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# Microwave Assisted Synthesis of a Small Library of Substituted *N*,*N*′-Bis((8-hydroxy-7-quinolinyl)methyl)-1,10-diaza-18-crown-6 Ethers

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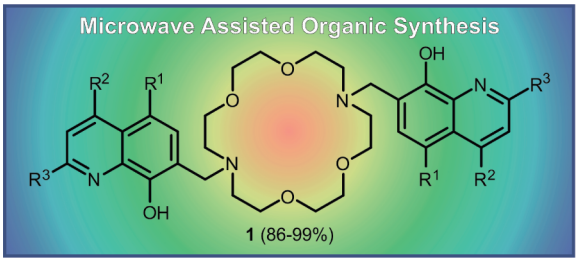
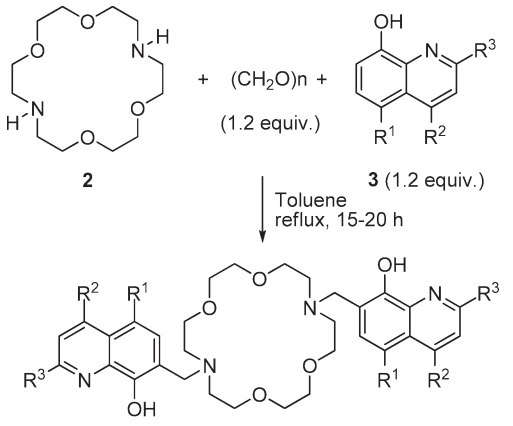



*N*,*N*′-Bis-((8-hydroxy-7-quinolinyl)methyl)-1,10-diaza-18-crown-6 ether **1a** and its analogue **1c** are known as fluorescent sensors of magnesium in living cells. With the aim to investigate the effects of the substitution pattern on the photophysical properties of ligands **1** and their metal complexes, we developed an efficient microwaves enhanced one-pot Mannich reaction to double-armed diaza-crown ligands **1** carrying a variety of substituents. This new protocol is characterized by shorter reaction times, enhanced yields, and improved product purities with respect to the use of conventional conductive heating.

1,10-Diaza-18-crown-6 ethers of general structure **1** (Scheme 1), bearing two 8-hydroxy quinoline side arms, have been recently proposed and applied as fluorescent chemosensors for a variety of metal ions, including magnesium(II) in living cells,[1] cadmium(II),[2] and mercury(II).[3] While undertaking a structural study on the effects of substituents on 2, 4, and 5 position of the quinoline ring on the complexing power and selectivity of **1**, the task of synthesizing good amounts of **1** of good purity proved to be somewhat troublesome. Indeed, the most practical synthetic approach to **1** was a thermal three-component classic Mannich reaction of 1,10-diaza-18-crown-6 ether **2** (0.02−0.045 M in toluene) with a slight excess of paraformaldehyde and the appropriate 8-hydroxyquinoline **3** (1.2 equiv). After refluxing in toluene for 15−20 h, the desired products were obtained in moderate to good yields (Scheme 1).[4] However, in our hands, complex crude reaction mixtures were obtained, from which pure **1** was recovered in a low amount after a sluggish purification by crystallization.

**SCHEME 1. Thermal One-Pot Mannich Synthesis of Ligands 1**




Since the pioneering works of Gedye, Giguere, and Majetich,[5] microwave-assisted organic synthesis (MAOS) has received an ever increasing attention and has now become a well-established and reproducible technique.[6]

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Even if the so-called "specific" or "non-thermal" microwave effects have not been definitely established up to date, a large number of publications demonstrate that microwave heating often results in enhanced reaction rates, as well as reduced formation of byproduct, with respect to conventional conductive heating. Microwave heating has now been successfully applied to a number of one-pot multicomponent procedures,[7] including Mannich and related reactions.[8]

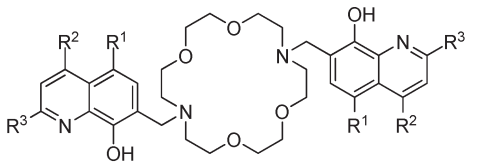
Here we report how the one-pot three-component Mannich synthesis of ligands **1** can be greatly improved by the use of microwave heating, with a reduction of the reaction time, an increase of the yield, and the minimization of side reactions, allowing much easier purification operations.

We started optimizing the reported procedure[4] for ligands **1a**−**d** using microwave heating in different solvents. Using the same reagent ratios and concentrations adopted in thermal reactions (**2** 0.045 M in toluene or 1,4-dioxane as the solvent), an encouraging improvement of the efficiency was observed, as shown in entries 1 and 2 of Table 1. The procedure was further optimized using a 5-fold increase in the concentration of **2** (0.25 M), a slight increased excess of paraformaldehyde (1.5 equiv) and an equimolar amount of hydroxyquinolines **3**. Using a power of 600 W for 2 h at atmospheric pressure, products **1a**−**d** were obtained in excellent yields in all the cases examined (entries 3−10, Table 1).

