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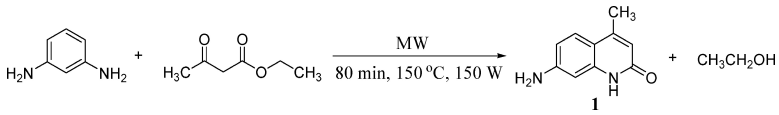

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Design, Microwave-Assisted Synthesis, and Photophysical Properties of Small Molecule Organic Antennas for Luminescence Resonance Energy Transfer

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A highly efficient microwave-assisted method was successfully developed for the synthesis of a library of carbostyril analogues. The reaction time for synthesis of carbostyril analogues was drastically reduced from a reported 18−58 h to only 80 min. Compounds obtained directly from each synthesis were more than 90% pure and did not require any further purification. On the basis of the fluorescence spectra of the compounds in the initial library, four carbostyril analogues were designed. Two of these analogues showed very favorable fluorescence profiles and have the potential to be used as small molecule organic antennas for LRET studies.

Introduction

Luminescence resonance energy transfer (LRET) is a relatively new technique developed in the past decade for spectroscopic measurements in biomolecules.[1,2] LRET offers many technical advantages over conventional fluorescence resonance energy transfer (FRET).[3] In FRET, a fluorescent donor molecule transfers energy via a nonradiative dipole−dipole interaction to an acceptor molecule, which is usually a fluorescent molecule.[4] There are several drawbacks of FRET. First, the donor and acceptor molecules must be in close proximity, since FRET cannot be detected at distances greater than 80 Å. Second, the lifetime of commonly used donor fluorophores is only in the nanosecond range, limiting the time that FRET can be measured accurately. Third, background interference arising from the fluorescence of donor and the excitation of acceptor by the laser can result in a poor FRET signal-to-background ratio. Since both the donor and acceptor contribute to background photons, the sample must be labeled and purified such that it has only one donor and one acceptor.[3]

LRET overcomes many of the drawbacks of FRET. In LRET, the donor is a luminescent lanthanide chelate, and the acceptor is a standard organic dye.[3] LRET can be accurately measured at distances above 100 Å.[5] The sensitized emissions are sharply spiked in wavelength, have millisecond lifetimes following an excitation pulse,[6] and are unpolarized and have high quantum yields.[7] Background interference is minimal in LRET because fluorescence from the donor is eliminated spectrally by measuring fluorescence at wavelengths where the donor does not emit and background fluorescence from the acceptor is eliminated, since the sensitized emission occurs within a millisecond time scale.[8] Since the prolonged sensitized emission only arises from donor−acceptor pairs, incomplete labeling does not contribute to the LRET signal. Because of the technical advantages of LRET over FRET, LRET has been used previously for the study of molecular association of dystrophin with actin,[9] conformational changes in nucleic acids,[10] analysis of RNA polymerase complexes,[11] and protein−protein interactions in living cells.[12] LRET also has been incorporated into DNA hybridization assays[13,14] and high-throughput screening assays.[15]

To develop a system for achieving LRET, an organic antenna is attached to a chelating agent and a lanthanide metal, such as terbium or europium.[8] 7-Amino-4-methylquinolin-2(1*H*)-one, commercially known as Carbostyril-124, has been used extensively as an organic antenna for the formation of lanthanide chelates.[16] Apart from being an organic antenna, carbostyril derivatives have shown therapeutic potential as dopamine receptor agonists,[17] positive inotropic agents,[18] β-adrenergic agonists,[19] and anti-herpes[20] and anti-HIV agents.[21,22]

In this study, we have developed a highly efficient microwave-assisted synthetic method for carbostyril analogues. On the basis of spectral results of the synthesized carbostyril analogues library, four new carbostyril analogues were then designed, and two of them maximally absorbed UV above 370 nm.

Results and Discussion

Development of Microwave-Assisted Synthesis. Carbostyril-124, having a quinolinone core, is a highly fluorescent organic compound. This fluorescence property has made it a suitable organic antenna for performing LRET. Carbostyril-124 has a maximum UV absorbance of ∼350 nm and must be excited at a wavelength in the ultraviolet region. However, ultraviolet light exposure causes undesirable responses in living cells and is not suitable for carrying out in vivo studies. Therefore, organic antennas with longer maximum absorbance wavelengths are needed to perform LRET in living cells.

