Günther Lahm, BSc

Syntheses of Heterocycles with Fluorescence Optical Properties

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Master Thesis

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Institute of Chemistry / Organic and Bioorganic Chemistry Karl-Franzens-University of Graz, Austria

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Acknowledgments

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Abstract

Title: Syntheses of Heterocycles with Fluorescence Optical Properties

Author: Günther Lahm

1st keyword: Quinolones
2nd keyword: Carbostyrils
3rd keyword: Fluorescence

Carbostyrils are stable fluorophors with excellent photophysical properties. In this thesis a number of 6-methoxycarbostyrils were investigated to show the influence of position 3 on the fluorescence properties.

6-Methoxy-carbostyril was synthesized starting from p-anisidine which gave with malonic acid 3-unsubstituted carbostyril. A nitration gave 3-nitrocarbostyril which was reduced to 3-acetamidocarbostyril.

Via reactive 4-chloroquinolones we introduced the sulfone substituent in all derivates by using lithium p-toluenesulfinate.

A donor in position 3 of 4-sulfonated carbostyril shifted down the absorption maxima to 356 nm compared to 3-unsubstituted 4-sulfonated carbostyril with absorption maxima at 385 nm.

Kurzfassung

Titel: Synthese von Heterocyclen mit fluoreszenzoptischen Eigenschaften

Autor: Günther Lahm

1. Stichwort: Chinolone
2. Stichwort: Carbostyrile
3. Stichwort: Fluoreszenz

Carbostyrile sind stabile Fluorophore mit hervorragenden photophysikalischen Eigenschaften. In dieser Arbeit wurden 6-Methoxycarbostyrile mit unterschiedlichen Substituenten in Position 3 untersucht, um den Einfluss von Position 3 auf die Fluoreszenz zu zeigen.

6-Methoxycarbostyril wurden ausgehend von p-Anisidin und Malonsäure hergestellt. Man erhielt ein 3-unsubstituiertes Carbostyril. Durch Nitrierung erhielt man 3-Nitro-carbostyril das anschließend zu 3-Acetamido-carbostyril reduziert wurde.

Ausgehend von den 4-Chlorochinolonen führten wir mit Lithium-p-toluolsulfinat den Sulfon–Substituenten in alle Derivate ein.

Durch einen Donor in Position 3 verschob sich das Absorptionsmaximum des 4-Sulfonyl-Carbostyrils zu kürzeren Wellenlängen. Im Vergleich zum unsubstituierten Carbostyril-Sulfon verschob sich das Absorptionsmaximum von 385 nm zu 356 nm.

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1. Introduction

1.1 Aim of the Stadlbauer / Uray Group

The use of fluorescence as a tool especially in biological science dramatically increased in the last 20 years. For most biochemical measurements fluorescent detection can replace the difficulties of handling and expensive radioactive tracers. [1]
Fluorescence is used in many different areas such as fluorescent lamps, chemical sensors, dyes, fluorescent labeling, mineralogy and gemmology.
Coumarins are studied extensively as fluorescence dyes. Through variation of the substituents at the positions shown in **Figure 1** the electronic properties were tuned. [2]

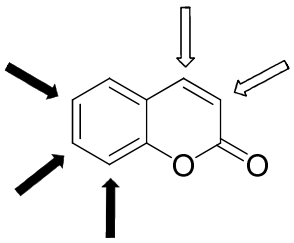


Figure 1: Positions for the substituent variation on the coumarin scaffold

A great number of fluorescence applications are reported for coumarins. They are used as laser dyes [3], as fluorescence labels in biological applications [4], and as optical brighteners [5]. Especially the use as optical brighteners should be emphasized because it is used in industrial scale.

The research of the Stadlbauer / Uray Group deals with the aza-analog the so called carbostyrils. In contrast to coumarins less attention has been paid to the carbostyrils probably because of their shorter absorption and emission wavelengths [6]. However many aspects speak in favour of carbostyrils. Compared to coumarines [7] and fluorescein-type dyes they are highly stable against chemicals. Furthermore they are insensitive to oxygen quenching compared to 1,10-phenantroline complexes and they are much more stable against photochemical and thermal stress relative to azodyes. All these properties predestine them as fluorescence markers for biochemical and medicinal applications.

Recently we have shown that we can improve carbostyrils in terms of absorption, emission and quantum yield by using the push-pull concept [8]. We used two donor substituents at position 6 and 7, and an acceptor at position 4.

So the aim of the Stadlbauer / Uray Group is to synthesize carbostyrils with all the advantages described above and without the disadvantage of shorter absorption and emission wavelengths. Furthermore we try to establish guidelines how to improve the fluorescence properties of carbostyrils for potential application in medicine and biochemistry.

1.2 Aim of the Master Thesis

Aim of the work was the synthesis of 4-sulfonated **I** and 4-sulfinated carbostyrils **II**. It is not clarified yet if we really get a sulfinated carbostyril or the sulfone isomer **III**. This fact will be described in more detail in chapter **2.9.1**. We made different derivates of each of these compounds to show the influence of position 3 on the fluorescence.

