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Fluorescence Properties of 6-Methoxy- and 6,7-Dimethoxyquinoline-3,4-dicarbonitriles

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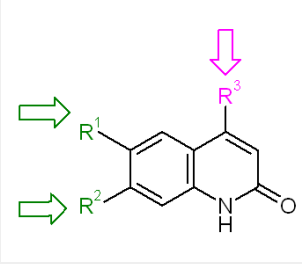
Abstract



A comparative study of 6-methoxy-, 7-methoxy- and 6,7-dimethoxycarbostyrils revealed, that both, the 6 and the 7-methoxy group (green arrows), have different effects to the fluorescence properties of carbostyrils such as fluorescence wavelength, quantum yield and Stoke's shift. A further important influence is visible from the electron acceptor properties of the substituent in position 4 (pink arrow).

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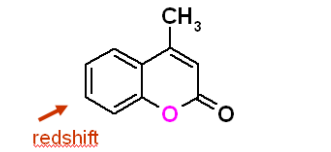
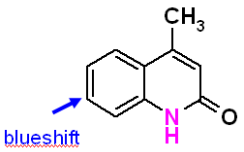
Introduction

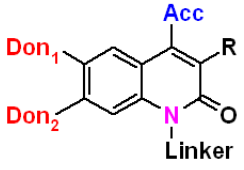


4-Methylcoumarin
7-H abs 330 nm flu 380 nm Φ 0.002
7-MeO 320 415 Φ 0.6



4-Methylcarbostyril
7-H abs 330 nm flu 375 nm; Φ 0.02
7-MeO 340 370 Φ 0.05

2x Don: MeO
Acc: CF_3

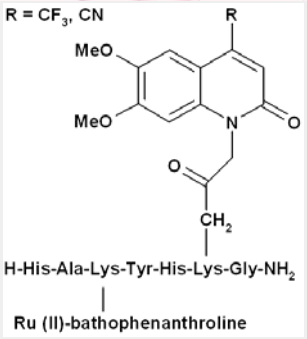


abs: 370 nm flu: 430 nm; Φ 0.5

Compared with similar coumarin fluorophors, 7-methoxycarbostyrils show similar emission wavelengths, but have the big disadvantage of much lower quantum yields, as shown in the adjacent scheme. On the other hand, the advantages of carbosytril systems are high stability against chemicals, thermal and photochemical stress, insensitivity to O_2 quenching, independence of luminescence in a broad pH region. Such properties make them particularly interesting to be used as probes in biological, biochemistry and medicine applications [1].

When the carbostyril moiety can be combined with highly fluorescence and photophysical properties, it offers subsequent reactions for the construction of labelled biological material such as peptides, proteins and carbohydrates [2,3].
Recently we have reported on the vastly improved luminescence properties of a big number of carbostyril (2-quinolone) systems [4]: the fluorescence is comparable with umbelliferon, but the molecules have much better stability. These properties we achieved by suitable substituents, e.g. acceptor groups such as trifluoromethyl in position 4 (Acc, blue) and donor groups such as methoxy or amino in position 6 and 7 (Don1 and

Don2, red). The molecules with methoxy groups gave excitation / emission maxima at ~370/430 nm, together with large Stoke' s shifts and sufficient quantum yields [2-4]. 6,7-Amino groups show longer wavelengths and better quantum yields, but have the big disadvantage of pH sensitivity. 4-Cyano substituents improved the fluorescence properties with longer wavelengths and better quantum yields [3].

