of dimethyl glutamate under basic conditions (Scheme 3). This reaction provided the intermediate **11** albeit in low yield. A critical side reaction, likely due to hydrolysis of the ester function, gave 2-carbomethoxy-4-methoxy-9-methoxymethyl-1,10-phenanthroline **12** as the major product (65%). The possible intramolecular lactamization of the dimethyl glutamate presumably generated the methanol responsible for the displacement of the bromine.

All attempts to improve the yields using other bases including Hünig's base, azeotropic distillation, and rigorously anhydrous conditions failed. Hydrolysis of the tetraester **11** was achieved under basic conditions affording the target tetra-acid derivative **13** in excellent yield.

The potential of ligand **13** to form stable lanthanide complexes was investigated using absorption and luminescence spectroscopy in aqueous solution in a TRIS/HCl buffer at pH 7.0 using stoechiometric amounts of the lanthanide nitrate salts. Basically, the free ligand exhibits intense π–π* and n–π* transitions in water corresponding to the aromatic phenanthroline moieties (Table 2). As was expected both from the design and from related lanthanides-phenanthroline derivatives,[18,24] the less energetic absorption band spanned from 300–370 nm (Figure 2).

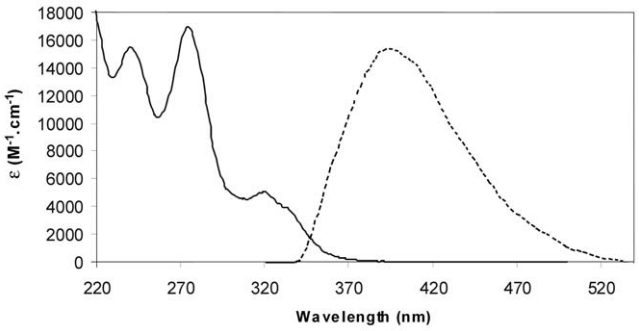
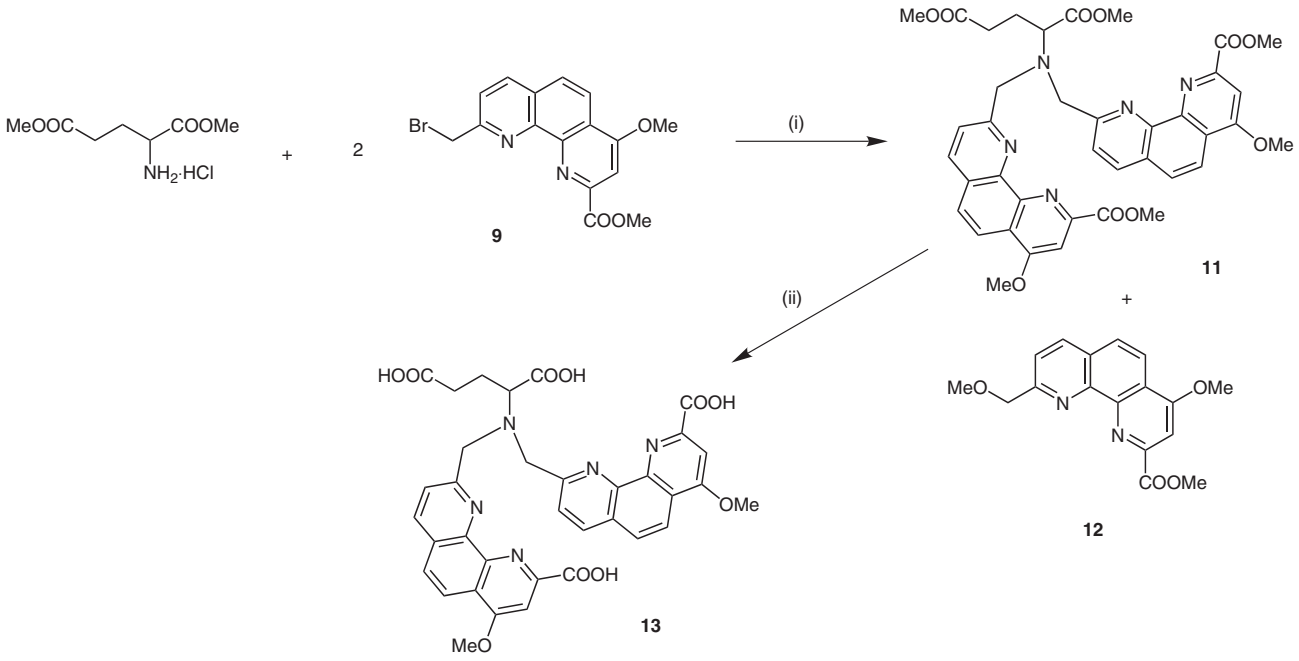