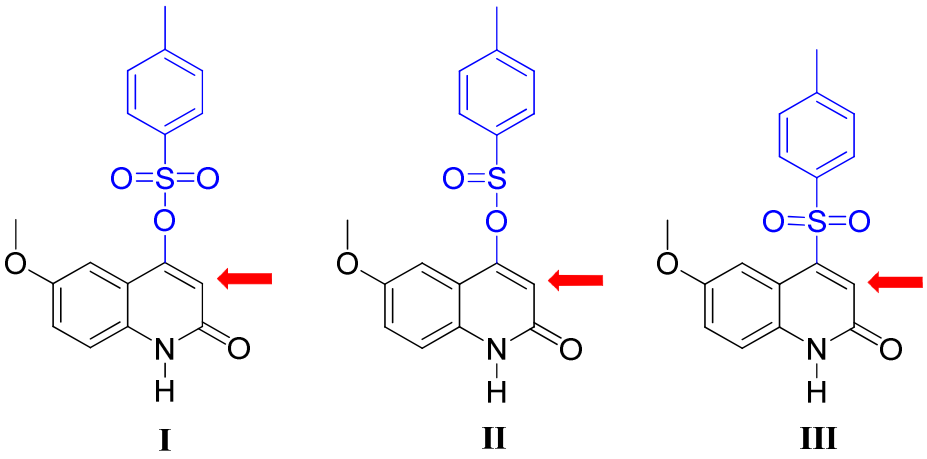


Figure 2: Position for the substituent variation on the carbostyril scaffold

The nitro group was tried to be used as an acceptor in position 3 and the acetamido group as a donor.

We have chosen sulfonates and sulfinates from two main reasons. They will probably show good fluorescent properties and they are good leaving groups and therefore interesting intermediates.

1.3 Applications already containing our carbostyrils

1.3.1 FRET (fluorescence resonance energy transfer)

"Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon [9]." FRET systems are powerful tools for distance determinations on nanometer scale especially for the characterization of biochemical events. In comparison to microscopy FRET systems have a higher resolution.

For FRET three primary conditions must be fulfilled.

1. Donor and acceptor must be close to each other (10–100 Å).
2. There must be an overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor.
3. The transition dipole orientations of acceptor and donor must be approximately parallel.

Recently the Bannwarth Group has shown that our carbostyrils are well suited as donors for FRET applications. **Figure 3** shows our donor chromophores in the FRET system.

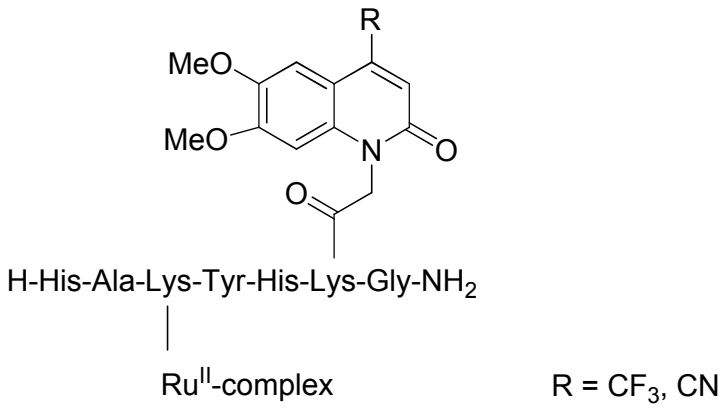


Figure 3: FRET Peptides with our carbostyril donors

Prof. Uray has shown the use of our carbostyrils in optical sensors for potassium ions [10]. Synthetic valinomycin-carbostyril-rhodamin changes his conformation after complexation with K^+. The distance change between carbostyril and rhodamin can be measured with FRET.

1.3.2 Antenna mediated lanthanide luminescence

Luminescence lanthanides have many advantages compared to standard fluorescent dyes. Every fluorescence application in biological media has difficulties caused by background signals. In most cases the emission lifetime of the luminescence lanthanides is longer so their emission can be measured time-resolved. Applications for luminescence lanthanides arise because of their large Stokes shifts, their good solubility in aqueous solvents and the already mentioned possibility of time-resolved measurements [11].

The use of our carbostyrils as antenna in luminescent lanthanides is already published in different journals [11, 12].

2. Synthesis of 6-methoxyquinolin-2(1*H*)-ones

2.1 Synthesis of 4-hydroxy-6-methoxyquinolin-2(1*H*)-one (3)

We have chosen the cyclocondensation of p-anisidine **(1)** and malonic acid **(2)** to 4-hydroxy-6-methoxyquinolin-2(1H)-one **(3)** as starting reaction in a sequence of reactions. Due to the hydroxy group in position 4 compound **3** is an useful intermediate.