**TABLE 1. Microwave-Assisted Synthesis of Ligands 1a−d**

| entry | solvent | **1**, $Y^a$ (%) |
|---|---|---|
| 1[b] | toluene | **1a**, 83 |
| 2[b] | 1,4-dioxane | **1a**, 80 |
| 3[c] | toluene | **1a**, 90 |
| 4[c] | 1,4-dioxane | **1a**, 88 |
| 5[c] | toluene | **1b**, 98 |
| 6[c] | 1,4-dioxane | **1b**, 95 |
| 7[c] | toluene | **1c**, 98 |
| 8[c] | 1,4-dioxane | **1c**, 99 |
| 9[c] | toluene | **1d**, 98 |
| 10[c] | 1,4-dioxane | **1d**, 96 |

[a]Isolated yields. [b]Reactions were run on 0.5 mmol of diazacrown **2**, 1.2 mmol of paraformaldehyde, and 1.2 mmol of 8-hydroxy-quinoline in 11 mL of solvent, using a power of 600 W for 2 h. [c]Reactions were run on 0.5 mmol of diazacrown **2**, 1.5 mmol of paraformaldehyde, and 1.0 mmol of 8-hydroxy-quinoline in 2 mL of solvent, using a power of 600 W for 2 h.



**1e**: $R^1 = R^2 = H$, $R^3 = CH_3$ **1f**: $R^1 = Ph$, $R^2 = R^3 = H$
**1g**: $R^1 = CH_2OBn$, $R^2 = R^3 = H$ **1h**: $R^1 = CH_2OCH_3$, $R^2 = R^3 = H$
**1i**: $R^1 = CH_2OCH_2C(CH_3)_2(C{=}O)OCH_3$, $R^2 = R^3 = H$
**1j**: $R^1 = CH_2On\text{-}C_8H_{17}$, $R^2 = R^3 = H$ **1k**: $R^1 = R^3 = H$, $R^2 = OCH_3$

**FIGURE 1.** Differently substituted ligands **1** synthesized.

**TABLE 2. Microwave-Assisted Synthesis of Ligands 1e−k[a]**

| entry | $t$ (h) | *1*, $Y^b$ (%) |
|---|---|---|
| 1 | 2 | **1e**, 95 |
| 2 | 4 | **1f**, 92 |
| 3 | 3 | **1g**, 98 |
| 4 | 3 | **1h**, 95 |
| 5 | 4 | **1i**, 86 |
| 6 | 3 | **1j**, 91 |
| 7 | 3 | **1k**, 90 |

[a]Reactions were run on 0.5 mmol of diazacrown **2**, 1.5 mmol of paraformaldehyde, and 1.0 mmol of 8-hydroxy-quinoline in 2 mL of 1,4-dioxane, using a power of 600 W for the reported time. [b]Isolated yields.

In terms of product quality, the use of stoichiometric amounts of **3** had a significant impact on the crude reaction mixture purity that in all cases was >95% as judged by its $^1$H NMR analysis. Thus, solvent removal under vacuum delivered ligands **1a**−**d** as solid compounds that were purified simply by washing with diethyl ether. If necessary, **1a**−**d** can be recrystallized from dichloromethane/diethyl ether mixtures.

With this simple and efficient procedure in our hands, we synthesized a new group of ligands (**1e**−**k**, Figure 1), to investigate their capability to bind magnesium(II) cation in water and living cells or to modulate their solubility and distribution among the cellular compartments.

The results obtained using the microwave enhanced protocol in 1,4-dioxane as solvent are reported in Table 2. All the new compounds **1e**−**k** were obtained in very good yields and purity according to the microwave-assisted protocol.

Concerning the synthesis of new ligands, it is worth underlining that, while 2-methyl-8-hydroxy-quinoline **3e** is a commercial product, quinolines **3f**−**k** were prepared following reported literature procedures. 5-Phenyl-8-hydroxy-quinoline (**3f**) was prepared by a Suzuki cross-coupling, starting from commercial 5-chloro-8-hydroxy-quinoline **3c**, as reported by Hormi.[9]

5-Alkyloxymethyl-8-hydroxy-quinolines (**3g**−**j**) were synthesized with a slighlty modified procedure with respect to the one reporteb by Burckhalter and Leib (Scheme 2, see Supporting Information).[10]

Finally, 4-methoxy-8-hydroxy-quinoline **3k** was prepared from the commercially available xanthurenic acid, as recently reported by Hormi.[11]