Figure 2 Absorption (—) and emission (---) spectra (upon excitation at 270 nm) of ligand **13** in 0.01 M TRIS/HCl buffer (pH 7.0)

Upon excitation in the 270 nm band, an intense fluorescence was observed around 390 nm. The short excited state lifetime is consistent with the singlet emissive state usually found in the oligopyridine series. Complexation



Scheme 3 *Reagents and conditions*: (i) K_2CO_3, MeCN, reflux, 14% of **11,** 65% of **12**; (ii) NaOH, MeOH–H_2O, 70 °C, 98%.

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with Eu^{3+} and Tb^{3+} resulted in a hyperchromic shift of the most intense absorption band (Figure 3) and no significant shift of the band around 320 nm. This is probably due to the rigidity of the phenanthroline core. It could be seen that, in the bipyridine case, complexation with lanthanides usually induced a bathochromic shift depending of the strength of the interaction and is prone to a *cis-trans* internal conversion of the two pyridine rings during the complexation process. In both lanthanide complexes of ligand **13**, excitation at 277 nm resulted in dual emission from the ligand and lanthanide (Figure 4).

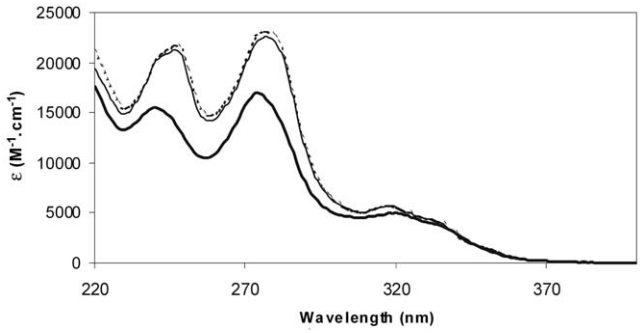


Figure 3 Absorption spectra of ligand **13** (—), [Eu(**13**-4H)]Na (–) and [Tb(**13**-4H)]Na (---) in 0.01 M TRIS/HCl buffer (pH 7.0)

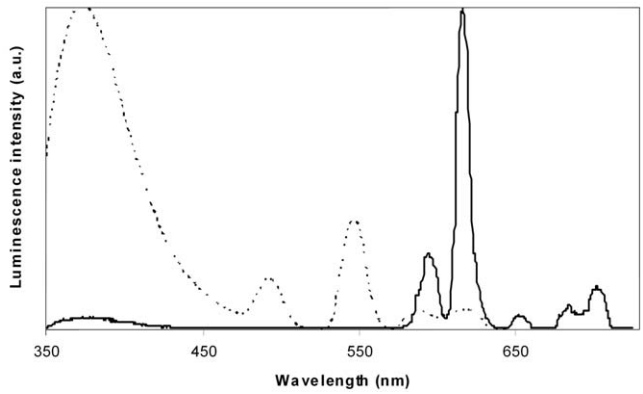


Figure 4 Normalized emission spectra of [Eu(**13**-4H)]Na (–) and [Tb(**13**-4H)]Na (---) in 0.01 M TRIS/HCl buffer (pH 7.0).