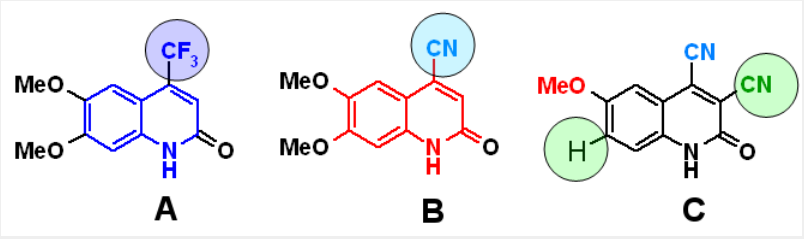


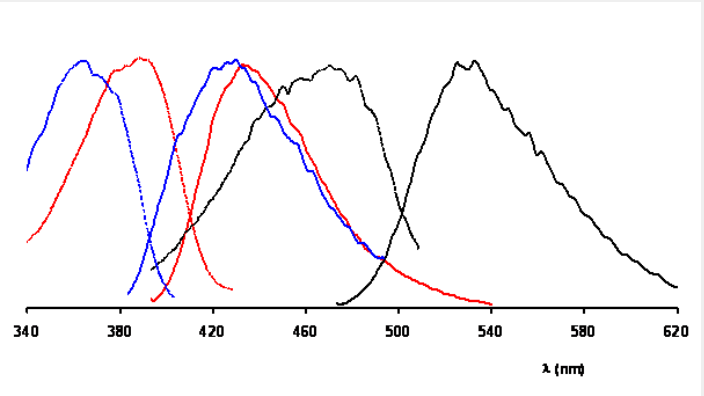
The group of Bannwarth [5] already used our fluorescent carbostyrils, incorporated in peptides, in a Fluorescence-Resonance-Energy Transfer (FRET)-system for distance determinations.

Aim of this contribution

In this contribution, we describe the study on different methoxy substituted carbostyril systems in position 6 and 7, and the influence of one or two cyano groups in position 3 and 4 on the fluorescence properties compared with a 4-trifluoromethyl group.

Results and Discussion





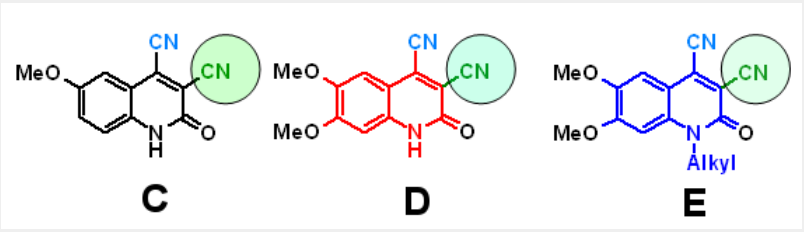
Comparison of 4-trifluoromethyl-, 4-cyano- and 3,4-dicyanocarbostyrils with different methoxy groups in positions 6 and 7

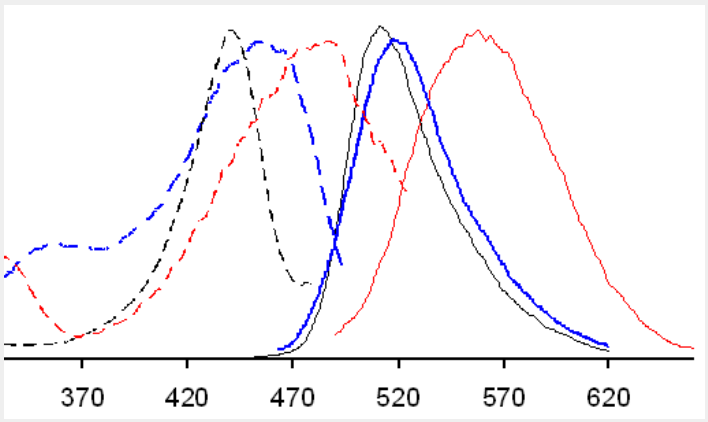
To study the influence of single methoxy groups, 6-methoxy-4-trifluoromethyl- and 7-methoxy-4-trifluoromethyl-carbostyrils were prepared according to literature procedures [2-4], and compared with 4-cyano-6-methoxy- and 4-cyano-7-methoxycarbostyrils. On the other hand, an additional cyano group was introduced at 3-position and the luminescence properties studied.

The results show, that the 4-trifluoromethyl-carbostyrils with the donor group in position 6 exhibit fluorescence at longer wavelength (460 nm) compared with the 7-methoxy derivative (400 nm), but both have low quantum yields (about 10%). The combination of both donor substituents (structure **A**, blue curve) give a small decrease of the wave length (to 420 nm) compared with the 6-methoxy derivative, which is counterbalanced by a big increase of the quantum yield (up to 30%). When the 4-trifluoromethyl group is exchanged against a 4-cyano group (structure **B**, red curve) the wavelength moves up to 440 nm; the quantum yield increases to about 50%.

When an additional cyano group was introduced at position 3 of 6-methoxy derivatives, a very big shift of the fluorescence wavelength of about 100 nm was observed (structure **C**, **black curve**); the fluorescence appears now in the green region (540 nm); however, the quantum yield decreases to only 15%.

