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## Fluorescent Carbostyrils as Labelling Compounds for Biopolymers

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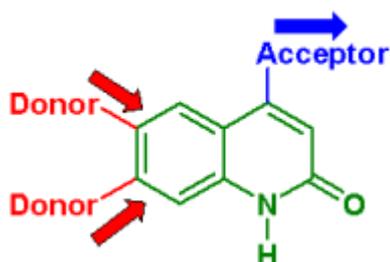
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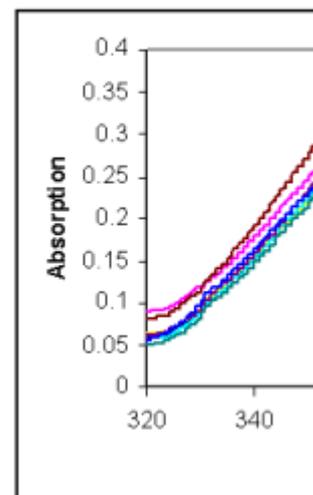
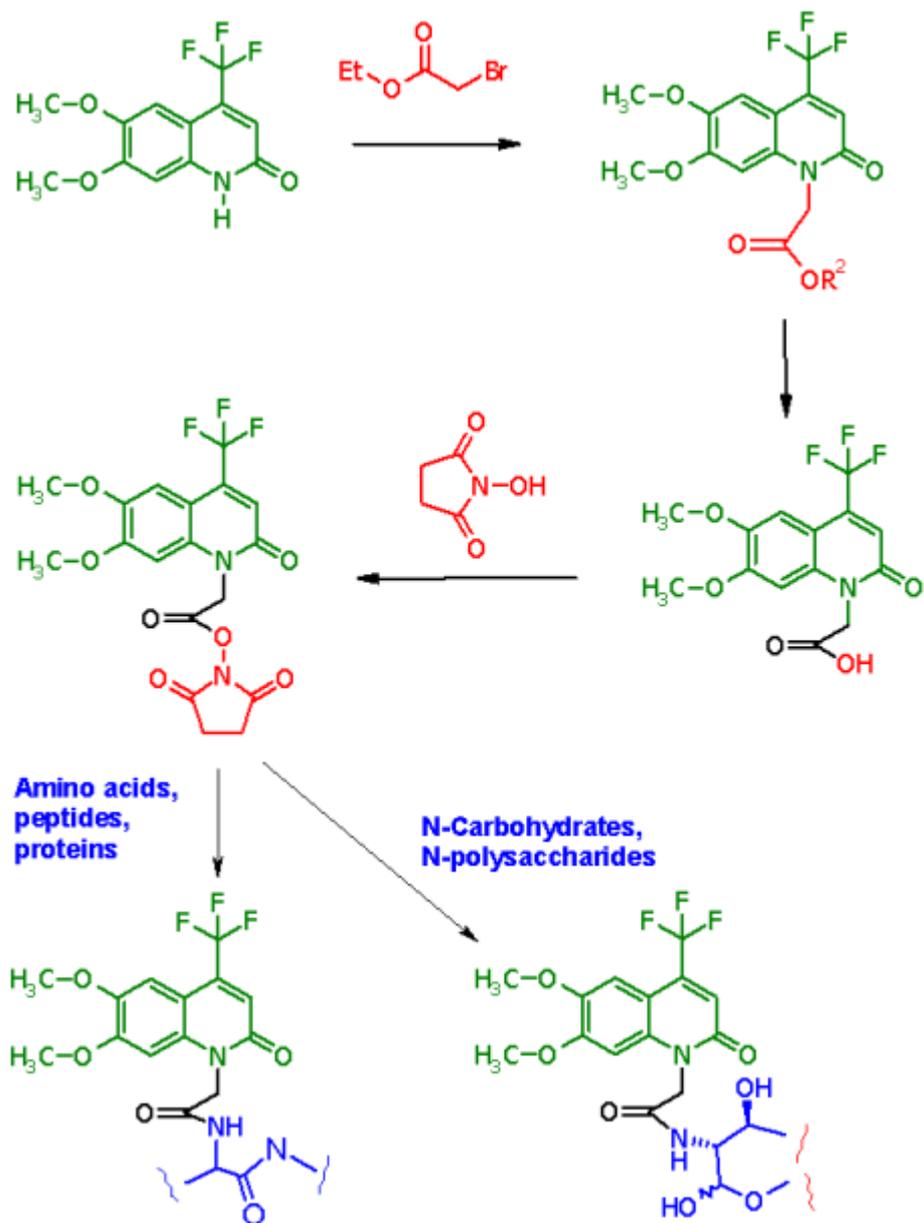
### General