The emission at 380 nm is likely due to ligand **13** (as found in Figure 2), whereas the sharper peaks are characteristic of Eu or Tb emission. The fluorescence of the ligand disappears if a 50 μs delay is applied, while the luminescence of the lanthanides persists. For both lanthanide complexes, the excitation spectrum matches the absorption spectra, allowing us to conclude that energy transfer is occurring from the phenanthroline subunits to the emissive states of the lanthanides. For all complexes, a single exponential decay is found, indicating that a single species is present in solution and responsible for the emissive state (Table 3). Nevertheless, the presence of remaining, ligand-centered fluorescence around 380 nm is likely attributed to low intersystem crossing efficiency. The weak luminescence of the Tb complex is understood by a poor positioning of the triplet state of the ligand, which favors a back energy transfer from the $^5D_4$ emissive state of the terbium.

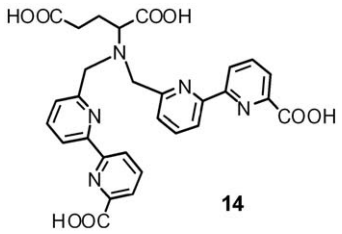
Table 2 Absorption Properties of Ligand **13**, [Eu(**13**-4H)]Na and [Tb(**13**-4H)]Na Complexes in 0.01 M TRIS/HCl Buffer (pH 7.0)

Compound	λ_{max} (nm)	ε_{max} ($M^{-1}\cdot cm^{-1}$)
13	320	5100
	274	17000
	240	15500
[Eu(**13**-4H)]Na	318	5700
	277	22600
	246	21300
[Tb(**13**-4H)]Na	318	5700
	277	23200
	247	21800

Further photophysical studies, in particular an examination of the excited state lifetimes in water and deuterated water for the Eu complex, also points to the presence of a single species in solution. By comparing the quantum yields and lifetimes in both solvents, an average value of one molecule of water was calculated to be present in the first coordination sphere of the Eu ($q = 1.2 \pm 0.2$).[25] These results can be favorably compared to ligand **14** (Figure 5). Unfortunately, this was not the case for the Tb complex for which weak luminescence was obtained due to unfavorable matching of the ligand and Tb excited states.

Table 3 Lifetimes and Quantum Yields of [Ln(**13**-4H)]Na and [Ln(**14**-4H)]Na (Ln = Eu, Tb) in 0.01 M TRIS/HCl Buffer (pH 7.0)

Complex	τ^{300K} (ms)		Φ^{300K} (%)	
	H_2O	D_2O	H_2O	D_2O
[Eu(**13**-4H)]Na	0.53	2.22	5.6	8.2
[Tb(**13**-4H)]Na	0.80	–	< 0.1	–
[Eu(**14**-4H)]Na	0.62	2.48	8	35
[Tb(**14**-4H)]Na	1.48	2.53	31	53



14

Figure 5

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In summary, we have shown that 8-nitroquinaldine can be converted through sequential reduction, cyclization, alkylation, bromination and N-alkylation to provide an easy entry into glutamate-based bis-phenanthroline carboxylate ligands. Complexation with Eu and Tb gives stable complexes in aqueous media. The Eu complex exhibits a lifetime of about 0.5 ms in water and a quatum yield of 5.6%. Further work is in progress to extend the scope of the present methodology to prepare terpyridine based molecular structures with the aim of obtaining highly luminescent complexes.