A complete set of results on the complexing and sensing abilities of the new ligands for divalent cations will be

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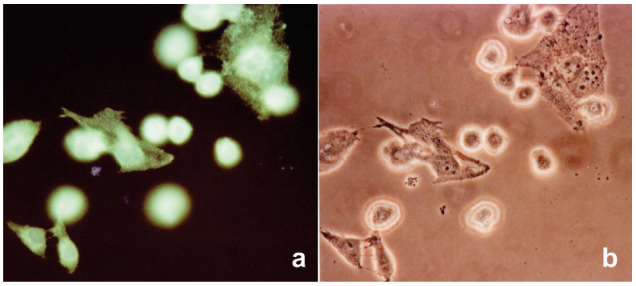
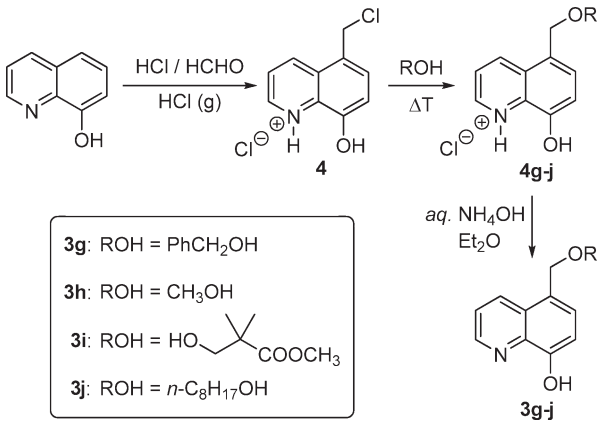



**FIGURE 2.** (a) SaOs-2 cells grown on a glass coverslip were stained for 5 min with **1a** dye (25 $\mu$M) dissolved in Dulbecco's phosphate-buffered saline (DPBS) and observed exciting the sample at 390 nm and collecting the emitted fluorescence around 500 nm. (b) The same field observed by phase contrast.

**SCHEME 2. Synthesis of Quinolines 3g−j**




reported in due course. Here we wish to report the photophysical properties of **1a** synthesized by the present methods, which allowed to reach a higher grade of purity compared to the method previously published.[4] We will particularly point out how this higher purity affects its sensing performance.

The probe **1a** was used to stain SaOs-2 cells as shown in Figure 2. It is clear, especially in the flattened attached cells, the ubiquitous distribution of the probe with a more intense fluorescence in the nuclear and perinuclear regions, as expected for the magnesium cellular distribution.

For a quantitative evaluation of the complexing ability of this species, the probe was dissolved in buffered water and titrated with $Mg^{2+}$. The titration was followed by spectrofluorimetric measurements and the fluorescence spectra are reported in Figure 3.

It has to be noted that the observed fluorescence enhancement of the dye **1a** obtained with this new synthetic method is remarkably higher than the one previously observed.[1a] This is due to the higher purity of the probe that results in a 5-fold lower initial signal in the absence of magnesium ions, allowing a lower detection limit.

The analytical capability of **1a** is thus maintained if not increased, as shown in Table 3, where the fluorescence data obtained with the probes obtained with the two synthetic methodologies are compared with atomic absorption determinations for different cell lines.

Finally, the more sensitive response to magnesium allowed to carry out the same applications at a lower concentration (20 $\mu$M compared to concentration ranging between 25 and 50 $\mu$M).

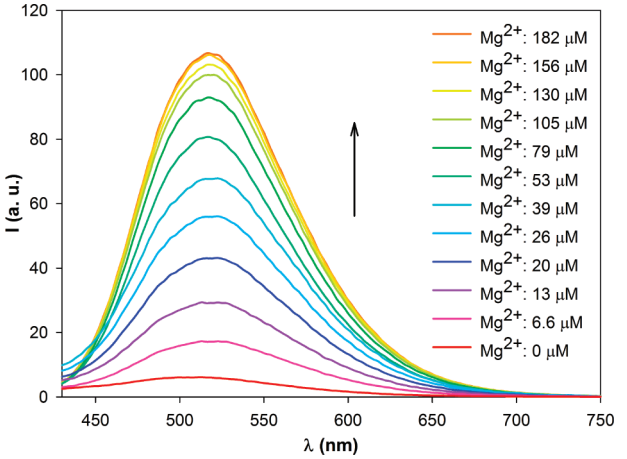



**FIGURE 3.** Fluorescence spectra ($\lambda_{ex}$ = 350 nm) of probe **1a** (25 $\mu$M) in Dulbecco's phosphate-buffered saline (DPBS) at room temperature upon addition of increasing amounts of $Mg^{2+}$ ions.