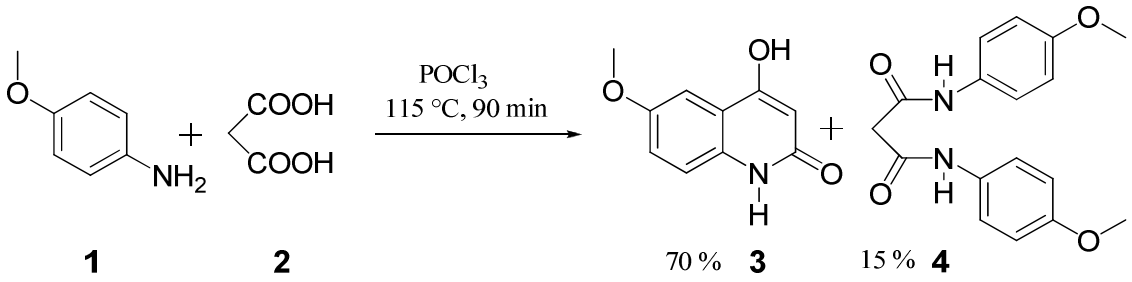
A Survey of the literature showed that there already exist some methods to synthesize 4-hydroxy-6-methoxyquinolin-2(1H)-one **(3)**. Shobana et al. [1] reported 1989 a method from p-anisidine and diethyl malonate using polyphosphoric acid as cyclization agent. This method gave a good yield of 84 %. A similar method was reported from Campbell et al. [2] in 1990 which gave only a yield of 11 %. Therefore the data from ref. [1] deserve a critical consideration. In 2010 Gao et al. [3] reported a one pot synthesis from Meldrum´s acid and p-anisidine using Eaton's reagent as cyclization agent. This method gave a yield of 61 %.

The following procedure presented in this work is a simple and fast alternative to the methods already described which gives a yield of 70 %.

4-Hydroxy-6-methoxyquinolin-2(1H)-one **(3)** was prepared from malonic acid **(2)** and p-anisidine **(1)** in phosphoryl chloride. Phosphoryl chloride was in this case both, a reagent and a solvent. In the first step of the reaction malonic acid **(2)** was probable converted to phosphorodichloridic anhydride. A vigorous reaction started abruptly at 95 °C, so it was important to stir adequately. Then the reaction mixture was heated to 115 °C and stirred for 90 minutes. Best results were achieved in the range of 110-115 °C under continued stirring. It was very important to check the temperature continuously, to avoid that the yield of **3** decreased. An increasing of the temperature would gave less by-product, *N,N'*-bis(4-mehoxyphenyl)malonamide **(4)** but **3** could be chlorinated.

Compounds **3** and **4** were characterized by ^{1}H-NMR. The ^{1}H-NMR spectrum of **3** showed a singlet at 5.73 ppm for C-3 proton and a singlet at 11.10 ppm for the NH proton. The ^{1}H-NMR spectrum of **4** showed two doublets at 6.87 ppm and 7.50 ppm which is typical for the para-substituted patterns of a benzene ring. The singlet from the methylen protons appeared at 3.39 ppm.

Scheme 1



2.2 Synthesis of 4-methylbenzenesulfinyl chloride (6)

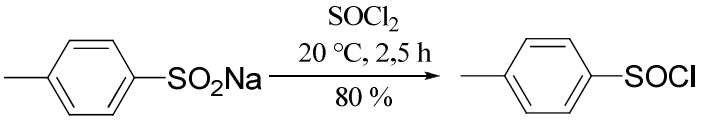
In order to introduce p-toluenesulfinate in compound **3** directly we needed 4-methylbenzenesulfinyl chloride **(6)** as a reagent. Direct sulfinylation has the advantage of less reaction steps compared to the introduction in 4-chloro carbostyril **12**. See chapter **2.9**.

4-Methylbenzenesulfinyl chloride **(6)** was prepared from *p*-toluenesulfinic acid sodium salt hydrate **(5)** in redistilled thionyl chloride according to ref. [4]. The sulfinyl chloride thus obtained was satisfactory for further preparative work. If sulfinyl chloride in higher quality is needed a distillation is recommended. The product should be stored under inert atmosphere.

Compound **6** was characterized by ^{1}H-NMR and infrared spectroscopy. The ^{1}H-NMR spectrum of **6** showed a singlet at 2.45 ppm from the methyl protons and AA',BB'-pattern at 7.77 ppm (J = 8.4 Hz) and at 7.40 ppm (J = 8.0 Hz) which is typical for the para-substituted patterns of a benzene ring. H_A and $H_{A'}$ was shifted downfield to 7.77 ppm due to the influence of SOCl.

The IR spectra showed a characteristic S=O band at 1175 cm^{-1}[5].