Reactions were performed under a dry atmosphere of argon using standard Schlenk tube techniques when stated. Solvents and raw materials were of analytical grade and were used as received. MeCN (Riedel-de Haën) was filtered over alumina (Merck) and distilled from P_2O_5 under an argon atmosphere immediately prior to use. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded at r.t. on a Bruker AC 200 spectrometer, ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at r.t. on a Bruker Avance 300 spectrometer. Shifts (δ) are reported in ppm relative to residual protons in the solvent. FT-IR spectra were recorded as KBr pellets on a Nicolet 210 spectrometer. UV-Vis absorption spectra were recorded on Uvikon 933 (Kontron Instrument) spectrophotometer. Emission and excitation spectra were recorded using a PerkinElmer LS 50B spectrofluorimeter equipped with a R928 (Hamamatsu) photomultiplier. Metal luminescence lifetimes were measured on a PTI QuantaMaster spectrofluorimeter. Metal luminescence quantum yields were measured using the procedure described by Haas and Stein,[26] using as standards $Ru(bpy)_3Cl_2$ ($\Phi = 0.028$ in aerated H_2O)[27] for Eu^{3+} and rhodamine 6G ($\Phi = 0.88$ in EtOH)[28] for Tb^{3+}. Fast atom bombardment (FAB, positive mode) mass spectra were recorded with ZAB-HF-VB analytical apparatus using *meta*-nitrobenzyl alcohol (*m*-NBA) as matrix. Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectra were recorded using a Perkin-Elmer/PerSeptive Biosystems Voyager-DE-RP mass spectrometer. Melting points were obtained on a Büchi Melting Point 535 capillary melting point apparatus in open-ended capillaries and are uncorrected. Chromatographic purifications were performed using 0.063–0.200 mm silica gel 60 (Merck) or aluminium oxide 90 (Merck). Thin layer chromatography (TLC) was performed on silica gel or aluminium oxide plates (Merck) coated with fluorescent indicator. All mixtures of solvents are given in v/v ratio.

2-Carbomethoxy-9-hydroxymethyl-1,10-phenanthroline (3)

A mixture of 2,9-dicarbomethoxy-1,10-phenanthroline **2**[19] (0.100 g, 0.338 mmol) and $NaBH_4$ (0.029 g, 0.744 mmol) was refluxed in MeOH (5 mL) for 1 h. The solution was evaporated to dryness and the residue was purified by column chromatography (Al_2O_3, CH_2Cl_2–MeOH, 97:3).

Yield: 0.020 g, 22%; pale-yellow solid; $R_f = 0.25$ (Al_2O_3, CH_2Cl_2–MeOH, 97:3).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.09 (s, 3 H), 5.13 (s, 2 H), 7.70 (d, ^3J = 8.5 Hz, 1 H), 7.77–7.90 (m, 2 H), 8.23 (d, ^3J = 8.5 Hz, 1 H), 8.35–8.44 (m, 2 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 53.0, 65.6, 121.3, 123.3, 125.5, 128.0, 128.8, 130.5, 136.9, 137.4, 145.0, 147.4, 165.9.

HRMS (MALDI-TOF): *m/z* [M + H]$^+$ calcd for $C_{15}H_{13}N_2O_3$: 269.0926; found: 269.0913.

2-Methyl-8-aminoquinoline (5)

2-Methyl-8-nitroquinoline (2.11 g, 11.2 mmol) was dissolved in aq HI (57%, 34 mL). The solution was heated to 90 °C for 2 h then, after cooling to r.t., sat. aq $NaHCO_3$ (100 mL) was added and the aqueous phase was extracted with EtOAc (560 mL). The organic layer was washed with sat. aq $Na_2S_2O_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography (SiO_2, CH_2Cl_2–MeOH, 100:0 to 99:1) to give compound **5** (1.68 g, 95%) as a crystalline orange powder.

All analyses correspond to those described in the literature.[21]

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.72 (s, 3 H), 4.98 (br s, 2 H), 6.90 (d, ^3J = 7.5 Hz, 1 H), 7.12 (d, ^3J = 8.0 Hz, 1 H), 7.25 (d, ^3J = 8.5 Hz, 1 H), 7.28 (t, ^3J = 8.0 Hz, 1 H), 7.94 (d, 8.5 Hz, 1 H).

2-(2-Methylquinolin-8-ylamino)butenedioic Acid Dimethyl Ester (6)

2-Methyl-8-aminoquinoline **5** (1.68 g, 10.6 mmol) and dimethyl acetylenedicarboxylate (2 mL, 16.3 mmol) were dissolved in MeOH (30 mL) and the solution was stirred at r.t. for 20 h in the dark. The solvent was evaporated and the residue was purified by column chromatography (SiO_2, CH_2Cl_2) to give compound **6** (3.18 g, 100%) as a yellow oil.