**TABLE 3. Intracellular Magnesium Content (nmol/$10^6$ Cells) Evaluated by Atomic Absoption Spectroscopy (AAS) and Fluorescence Spectroscopy (FS) Using 1a**

| cell line | AAS | FS |
|---|---|---|
| undifferentiated HL60 | 11.2 ± 4.9 | 11.9 ± 1.5[a] |
| DMSO-differentiated HL60 | 6.6 ± 3.5 | 7.2 ± 1.8[a] |
| Jurkat-6 | 26.5 ± 8.9 | 25.4 ± 8.2[b] |
| A 2780 | 39.4 ± 5.9 | 37.2 ± 2.7[b] |

[a] **1a** synthesized according to ref 4. [b] **1a** synthesized by the herein proposed method.

In conclusion, *N*,*N*′-bis-((8-hydroxy-7-quinolinyl)methyl)-1,10-diaza-18-crown-6 ether (**1a**) and 10 analogues carrying one more substituent on position 2, 4, or 5 of the quinoline ring system (**1b**−**k**) have been efficiently synthesized via a simple catalyst-free three-component bidirectional Mannich reaction under microwave heating, using toluene or 1,4-dioxane as solvent. The new reaction protocol benefited from a remarkable improvement with respect to the analogous thermal reaction, in terms of reaction time, yield, and suppression of side reactions, resulting in an increased purity of the crude products. Previous results on the use of **1a** and **1c** as fluorescent chemosensors for magnesium ions in water and living cells were confirmed by a preliminary assessment of the photophysical properties of the new ligands. The results obtained clearly indicate that the higher purity ensured by this new synthetic methodology has a beneficial effect also on the analytical performance of the probes.

## Experimental Section

**Microwave Assisted Synthesis of 1,10-Bis((2-methyl-8-hydroxy-7-quinolinyl)methyl)-1,10-diaza-18-crown-6 Ether (1e, Table 2, Entry 1).** A mixture of 2-methyl-8-hydroxy-quinoline **3e** (0.16 g, 1.0 mmol), 1,10-diaza-18-crown-6 ether **2** (0.131 g, 0.5 mmol), and paraformaldehyde (0.045 g, 1.5 mmol) in 1,4-dioxane (2 mL) was irradiated under inert atmosphere at 600 W for 2 h in a MILESTONE MicroSYNTH Labstation microwave apparatus. The internal temperature was monitored using a fiber-optic temperature sensor. The solution was allowed to reach room temperature, the solvent was evaporated at reduced pressure and the crude residue was purified by washing with anhydrous diethyl ether. The title compound was obtained as a faint yellow solid after solvent removal under vacuum (0.288 g, 0.48 mmol,

95%): mp = 163−164 °C; $^1$H NMR (200 MHz, $CD_3OD$) $\delta$ 7.97 (d, $J$ = 8.5 Hz, 2H), 7.24 (d, $J$ = 8.3 Hz, 2H), 7.20−7.11 (m, 4H), 4.00 (s, 4H), 3.72 (t, $J$ = 5.3 Hz, 8H), 3.62 (s, 8H), 2.95 (t, $J$ = 5.4 Hz, 8H), 2.72 (s, 6H); $^{13}$C NMR (50 MHz, $CD_3OD$) $\delta$ 158.0, 152.5, 138.6, 136.5, 127.1, 127.1, 122.5, 119.5, 117.6, 70.9, 69.4, 58.1, 53.8, 24.9. Anal. Calcd for $C_{34}H_{44}N_4O_6$ (604.74): C, 67.53; H, 7.33; N, 9.26. Found: C, 67.16; H, 7.20; N, 9.15.

**Cell Culture and Staining.** The cells were grown in RPMI-123 medium supplemented with 2 mM glutamine and 10% foetal calf serum, at 37 °C and in presence of 5% $CO_2$. For microscope examination, SaOs-2 cells were seeded on glass coverslip and allowed to growth for 24 h, then rinsed in Dulbecco's phosphate buffered saline without calcium and magnesium (DPBS) and stained in the dark for 5 min with **1a** dye 25 $\mu$M dissolved in DPBS. The sampled were gently blotted to remove the excess of liquid, put on a microscope slide and observed with a Axioplan fluorescence microscope (Zeiss, Germany) exciting the sample around 390 nm and collecting the emitted fluorescence around 500 nm.

**Cellular Magnesium Determination.** Concentrations of cell magnesium contents were calculated by fluorescence spectroscopy and by atomic absorption spectroscopy as previously reported on sonicated cells samples.[1]

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**Supporting Information Available:** Characterization data, experimental details, and copies of $^1$H and $^{13}$C NMR spectra of compounds synthesized. This material is available free of charge via the Internet at http://pubs.acs.org.