Scheme 2



2.3 Introduction of p-toluenesulfinate in position 4 of compound 3

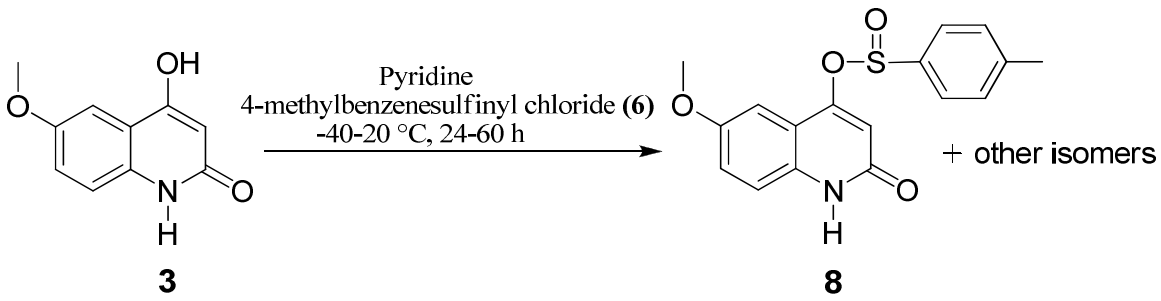
The direct sulfinylation was performed according to ref. [6] under different reaction conditions, as described in **Table 1**. We always got a mixture of 3 fluorescent compounds even at -40 °C probably containing O- and N- mono and diacylated derivates. Two fluorescent compounds had the same ratio at all reaction conditions. Performing the reaction at even lower temperature we would probably get just two fluorescent compounds. All our separation attempts with chromatographic methods failed. As the direct sulfinylation had a low selectivity we introduced p-toluenesulfinate in 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)**. See chapter **2.9**.

solvent	temperature (°C)	time (h)	mixing ratio
pyridine	20	24	1.0 : 1.0 : 0.8
pyridine	0	30	1.0 : 1.0 : 0.7
pyridine	-40	60	1.0 : 1.0 : 0.5

Table 1: reaction conditions for the introduction of p-toluenesulfinate in position 4 of compound **3**

The mixture was characterized by ^{1}H-NMR which showed three singlets between 3.7-3.9 ppm. One of the singlets was probably from the methoxy protons of compound **8** and one of the other singlets was probably from the methoxy protons of the disulfinate analog of compound **10**.

Scheme 3



3 **8**

2.4 Synthesis of 6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzensulfonate (9)

The following procedure is a simple method to introduce p-toluenesulfonate directly in compound **3**. Direct sulfonation has the advantage of less reaction steps.

6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzensulfonate **(9)** was prepared from 4-hydroxy-6-methoxyquinolin-2(1*H*)-one **(3)** and 1.5 eq of p-tosyl chloride **(7)** in dry pyridine according to ref. [6]. We obtained the best ratio between compound **9** and **10**, when the reaction was performed at room temperature for 30 hours. Compound **9** and **10** were both fluorescent. Besides these 2 compounds we found traces of a very strong fluorescent compound during the fluorescent measurements of compound **9**. This compound had an absorption maximum at 650 nm and an emission maximum at 690 nm. However, all our separation attempts with preparative HPLC failed, so it was not possible to determine the structure. The main reason why it could not be separated was, that the highly fluorescent compound was only generated in traces during the reaction.

The structural elucidation of compound **9** was based on spectral analyses. The strong lactam carbonyl stretching was observed in the infrared spectrum at 1650 cm^{-1}. Furthermore we found a broad N-H band between 2700 and 3100 cm^{-1}.

The ^{1}H-NMR spectrum of **9** showed a singlet at 6.28 ppm for C-3 proton, a singlet at 11.95 ppm for the NH proton and AA',BB'-pattern at 7.92 ppm (J = 8.3 Hz) and at 7.48 ppm (J = 8.2 Hz) which is typical for the para-substituted patterns of a benzene ring. H_A and $H_{A'}$ was shifted downfield at 7.92 ppm due to the influence of the SO_3 group.

The ^{13}C-NMR spectrum showed 15 different peaks as expected. The peaks for the $C_{BB'}$ at 128.8 ppm and for the $C_{AA'}$ at 131.0 had twice the intensity as the other 13 peaks. The peak for the lactam carbon appeared at 161.5.