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Table 1. Solvent Selection for Microwave-Assisted Synthesis of Compound **1**

solvent	temperature (°C)	crude yield (%)[a]
no solvent	200	16
DMF	170	no product
NMP	210	no product
water	150	49

[a] Isolated yields.

Table 2. Temperature Optimization of Microwave-Assisted Synthesis

temperature (°C)	crude yield (%)[a]
120	ND[b]
130	ND[b]
140	ND[b]
150	49
160	46
170	32
180	23

[a] Isolated yields. [b] Not determined.

The synthesis of Carbostyril-124 was reported in several papers, and the synthetic conditions varied widely from 18 to 58 h of reflux of reactants.[23,24] Reported yields were also low and highly varied. The recent success of using microwave in the synthesis of heterocyclic compounds[25,26] prompted us to develop a microwave-assisted synthetic method to generate a library of carbostyril analogues.

The synthesis of Carbostyril-124 was attempted on the microwave reactor using equimolar quantities of 1,3-phenylenediamine and ethyl acetoacetate. Two independent reports stated that the synthesis was carried out neat and that temperatures up to 150 °C were used.[23,24] However, the product obtained was not pure, and further workup and purification procedures were required. To optimize the yield and purity of carbostyril-124, a suitable solvent needed to be chosen for its synthesis using the microwave reactor. Microwave-assisted reactions were carried out at 150 W for 30 min, and solvents tested included DMF, NMP, and water. Neat reactions were carried out as a control. Temperatures were set at 30−50 °C above the boiling point of the solvents, and all reactions were carried out in sealed tubes. Product yields obtained showed that the best solvent was water, which was used in all subsequent reactions (Table 1).

Next, the microwave-assisted reaction was carried out using water as solvent and optimized for temperature and time for synthesis of Carbostyril-124. 1,3-Phenylenediamine and ethyl acetoacetate were reacted for 30 min in the microwave reactor while optimizing for temperature. When the temperature was at or below 140 °C, reactions were not complete, sticky products were obtained (Table 2), and product yields could not be determined. When the temperature was raised to 150 °C, product yield was comparatively better and a white, powdery product was obtained. Temperatures above 150 °C did not give better yields and caused the product to darken. This darkening might be due to decomposition of the product. These results demonstrated that 150 °C is the optimal temperature for Carbostyril-124 synthesis.

To optimize the reaction time, reactions were performed at 150 W and 150 °C, while reaction times were increased from 30 to 100 min at 10-min intervals (Table 3). Increasing the reaction time from 30 to 80 min resulted in an increase in product yield. However, reaction times longer than 80 min caused the yield to decrease. This may be due to the formation of side products as the reaction time was increased.

Table 3. Time Optimization of Microwave-Assisted Synthesis

reaction time (min)	crude yield (%)[a]
30	49
40	49
50	55
60	55
70	55
80	58
90	49
100	46

[a] Isolated yields.

Microwave-assisted synthesis of Carbostyril-124 gave the highest yield when reacting 1,3-phenylenediamine and ethyl acetoacetate in the presence of water as a solvent, at 150 °C and 150 W for a period of 80 min. This microwave-assisted synthetic method is a great improvement over traditional synthetic methods, drastically reducing the reaction time from 18−58 h to only 80 min.

Microwave-Assisted Synthesis of a Library of Carbostyril Analogues. Three differently substituted phenylenediamines and five β-ketoesters were reacted in 15 individual reactions to yield a library of 15 carbostyril analogues. Reactions were "one-pot" syntheses in which two reactants were added into the same reaction vessel using water as the solvent. Reactions were microwave-irradiated at 150 W and 150 °C for 80 min to produce a powdery solid product of reasonable yield. The synthetic route for Carbostyril-124 (**1**) is shown in Scheme 1.