All analyses correspond to those described in the literature.[21]

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.76 (s, 3 H), 3.71 (s, 3 H), 3.79 (s, 3 H), 5.55 (s, 1 H), 6.93 (d, ^3J = 7.0 Hz, 1 H), 7.26–7.43 (m, 3 H), 7.99 (d, ^3J = 8.5 Hz, 1 H), 10.82 (s, 1 H).

2-Carbomethoxy-4-hydroxy-9-methyl-1,10-phenanthroline (7)

Compound **6** (1.38 g, 4.6 mmol) was dissolved in Ph_2O (50 mL) and the solution was refluxed at 260 °C for 20 min. After cooling to r.t., Et_2O (50 mL) was added and the solution was cooled to 0 °C to complete crystallization of **7**. The solid was filtered and washed with Et_2O yielding compound **7** (844 mg, 3.1 mmol). The mother liquor was evaporated to dryness and the residue was purified by column chromatography (SiO_2, CH_2Cl_2–MeOH, 100:0 to 95:5) to give **7** (174 mg, 0.6 mmol). Upon combination of the two fractions, compound **7** (1.02 g, 83%) was isolated as a yellowish powder.

All analyses correspond to those described in the literature.[21]

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.80 (s, 3 H), 4.08 (s, 3 H), 7.14 (s, 1 H), 7.47 (d, ^3J = 8.5 Hz, 1 H), 7.57 (d, ^3J = 9.0 Hz, 1 H), 8.09 (d, ^3J = 8.5 Hz, 1 H), 8.21 (d, ^3J = 9.0 Hz, 1 H).

2-Carbomethoxy-4-methoxy-9-methyl-1,10-phenanthroline (8)

In a Schlenk tube under argon were dissolved 2-carbomethoxy-4-hydroxy-9-methyl-1,10-phenanthroline **7** (2.04 g, 7.6 mmol), MeI (950 μL, 15.3 mmol) and anhyd K_2CO_3 (2.11 g, 15.2 mmol) in dry MeCN (60 mL). The solution was heated to 80 °C for 19 h then the mixture was evaporated to dryness, and the solid residue was partitioned between CH_2Cl_2 (100 mL) and H_2O (15 mL). The aqueous phase was further extracted with CH_2Cl_2 (4 × 15 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting solid was purified by column chromatography (Al_2O_3, CH_2Cl_2–MeOH, 99:1).

Yield: 2.05 g, 95%; yellow solid; $R_f = 0.54$ (Al_2O_3, CH_2Cl_2–MeOH, 98:2).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.91 (s, 3 H), 4.06 (s, 3 H), 4.12 (s, 3 H), 7.47 (d, ^3J = 8.5 Hz, 1 H), 7.77 (d, ^3J = 9.0 Hz, 1 H), 7.83 (s, 1 H), 8.08 (d, ^3J = 7.5 Hz, 1 H), 8.12 (d, ^3J = 9.0 Hz, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 25.8, 52.8, 56.2, 102.9, 118.7, 122.2, 123.9, 126.9, 127.4, 136.0, 145.2, 146.1, 148.6, 160.1, 163.2, 166.5.

MS (FAB$^+$): *m/z* (%) = 283.2 (100) [M + H]$^+$.

Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 67.92; H, 4.93; N, 9.78.

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2-Carbomethoxy-4-methoxy-9-bromomethyl-1,10-phenanthroline (9).

A solution of **8** (1 g, 3.5 mmol), NBS (630 mg, 3.5 mmol) and AIBN (30 mg, 0.2 mmol) in benzene (10 mL) was irradiated for 30 min with a 100 W halogen lamp. The mixture was evaporated to dryness and the residue was purified by a column chromatography (Al_2O_3, CH_2Cl_2–hexane, 50:50).