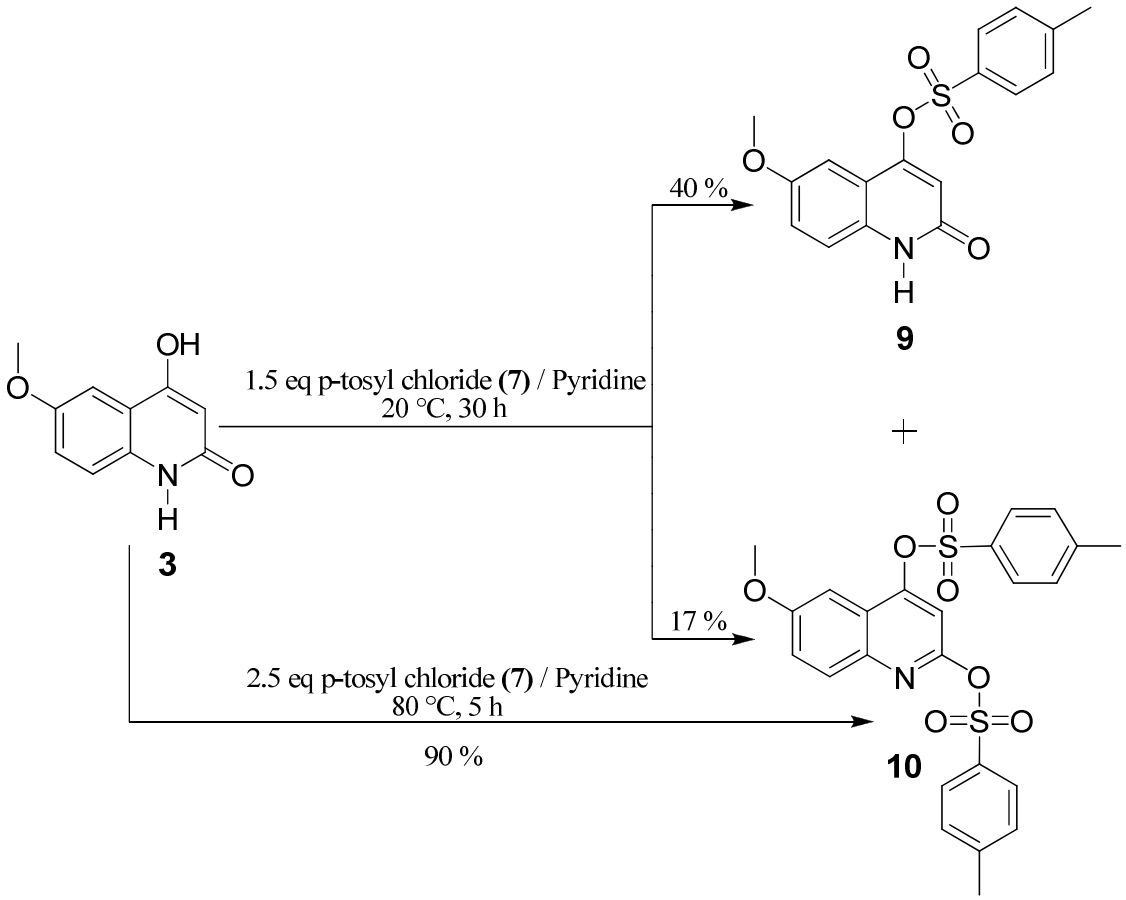
The mass spectrum showed with APCI positive and negative mode a base peak with 100 %.

The findings were confirmed by elemental analyses.

2.5 Synthesis of 6-methoxyquinoline-2,4-diyl bis (4-methylbenzenesulfonate) (10)

6-Methoxyquinoline-2,4-diyl bis(4-methylbenzenesulfonate) **(10)** was obtained during the work up of compound **9**. The compound was obtained in good yields of 90 %, when we used 2.5 eq of p-tosyl chloride (**7)** instead of 1.5 eq and when the reaction was performed at 80 °C for 5 hours. Compound **10** was found as a new category of fluorescent compounds. It showed similar fluorescent properties like compound **9** in reference to absorption and emission.

Scheme 4



The structural elucidation of compound **10** was based on spectral analyses. There was no lactam carbonyl stretching in the region of 1650 cm^{-1} and there was no characteristic broad band for the N-H stretching in the infrared spectrum.

The ^{1}H-NMR spectrum of **10** showed three singlets at 2.38 ppm, 2.43 ppm and at 3.75 ppm. The first two singlets could be dedicated to the two methyl protons and the other one to the methoxy protons. There was no peak for the N-H proton. Furthermore we found CC',DD'-

pattern at 7.93 ppm (J = 8.3 Hz) and at 7.50 ppm (J = 8.0 Hz) which is typical for the para-substituted patterns of a benzene ring. The AA',BB-pattern was found at 7.84 ppm (J = 8.3 Hz). The doublet from $H_{BB'}$ was overlapped by the C-7 proton from 7.42 to 7.46 ppm. Compared to compound **9** the C-3 proton was shifted more downfield, caused by the greater electronegativity of the sulfonate group in position 2.
The mass spectrum showed with APCI positive mode a base peak with 100 %.
The findings were confirmed by elemental analyses.

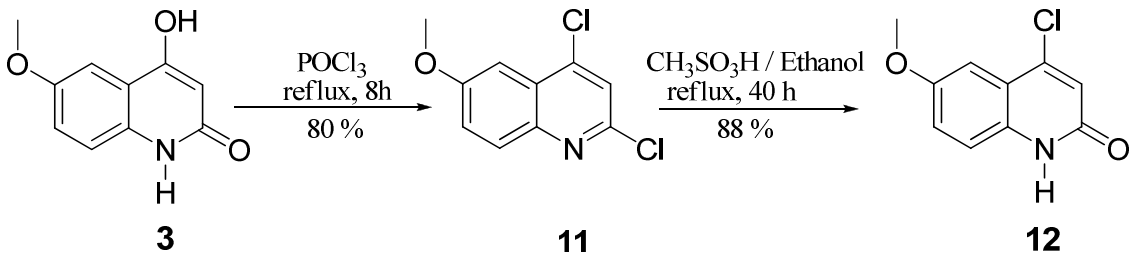
2.6 Synthesis of 2,4-dichloro-6-methoxyquinoline (11)

As the direct sulfinylation had a low selectivity we introduced p-toluenesulfinate in 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)**. See chapter **2.3** and **2.9**. In order to prepare 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)** we obtained first 2,4-dichloro-6-methoxyquinoline **(11)** because a selective mono-chlorination was not possible.