Microwave-assisted synthesis resulted in compounds with excellent purity (Table 4) and 7 of the 15 compounds were 100% pure (Figure 1), as determined by HPLC. The rest of the compounds were >90% pure. All of the compounds were collected by simple filtration and washed with ether and water. This method of synthesis is a great improvement over conventional synthesis of carbostyril analogues because the resulting products did not need to be purified further by flash column or recrystallization after each synthesis to obtain products of acceptable purity. Pure products can be obtained using this method because the reaction time is greatly reduced and side products cannot be formed during this shorter time frame. This method also saves time and resources because products obtained do not need to be purified extensively for further studies.

Isolated yields of carbostyril analogues varied widely from 10 to 94%. The highest yields were obtained from compounds with CF_3 substituents in the R_2 position. This higher yield may be due to the electron-withdrawing effect of CF_3, which may enhance the ring closure process during synthesis. The lowest yield was obtained from **14**, in which CH_3 substituents were found on R_1, R_2, and R_4 positions. The increased number of substituents might make ring closure difficult and cause the yield to drop.

UV Absorption and Fluorescence Analysis of Carbostyril Analogues. After synthesis, the 15 carbostyril

Scheme 1. Microwave-Assisted Synthesis of Compound **1**

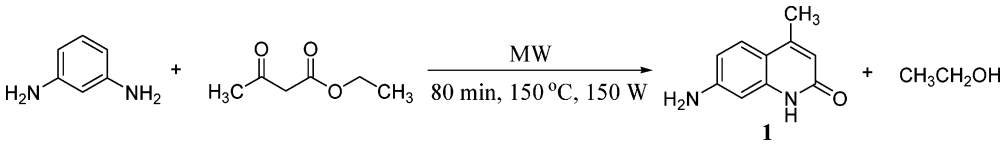

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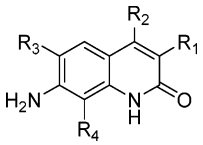
Table 4. Yields and Purities of a Library of 15 Carbostyril Analogues



compd	R1	R2	R3	R4	yield (%)[a]	purity (%)[b]
1	H	CH_3	H	H	58	94.2
2	H	C_2H_5	H	H	37	99.2
3	H	C_3H_7	H	H	30	100.0
4	CH_3	CH_3	H	H	19	96.8
5	H	CF_3	H	H	94	100.0
6	H	CH_3	CH_3	H	40	95.9
7	H	C_2H_5	CH_3	H	32	98.9
8	H	C_3H_7	CH_3	H	23	92.9
9	CH_3	CH_3	CH_3	H	15	96.4
10	H	CF_3	CH_3	H	76	100.0
11	H	CH_3	H	CH_3	27	100.0
12	H	C_2H_5	H	CH_3	14	100.0
13	H	C_3H_7	H	CH_3	12	100.0
14	CH_3	CH_3	H	CH_3	10	98.7
15	H	CF_3	H	CH_3	90	100.0

[a] Isolated yields. [b] HPLC purity.

analogues were scanned using a UV−visible spectrophotometer to record their UV absorbance spectra. The compounds were then excited at their maximum UV absorbance wavelengths (λ_{max}) using a fluorometer to observe their emission spectra (Table 5). Results showed that substituents at positions 4 and 6 of the quinolinone core in these analogues had the greatest effect on the λ_{max} values and the emission wavelengths. Compounds **5**, **10**, and **15**, which have a highly electron-withdrawing CF_3 substituent at position 4, showed a dramatic increment of λ_{max} values and emission wavelengths. An electron-donating substituent such as CH_3 at position 6 is also favorable. These observations are consistent with Uray's hypothesis that a "push-pull" effect due to substituents at positions 4 and 6 affects the fluorescence spectra of carbostyril analogues.[27] An electron-donating substituent at position 6 and an electron-withdrawing substituent at position 4 causes this "push-pull" effect and shifts both the λ_{max} and emission wavelengths toward the visible range.[28−30] Since visible light is not harmful to living cells, we should be able to use these carbostyril analogues as organic antennas in lanthanide chelates when used as fluorescent probes in cell studies. A comparison of the fluorescence spectra of **1** and **10** is shown in Figure 2.