Yield: 468 mg, 37%; yellow solid; $R_f = 0.55$ (Al_2O_3, CH_2Cl_2–MeOH, 99:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.06 (s, 3 H), 4.12 (s, 3 H), 4.93 (s, 2 H), 7.77 (d, ^3J = 9.0 Hz, 1 H), 7.83 (s, 1 H), 7.87 (d, ^3J = 8.5 Hz, 1 H), 8.17 (d, ^3J = 9.0 Hz, 1 H), 8.21 (d, ^3J = 8.5 Hz, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 34.6, 53.0, 56.3, 103.3, 120.2, 122.4, 123.7, 127.0, 128.1, 137.1, 144.5, 145.9, 148.9, 157.6, 163.3, 166.2.

MS (FAB): *m*/*z* (%) = 281.2 (30) [**9** – Br]$^+$, 361.2 (100) [**9** + H]$^+$, 363.2 (100) [**9** + H]$^+$.

Anal. Calcd for $C_{16}H_{13}BrN_2O_3$: C, 53.21; H, 3.63; N, 7.76. Found: C, 52.94; H, 3.26; N, 7.51.

2-Carbomethoxy-4-methoxy-9-dibromomethyl-1,10-phenanthroline (10)

Compound **10** was obtained in 8% yield as a by-product of the radical bromination of **8**.

$R_f = 0.73$ (Al_2O_3, CH_2Cl_2–MeOH, 99:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.09 (s, 3 H), 4.15 (s, 3 H), 7.06 (s, 1 H), 7.82 (d, ^3J = 9.0 Hz, 1 H), 7.86 (s, 1 H), 8.24 (d, ^3J = 9.0 Hz, 1 H), 8.27 (d, ^3J = 9.0 Hz, 1 H), 8.34 (d, ^3J = 8.5 Hz, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 42.4, 53.2, 56.4, 103.5, 121.2, 122.8, 122.9, 126.9, 128.9, 129.1, 138.2, 146.1, 149.1, 159.5, 163.4, 166.1.

MS (FAB): *m*/*z* (%) = 280.3 (15) [**10** – Br_2]$^+$, 359.2 (48) [**10** – Br]$^+$, 361.2 (50) [**10** – Br]$^+$, 441.1 (100) [**10** + H]$^+$.

Anal. Calcd for $C_{16}H_{12}Br_2N_2O_3$: C, 43.67; H, 2.75; N, 6.37. Found: C, 43.40; H, 2.63; N, 6.05.

Dimethyl *N*,*N*-[(7-Methoxy-9-carbomethoxy-1,10-phenanthrol-2-yl)methyl]glutamate (11)

In a Schlenk tube under argon were dissolved dimethyl glutamate hydrochloride (96 mg, 0.45 mmol) and anhydrous K_2CO_3 (250 mg, 1.81 mmol) in dry MeCN (15 mL). The solution was heated to 80 °C for 10 min then compound **10** (360 mg, 1 mmol) was added. The solution was heated to 80 °C for 18 h then a further portion of **10** (52 mg, 0.14 mmol) was added and the solution was heated to 80 °C for 24 h. The mixture was evaporated to dryness, and the solid residue was partitioned between CH_2Cl_2 (30 mL) and H_2O (10 mL). The aqueous phase was further extracted with CH_2Cl_2 (4 × 30 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting solid was purified by column chromatography (Al_2O_3, CH_2Cl_2–MeOH, 100:0 to 99.3:0.7).