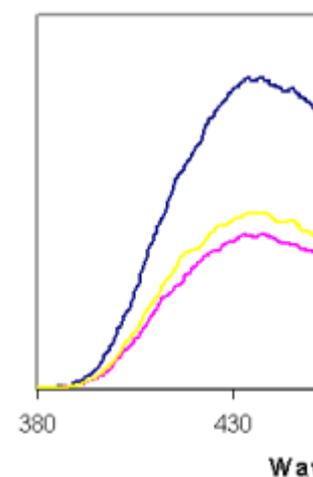
Fluorescent 6,7-donor - and 4-acceptor substituted carbostyrils have been found to have largely pH independent properties, large Stokes shifts and medium to high quantum yields [1]. Functionalized with O-succinimidyl (OSu) esters labelling of peptides and carbohydrates could be achieved.

### N-1 Functionalized Carbostyrils

Regioselective N-alkylation of 2-(trifluoromethyl)-2-quinolone under phase transfer conditions using a trifluoromethyl group leads to O-succinimidyl esters readily in aqueous solution. The presence of aliphatic amino groups of 2-(trifluoromethyl)-2-quinolone N-carbohydrates (e.g. aminoglycosides, chitosan) (e.g. chitosan).

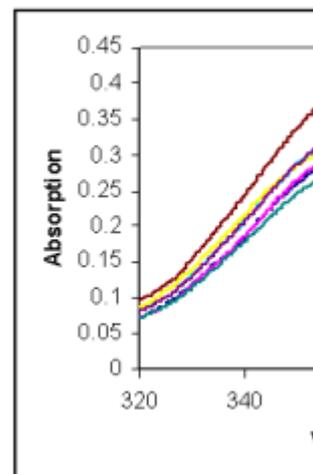
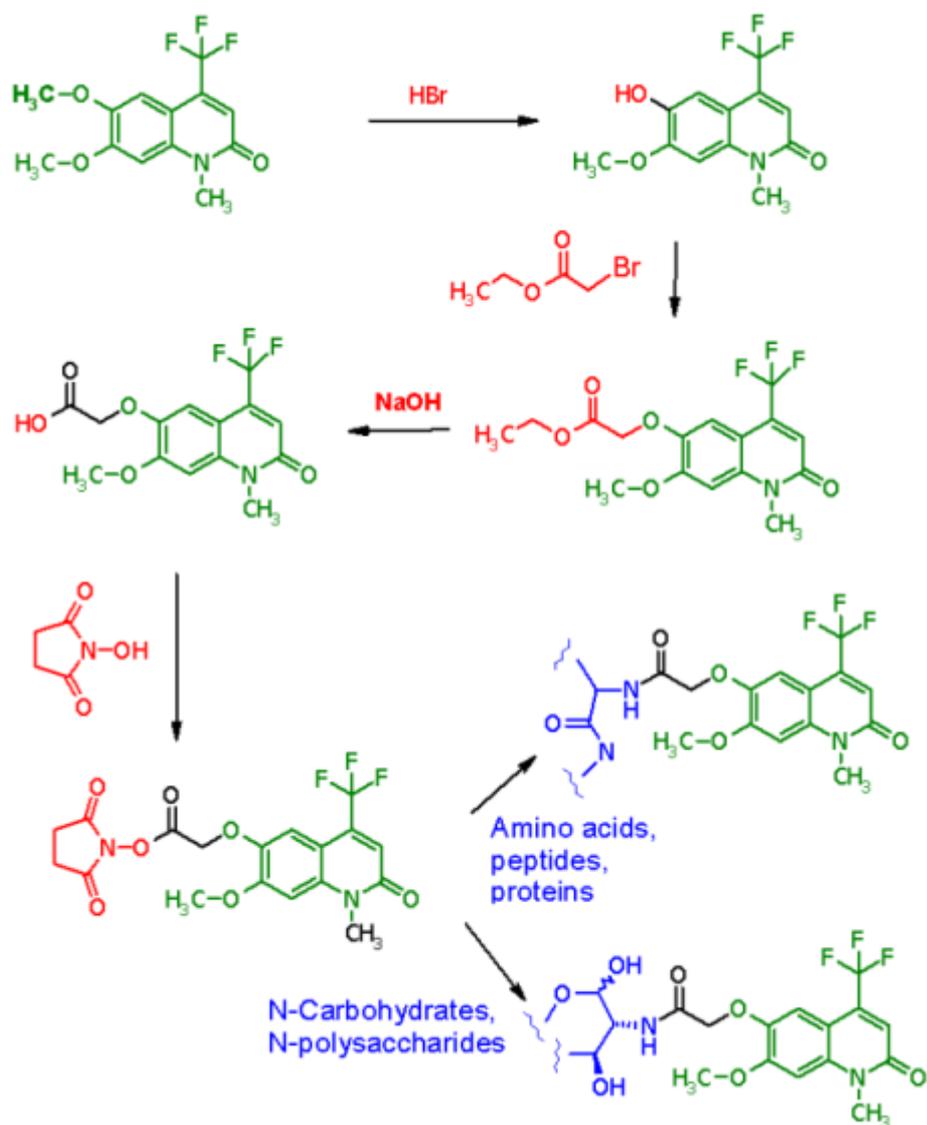