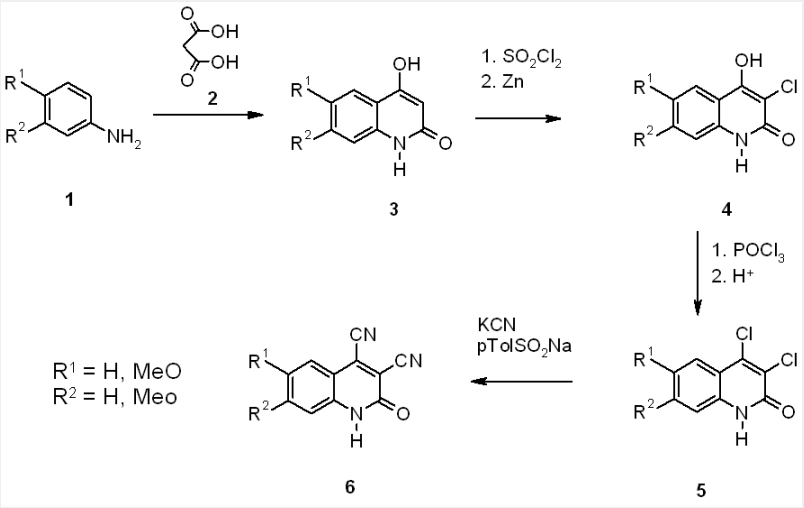
Comparison of 3,4-dicyanocarbostyrils having different methoxy groups





When into 3,4-dicyano-6-methoxycarbostyril (structure **C**, red curve) a further 7-methoxy group was introduced, the resulting 3,4-dicyano-6,7-dimethoxy-carbostyril (structure **D**, blue curve) suffers a little blue shift and has now an absorption wavelength at 450 nm and the emission wavelength at 520 nm, however, with an excellent quantum yield of about 50 % (in acetonitrile); the values are similar to fluorescein at pH 3.3 (**black curve**).

The introduction of simple alkyl or alkyl linker groups (e.g. CH_2-COOX, structures **E**) at N-1 did not big affect the fluorescence properties and gave similar values with absorption maxima of about 450 nm and emission maxima of about 520 nm; the quantum yield, however, decreased to about 20 %.



Synthetic procedure

The synthesis of the title compounds, 3,4-dicyanocarbostyrils (**6**) starts with a ring closure reaction of anilines **1** having the methoxy group at the correct position, with malonic acid **2**, which gave the corresponding 4-hydroxy-carbostyrils **3**. The precursors **5** were obtained by subsequent electrophilic bis-chlorination of **3** with sulfuryl chloride at position 3 (forming a 3.3-dichloroquinolin-2,4-dione structure), followed by reduction of one 3-chloro atom with zinc to give 3-chloro-4-hydroxy-carbostyrils **4**.

Nucleophilic chlorination of 3-chloro-4-hydroxy-carbostyrils **4** with phosphoryl chloride in position 2 and 4 gave 2,3,4-trichloroquinoline structures, which formed by regioselective hydrolysis in position 2 the 3,4-dichloro-2-quinolones **5**. These precursors react in the case of the 6-methoxy- and 6,7-dimethoxyderivative with potassium cyanide and p-toluene sulfinate to the dicyano product, 2-oxoquinoline-3,4-dicarbonitriles **6**. 7-Methoxyderivative of structure **5** gave only the 4-mono-cyano product, 2-oxoquinoline-4-carbonitrile.

Conclusion

6,7-Dimethoxycarbostyril with 2 cyano-substituents in position 3 and 4 (6,7-dimethoxy-2-oxo-quinoline-3,4-dicarbonitrile) shows excellent fluorescence properties: 520 nm emission maximum, excellent quantum yield (50% in acetonitrile), a good Stoke' s shift of 90 nm, good stability and no pH dependence. <

These characteristics make these structure as a useful alternative to radioactive probes, to other fluorescent dyes, for biological investigations and also as donor in energy transfer experiments.

Experimental

Spectral measurements

UV/vis spectra: Shimadzu UV/VIS scanning spectrophotometer UV-2101 PC; concentration: 0.01 mg/mL.
Excitation and emission spectra: Perkin-Elmer LS50B luminescence spectrofluorometer. concentration: 1x 10^{-5} M, DMSO or MeCN.
Determination of quantum yields: emission signals were set in relation to the known signal of 6,7-dimethoxy-1-methyl-4-trifluoromethyl carbostyril under the same conditions.

General procedure for 6,7-dimethoxy-2-oxo-quinoline-3,4-dicarbonitrile (6)

One equivalent of the appropriate 3,4-dichlorocarbostyril **5**, one equivalent of sodium p-toluenesulfinate, and 2.5 equivalents of potassium cyanide in dimethylformamide were heated for several hours with vigorous stirring, cooled to room temperature, quenched with ice/water, and acidified with hydrochloric acid. The precipitate was filtered by suction, washed with water and dried to afford red or yellow prisms of **6**.

Acknowledgement

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