2,4-Dichloro-6-methoxyquinoline **(11)** was prepared from 4-hydroxy-6-methoxyquinolin-2(1H)-one **(3)** in phosphoryl We obtained the compound in a good yield of 80 %.

Compound **11** was characterized by ^{1}H-NMR. Comparison of the ^{1}H-NMR data of compound **3** and **11** showed that the signal of NH was not observed in **11** which appeared in 4-hydroxycarbostyril **3**. The signal for the C-3 proton of compound **11** was shifted downfield due to the strong electronegativity of the chloro-atoms in position 2 and 4. It appeared at 7.93 ppm compared to 5.74 ppm for compound **3**.

Scheme 5



2.7 Synthesis of 4-chloro-6-methoxyquinolin-2(1*H*)-one (12)

In order to introduce p-toluenesulfinate we needed 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)** as starting material. Compared to the direct sulfinylation we have the disadvantage of two additional reaction steps but the advantage of higher selectivity. See chapter **2.3** and **2.9**.

4-Chloro-6-methoxyquinolin-2(1*H*)-one **(12)** was prepared from 2,4-dichloro-6-methoxy-quinoline **(11)** and 70 % methanesulfonic acid in ethanol. We obtained the compound in a good yield of 88 %.

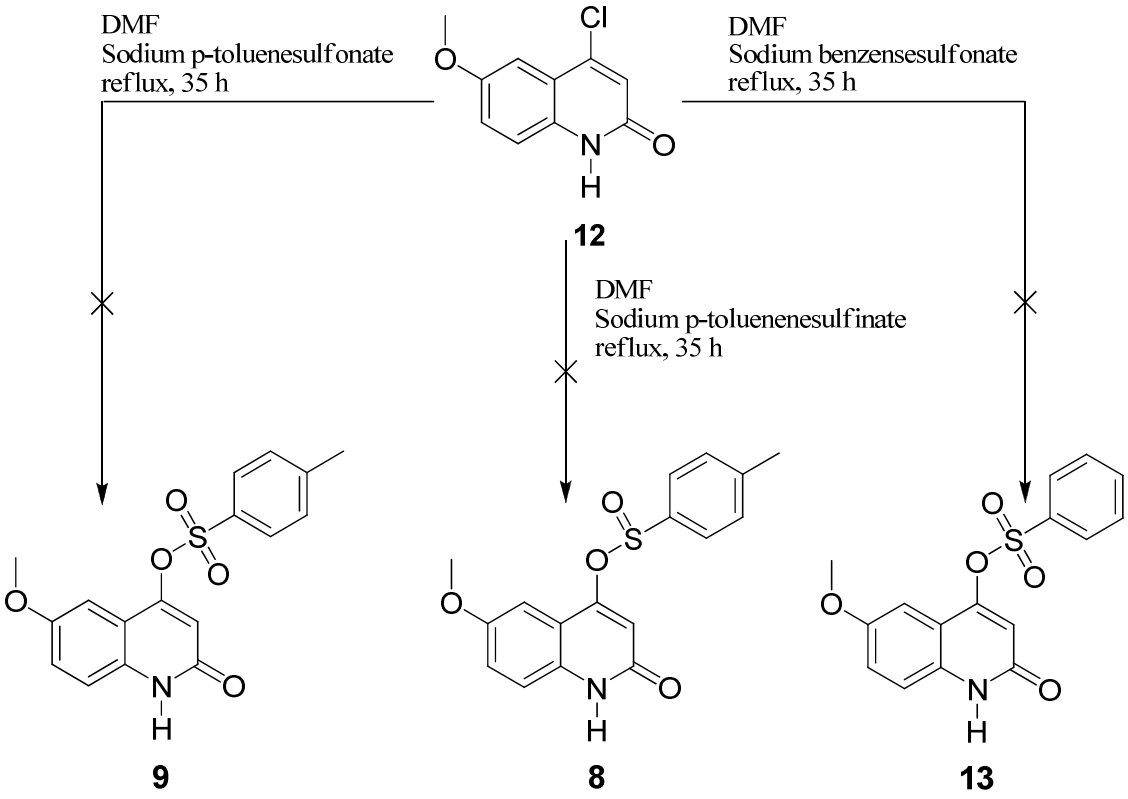
Compound **12** was characterized by ^{1}H-NMR. Comparison of the ^{1}H-NMR data of compound **11** and **12** showed that a signal for NH was observed in **12** which was not present in compound **11**. The signal for the C-3 proton of compound **12** was shifted upfield. It appeared at 6.82 ppm compared to 7.93 ppm for compound **11** due to the influence of just a single chloro atom.

2.8 Attempts of the introduction of p-toluenesulfonate, benzenesulfonate and p-toluenesulfinate in position 4 of compound 12 by using the appropriate sodium salt

Actually we just wanted to introduce p-toluenesulfonate in compound **12** by using the appropriate sodium salt to get a higher yield compared to the direct sulfonation described in chapter **2.4**. After this attempt failed we decided to find out whether the sulfonate was not reactive enough or if the salt was responsible for the low reactivity. Therefore we investigated different reagents.