Absorption spectra of all N (including the derivatives) are similar with a maximum values between 9000 and 13500. Emission spectra show Stokes shift sufficient quantum yields (0.2-0.35) in water.

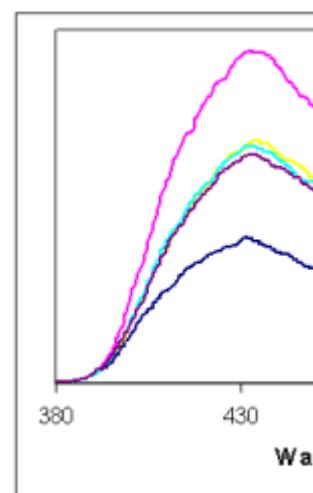


## 6-O Functionalized Carbostyrils

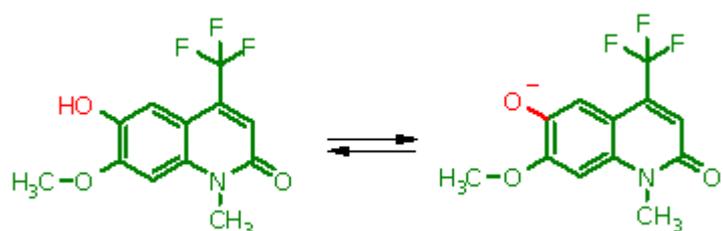
The regioselective introduction of the O position was achieved by the presence of a methoxy group of 1-alkyl-6-oxo-1,2,3,4-tetrahydroquinolin-3(1H)-one, and functionalized with the O-succinimidyl (OSu) group, which is used to label amino acids, serum albumine, amino-carbohydrates (aminoglucose) or amino-polysaccharides in aqueous solution. The UV spectra were similar (maximum about 370 nm, and 13500).



Emission spectra show a  $\lambda_{\text{em}}$  of 430 nm, however quantum yield is very low (0.02-0.035 in water).

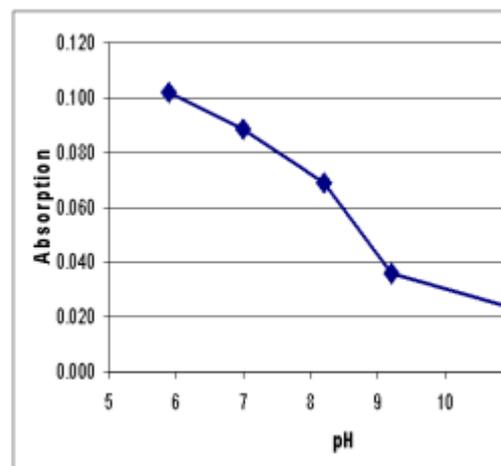
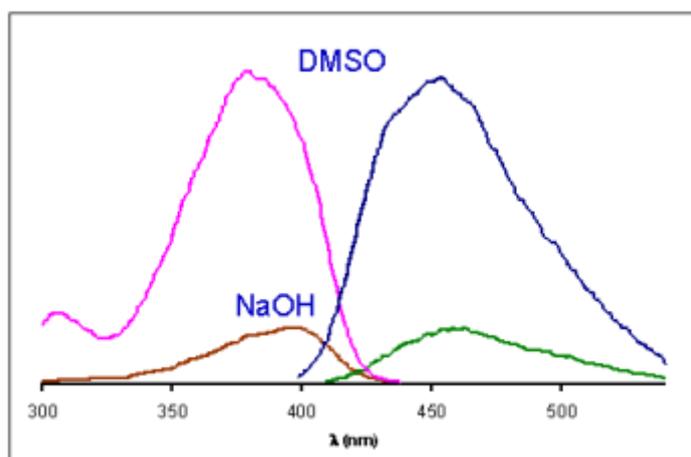