Table 5. UV Absorption and Fluorescence Data of the Library of 15 Carbostyril Analogues

compd	λ_{max} (nm)	emission λ (nm)
1	348.0	381.0
2	348.0	382.2
3	348.0	382.2
4	346.0	387.1
5	364.0	429.3
6	353.0	384.0
7	353.0	384.3
8	353.5	386.1
9	351.0	389.5
10	371.0	429.0
11	348.0	385.7
12	348.0	389.5
13	348.5	388.0
14	346.5	395.0
15	364.0	443.4

The λ_{max} and emission wavelengths of compounds **5**, **10**, and **15**, which have the same CF_3 substituent in position 4 of the quinolinone core, were near the visible range (Figure 3). On the basis of these observations, new compounds were designed with a strongly electron-withdrawing CF_3 substituent conserved at position 4 and with different substituents in position 6.

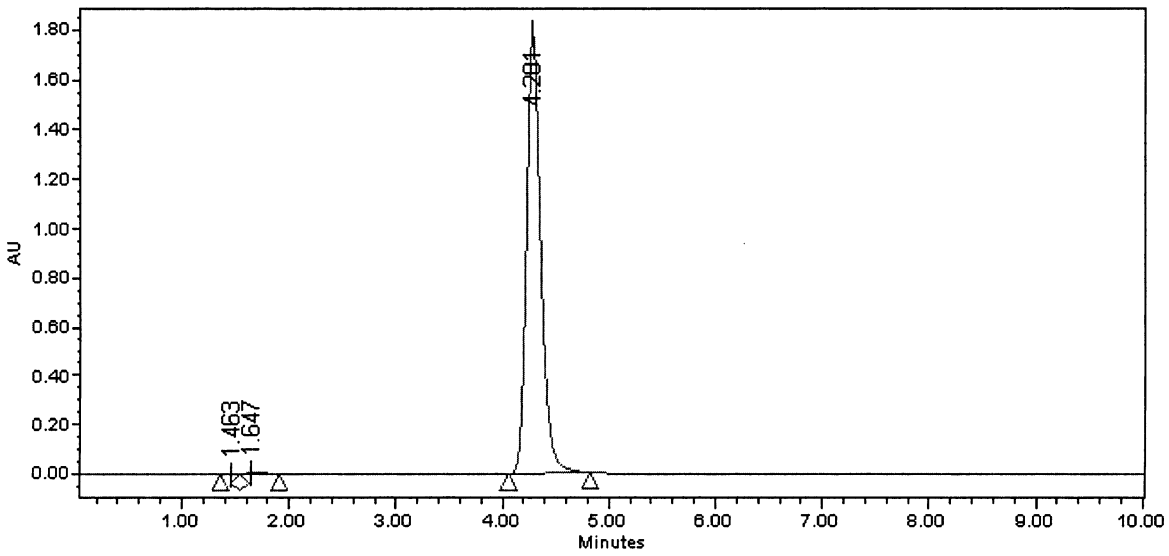


Figure 1. HPLC chromatogram of compound **5**.