Yield: 46 mg, 14%; yellow-orange powder; $R_f = 0.31$ (Al_2O_3, CH_2Cl_2–MeOH, 95:5).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.17–2.28 (m, 2 H), 2.61 (t, ^3J = 7.5 Hz, 2 H), 3.44 (s, 3 H), 3.71 (t, ^3J = 7.5 Hz, 1 H), 3.83 (s, 3 H), 4.06–4.19 (m, 12 H), 4.45–4.70 (m, 4 H), 7.82 (d, ^3J = 9.0 Hz, 2 H), 7.86 (s, 2 H), 8.13 (d, ^3J = 8.0 Hz, 2 H), 8.18 (d, ^3J = 9.0 Hz, 2 H), 8.24 (d, ^3J = 8.5 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.0, 29.7, 31.0, 51.4, 51.7, 53.0, 53.4, 56.3, 58.1, 62.4, 62.9, 103.1, 119.4, 122.3, 122.7, 122.8, 127.3, 127.4, 127.9, 136.8, 145.2, 146.1, 148.6, 149.0, 161.1, 161.3, 163.2, 163.3, 166.0, 166.4, 173.1, 173.6.

HRMS (MALDI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_{39}H_{38}N_5O_{10}$: 736.2619; found: 735.2608.

2-Carbomethoxy-4-methoxy-9-methoxymethyl-1,10-phenanthroline (12)

Compound **12** was obtained in 65% yield as a by-product of the alkylation of dimethyl glutamate hydrochloride with bromomethylphenanthroline **10**.

$R_f = 0.62$ (Al_2O_3, CH_2Cl_2–MeOH, 95:5).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.98 (s, 3 H), 4.04 (s, 3 H), 4.09 (s, 3 H), 5.26 (s, 2 H), 7.40 (d, ^3J = 8.0 Hz, 1 H), 7.71 (d, ^3J = 9.0 Hz, 1 H), 7.76 (s, 1 H), 8.07 (d, ^3J = 9.0 Hz, 1 H), 8.13 (d, ^3J = 8.0 Hz, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.4, 44.4, 52.7, 56.2, 103.1, 119.6, 120.3, 122.3, 127.0, 127.9, 136.8, 145.2, 146.0, 148.6, 154.7, 163.0, 166.2.

MS (FAB): *m*/*z* (%) = 281.1 (38) [**12** – OCH_3]$^+$, 313.1 (100) [**12** + H]$^+$.

Anal. Calcd for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.27; H, 5.08; N, 8.79.

***N*,*N*-[(7-Methoxy-9-carboxy-1,10-phenanthrol-2-yl)methyl]glutamic Acid Tetrahydrochloride (13)**

A solution of compound **11** (29 mg, 0.04 mmol) and NaOH (8 mg, 0.2 mmol) in a mixture of MeOH (8 mL) and H_2O (2 mL) was heated to 70 °C for 8 h. The solution was evaporated to dryness, acidified with aq HCl (1N) and the resulting solution was again evaporated to dryness. The residue was solubilized in MeOH and the product was precipitated with Et_2O. Upon centrifugation, **13**·4HCl (32 mg, 98%) was isolated as a yellow-orange powder.

IR (KBr): 3436, 2924, 2853, 1635, 1507, 1454, 1384, 1235, 1070 cm^{-1}.

^{1}H NMR (300 MHz, CD_3OD): δ = 2.39–2.49 (m, 2 H), 2.84 (t, ^3J = 7.0 Hz, 2 H), 4.17 (t, ^3J = 7.5 Hz, 1 H), 4.35 (s, 6 H), 4.81–4.85 (m, 4 H), 7.87 (d, ^3J = 9.5 Hz, 2 H), 7.89 (s, 2 H), 8.16 (d, ^3J = 9.0 Hz, 2 H), 8.18 (d, ^3J = 8.5 Hz, 2 H), 8.65 (d, ^3J = 9.0 Hz, 2 H).

MS (FAB): *m*/*z* (%) = 340.5 (20) [**13** + 2 × H]$^{2+}$, 680.2 (80) [**13** + H]$^+$.

Anal. Calcd for $C_{35}H_{29}N_5O_{10}$·4HCl: C, 50.93; H, 4.03; N, 8.48. Found: C, 50.82; H, 4.15; N, 8.34.

UV/Vis (0.01 M TRIS/HCl buffer, pH 7.0): λ_{max} (ε) = 240 (15500), 274 (17000), 320 nm (5100).

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