### 6-Hydroxycarboxystyryls

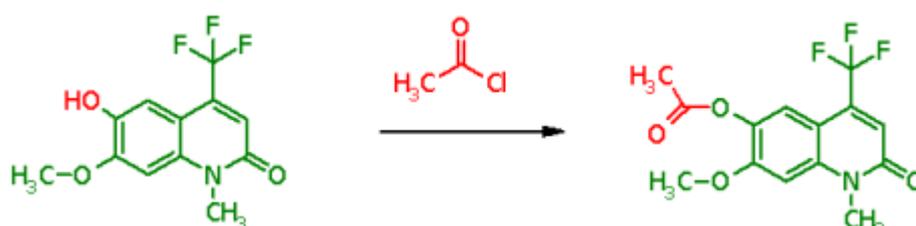


6-Hydroxycarboxystyryl showed a larger Stokes shift of 85 nm and sufficient emission in DMSO. Quantum yield in water was very low. The pH-dependence of the absorption of the hydroxy compound (362 nm) revealed a pKa of approximately 7.5.

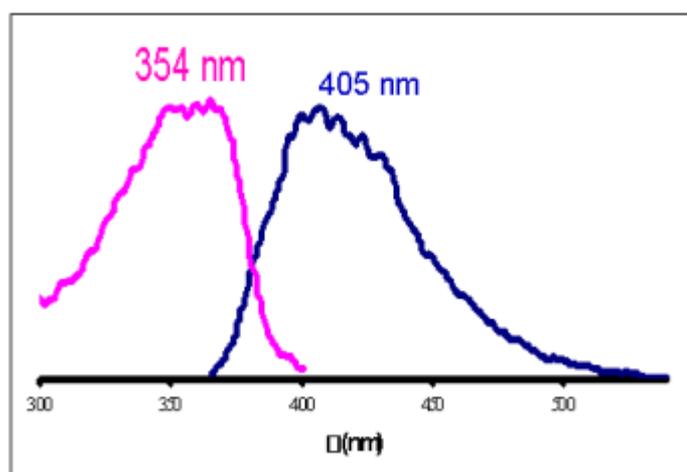
pH-Dependence of the absorption of the hydroxy compound (362 nm) revealed a pKa of approximately 7.5.



### 6-Acetoxy-Carbostryrils



6-Acyloxycarbostryrils were synthesized by acetylation of 6-hydroxycarbostryrils and explored as model compounds for labelling purposes. The spectral data of the 6-acetoxycarbostryril show that both excitation and emission spectra suffered a blue-shift of about 20 nm. The epsilon values are rather high (11400 in DMSO and 14600 in water), however the quantum yields are with 0.006-0.007 both in DMSO and water extremely low.



### Experimental

**Synthesis of N-1-alkylated carbostryrils:** N-Alkylation of 6,7-dimethoxy-4-trifluoromethyl-2-quinolone was performed by reaction with bromoacetate and sodium hydroxide in a binary solvent catalyzed by crown ethers at room temperature; the regioisomers were obtained as a 9:1 mixture and separated by dry flash column chromatography. The ester group was hydrolyzed by heating in aqueous sodium hydroxide solution and the acid reacted with N-hydroxysuccinimide in the presence of carbodiimide at room temperature to give O-succinimidyl (OSu) esters. The labeling reactions were performed with aqueous solutions of amino acids, peptides, proteins, amino sugars in slightly basic medium.

**Synthesis of O-6-alkylated carbostryrils:** Ether cleavage at the 6-O position was performed with hydrobromic acid under reflux, and etherification with bromoacetate in DMF/potassium carbonate at room temperature. The ester group was hydrolyzed by heating in aqueous sodium hydroxide solution and the acid reacted with N-hydroxysuccinimide in the presence of carbodiimide at room temperature to give O-succinimidyl (OSu) esters. The OSu ester was used to label in aqueous solutions amino acids, peptides, proteins, amino sugars in slightly basic medium.

**Synthesis of 6-O-acetyl carbostryril:** Acylation at O-6 was performed with acetyl chloride in DMF/potassium carbonate at room temperature.

**UV/vis spectra:** Shimadzu UV/VIS scanning spectrophotometer UV-2101 PC; concentration: 0.01 mg/mL.

**Excitation and emission spectra:** Shimadzu RF-5001 PC spectrofluorometer (150-W Xe lamp, 6 selectable slits: 1.5, 3, 5, 10, 15, 20 nm, R452-01 photomultiplier; monochromator: ion-blazed holographic concave grating F/2.5); concentration: 0.001 mg/mL.

Determination of quantum yields: emission signals were set in relation to the known signal of quinine under the same conditions.

## Acknowledgement

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## References

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