Attempts to synthesize 6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzene sulfonate **(9)**, 6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-benzenesulfonate **(13)** and 6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate **(8)** by using the appropriate sodium salt according to ref. [6] failed. The reaction process was monitored by TLC. After no reaction could be detected, we increased the temperature, added the appropriate salt in excess and added 15-crown-5 to the reaction mixture to force the reaction. All these attempts failed. The purified and isolated product could be identified as starting material **12** by ^{1}H-NMR.

Scheme 6



The donor character of the methoxy group in position 6 of compound **12** by the inductive and mesomeric effect influenced the reactivity.

Inductive effect:

The electron arrangement between two different atoms is not uniform. This effect is closely related to the electronegativity of the substituents. There are two different effects: **- *I* effect** (electron-withdrawing inductive effect) and the **+ *I* effect** (electron releasing inductive effect)

Mesomeric effect:

Substituents take part at the mesomerism. There are two different effects: **- *M* effect** (electron withdrawing effect caused by double or triple-bond of the substituent) and **+ *M* effect** (electron releasing effect caused by a free electron-pair of the substituent) [7, 8]

In the case of the methoxy group, the **+ *M* effect** assigned the donor character.

Therefore compound **12** was less electrophile against nucleophiles.

We performed successfully the introduction of p-toluenesulfinate in position 4 of compound **12** by using lithium p-toluenesulfinate, instead of the analog sodium salt. The higher reactivity of the lithium salt could be probably illustrated by the two following explanations.

Explanation 1:

The solubility of lithium salts often differs distinctly from those of sodium salts. Although the charge to radius ratio of lithium is comparable to those of magnesium and to those of sodium they behave often like their magnesium analogs. For example LiBr, LiCl and especially LiI are soluble in oxygen and nitrogen containing organic solvents, like their magnesium analogs. The appropriate sodium salts are not soluble in those organic solvents. [9]

Explanation 2:

Our situation is comparable to those of a latex-cation-exchanger with sulfonate groups as exchanger.

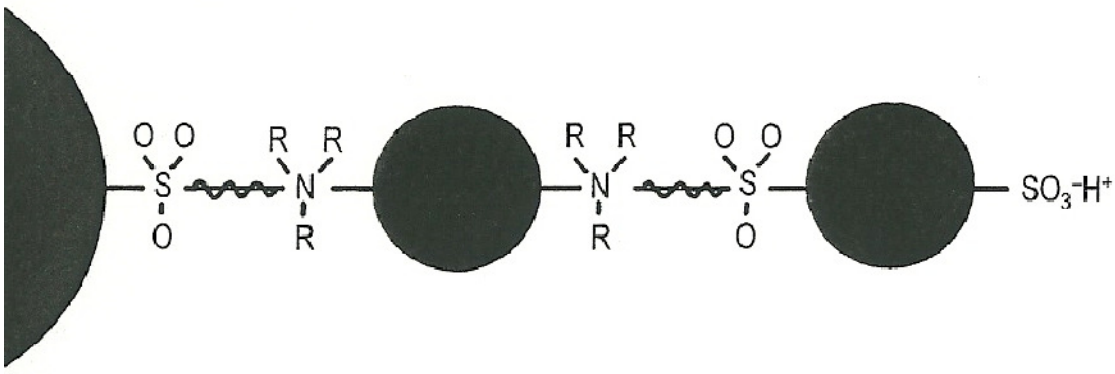


Figure 4: latex-cationen-exchanger [10]

The exchange site generally is not well hydrated. “Less hydrated cations are better able to enter the more hydrophobic stationary phase than more highly hydrated cations which prefer to partition in aqueous eluents [11].” Lithium p-toluenesulfinate is more reactive than sodium p-toluenesulfinate because lithium is better hydrated and therefore we have more free anions [11, 12]. The reaction was performed in dry DMF but not under inert conditions and the lihium salt was not totally dry, so we had a small amount of water in the system.

Compared to explanation 1, the effect illustrated in explanation 2 was probably less important.

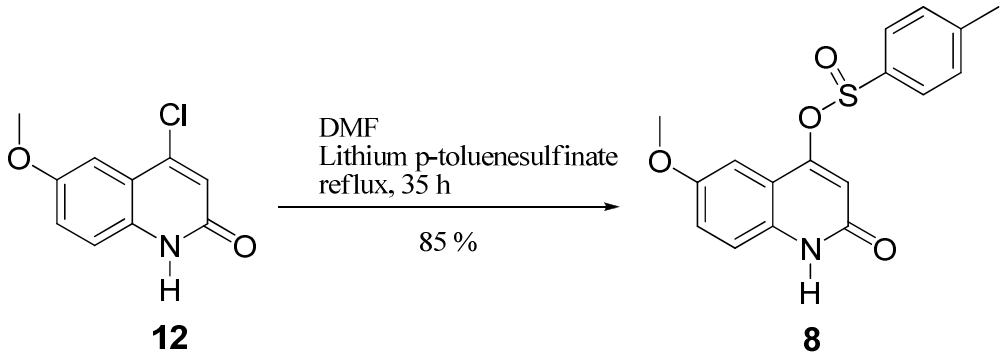
2.9 Synthesis of 6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate (8)

As the direct sulfinylation had a low selectivity we introduced p-toluenesulfinate in 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)**. Compared to the direct sulfinylation we have the disadvantage of two additional reaction steps but the advantage of higher selectivity. See chapter **2.3**.