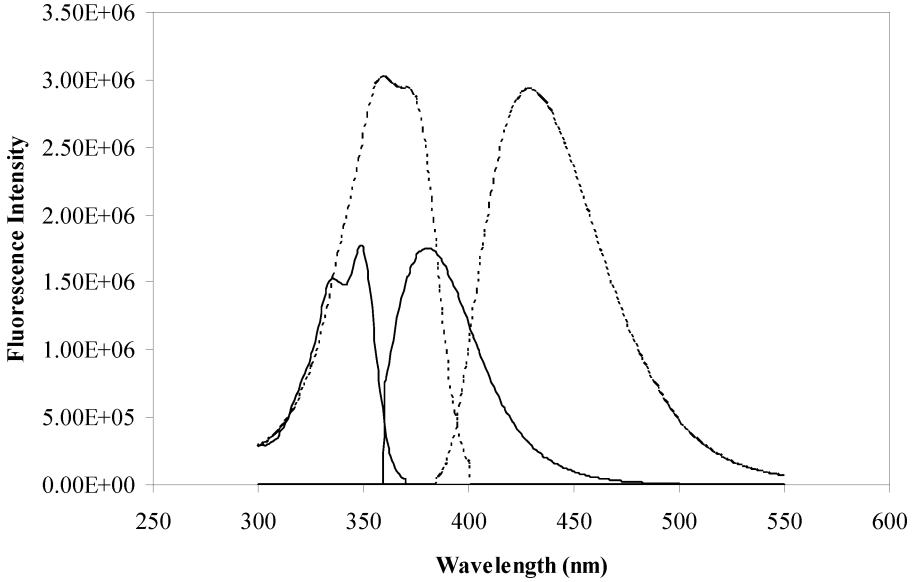


Figure 2. Excitation and emission spectra of compounds **1** (solid lines) and **10** (dotted lines).

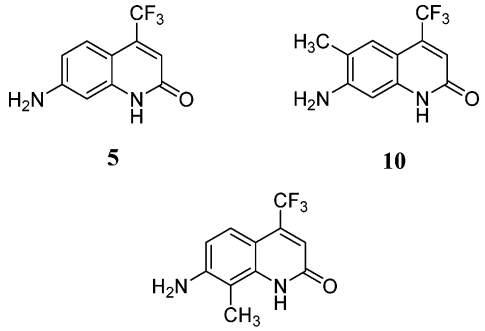
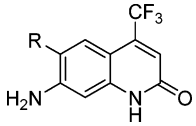


Figure 3. Chemical structures of compounds **5**, **10**, and **15**.

Table 6. Yields, Purities, UV Absorption, and Fluorescence Data of Compounds **16**−**19**



compd	R	yield (%)[a]	purity (%)[b]	λ_{max} (nm)	emission λ (nm)
16	F	41	98.7	368.5	414.0
17	Cl	25	67.1	369.0	417.0
18	OCH_3	65	100.0	373.5	438.6
19	OH	86	100.0	378.5	443.0

[a] Isolated yields. [b] HPLC purity.

Design of Carbostyril Analogues with More Favorable Photophysical Properties. Four carbostyril analogues were designed and synthesized using the synthetic conditions described above. UV absorbance and fluorescence spectra of the compounds were recorded (Table 6). The λ_{max} values and emission wavelengths of these four compounds were slightly longer than compounds **1**−**15**. Of the four compounds, the λ_{max} values and fluorescence spectra of **18** and **19** showed the greatest shift in maximum absorbance and emission wavelengths. This shift is due to the presence of a strongly electron-withdrawing substituent at position 4 (CF_3) and strongly electron-donating substituent at position 6 (OCH_3 and OH, respectively).

Compound **19** showed an excellent fluorescence spectrum, as compared to **1**. The maximum emission wavelength for **19** was 65 nm longer than the excitation wavelength (λ_{max}), which is 2-fold greater than the 33-nm difference seen for **1** (Figure 4). This increase in the difference between excitation and emission wavelength could serve as a great advantage for **19** when performing LRET because background photons resulting from excitation can be completely blocked if the signal is read near 443 nm, which is the maximum emission wavelength of **19**.

Conclusion

Carbostyril-124 has been extensively used as a small molecule organic antenna for LRET but has been synthesized under conditions that varied widely and resulted in low product yield and purity. In this study, a highly efficient and economical microwave-assisted synthetic method was developed that produced a library of 15 carbostyril analogues. Reactions were carried out in a microwave reactor at 150 °C and 150 W for 80 min, decreasing the time of synthesis from 18−58 h to just 80 min. Purity of the products obtained was higher than 90%, and further purification was not required for each product, saving time and resources.

Three out of 15 carbostyril analogues synthesized in the library showed encouraging UV absorbance and fluorescence spectra and were selected as lead compounds for further design of analogues. The CF_3 substituent in position 4 of the quinolinone core of carbostyril was conserved while position 6 was varied and four other analogues were designed and synthesized. Of these four, two of the analogues (**18** and **19**) proved to be potential candidates for chelate formation and LRET studies due to their long and favorable λ_{max} values and the wide difference in their excitation and emission wavelengths. Further studies on the formation of chelates and complexation with lanthanide metals and tagging biological molecules with these new antennas for LRET studies are currently in progress.

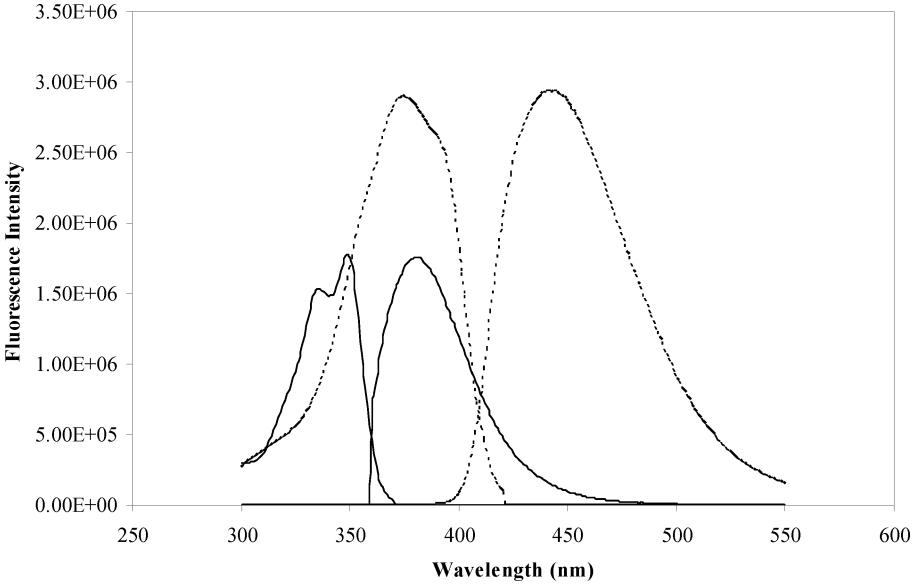


Figure 4. Excitation and emission spectra of compounds **1** (solid lines) and **19** (dotted lines).

Experimental Section

Chemicals. All chemicals were purchased from Aldrich Chemical Co. Anhydrous ethyl ether was purchased from EMD Chemicals, and all the HPLC grade solvents were purchased from Mallinckrodt Chemicals.

Synthesis. All organic antennas were synthesized using the general procedure described below involving a CEM Corp. Discover laboratory microwave with Explorer unit.

General Procedure for Microwave Synthesis of Organic Antennas. A mixture of substituted phenylenediamine (2 mmol), β-ketoester (2 mmol), and water (1 mL) was added into a 10-mL glass tube with a magnetic stirring bar and covered with a plastic cap. The synthesis was carried out at 150 °C for 80 min under 150 W of microwave irradiation using a CEM Corp. Discover laboratory microwave with an automated Explorer unit. Completion of the reaction was checked by TLC at the end of 80 min. All products were left at 4 °C overnight, collected by suction filtration, washed with ether and then water, and dried.

Compound Characterization. Mass spectral analyses were recorded on a Waters Alliance HT/Micromass ZQ system (ESI). ^{1}H NMR spectra were recorded on a Varian VNMR 400-MHz spectrometer and processed using the MestRe-C version 3.7.9.0 software. All the compounds were dissolved in DMSO-d_6 during NMR analysis.

Proof of Purity of Carbostyril Analogues. Purity of carbostyril analogues was determined using a Waters 2690 HPLC separations module coupled to a Waters 996 photodiode array detector with a Nova-Pak 60-Å C18 column (3.9 i.d. × 150 mm, 4 μm). The mobile phase consisted of 70% water with 0.1% TFA and 30% acetonitrile. Flow-rate was set at 0.8 mL/min, and each run took 10 min to complete. The detection wavelength was set at 328 nm.

UV and Fluorescence Analysis of Carbostyril Analogues. UV scans from 200 to 500 nm were recorded on a Shimadzu UV-1601 UV-visible spectrophotometer. Compounds for UV scans were dissolved in methanol. Fluorescence spectra of carbostyril analogues were recorded using a Photon Technology International (PTI) QM-2000-6 fluorescence spectrophotometer. Compounds for fluorescence measurements were dissolved in acetonitrile.

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Supporting Information Available. ^{1}H NMR spectra, HPLC chromatograms, MS spectra, and fluorescence spectra of carbostyril analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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