6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate **(8)** was prepared from 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)** and lithium p-toluenesulfinate in DMF. We obtained the compound in a good yield of 85 %. This compound showed excellent fluorescence properties. The strong fluorescence was even obvious by checking the TLC. Compared to the method described in ref. [6] we used the lithium salt instead of the sodium salt and found out that this was more reactive. All our attempts by using the sodium salt, as described in **2.8** failed.

The structural elucidation of compound **8** was based on spectral analyses. The ^{1}H-NMR spectrum of **8** showed two singlets at 2.38 ppm, and 3.73 ppm. They could be dedicated to the methyl protons and to the methoxy protons. Furthermore we found AA',BB'-pattern at 7.95 ppm (J = 8.2 Hz) and at 7.48 ppm (J = 8.4 Hz) which is typical for the para-substituted patterns of a benzene ring. H_A and $H_{A'}$ was shifted downfield at 7.95 ppm due to the influence of SO_2. In the area of 7.25-7.47 ppm was an overlap between the protons of 3-H, 5-H, 7-H and 8-H so it was not easy to distinguish between the signals of each proton.
Compound **8** showed compared to the sufonate analog **9** better fluorescence properties. This could be explained by the stronger withdrawing character of the sulfinate group. This fact was verified by the ^{1}H-NMR spectra. The ^{1}H-NMR spectrum of **9** showed a singlet at 6.28 ppm for the C-3 proton which is 0.97 ppm lower compared to the signal of compound **8** for the C-3 proton which appeared at 7.25 ppm.

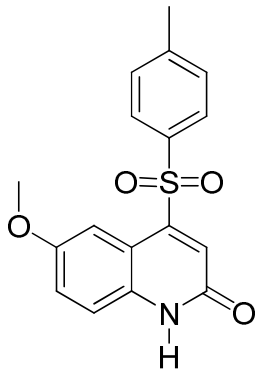
Scheme 7



12 **8**

2.9.1 Alternative structure for compound 8

Compound **8** could also have the following isomeric structure **8X**.



8X

In this case we got a sulfone instead of a sulfinate. That means the substitution took place on the sulphur and not on the oxygen as outlined for structure **8**.
The following aspect indicates the generation of compound **8**, which means substitution on the oxygen.

After treating compound **8** or **8X** with dry potassium cyanide in dry DMF, the functional group in position 4 was easily exchanged against cyano group [13]. Sulfinates are compared to sulfones good leaving groups for nucleophilic substitution [14]. Therefore an easy substitution of the functional group in position 4 suggests that the substitution took place on the oxygen.

The following aspects indicate the generation of compound **8X**, which means substitution on the sulphur.
We found two characteristic bands in the IR spectra at 1324 cm^{-1} and at 1135 cm^{-1} which indicate a sulfone [15].

Sulfones are better withdrawing groups than sulfinates, what would explain the strong fluorescence compared to the sulfonated compound **9**.

K. Schank postulated 1967 that alkali metal sulfinates would react with alkyl halides exclusive to sulfones [15, 16]. One can conclude that sulfinates would react with heteroaryl halides to sulfones too.

A. Schörbel and A. Wagner described a method to prepare diaryl sulfones with sodium sulfinate and aryl halides. In their description the aryl halide must be activated by nitro-groups [17].

A clarification will bring the X-ray crystallography which is in process.

2.10 Synthesis of 4-hydroxy-6-methoxy-3-nitroquinolin-2(1H)-one (14)

In order to show the influence of position 3 on the fluorescence we prepared a 3-acceptor substituted carbostyril. Therefore 2-quinolone **3** was nitrated.

4-Hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)** was obtained in the reaction from 4-hydroxy-6-methoxyquinolin-2(1*H*)-one **(3)** and concentrated nitric acid in glacial acetic acid. The electrophilic substitution was catalyzed by sodium nitrite to accelerate the velocity and to allow performing the reaction at room temperature. The catalytic effect could be explained by an initial nitrosation of compound **3** by the use of sodium nitrite [18]. Performing the reaction using nitric acid and boiling acetic acid should lead to a direct nitration of compound **3** which could be substituted by the hydroxy group [19, 20]. The benzo part of compound **3** could by nitrated by performing the reaction without the catalyst at reflux.

Compound **14** was characterized by ^{1}H-NMR. Comparison of the ^{1}H-NMR data of compound **3** and **14** showed that a signal for the C-3 proton was observed in **3** which disappeared in compound **14**. The signal for the N-H proton of compound **14** was shifted downfield. It appeared at 11.89 ppm compared to 11.10 ppm for compound **3** due to the electron withdrawing influence of the nitro group.

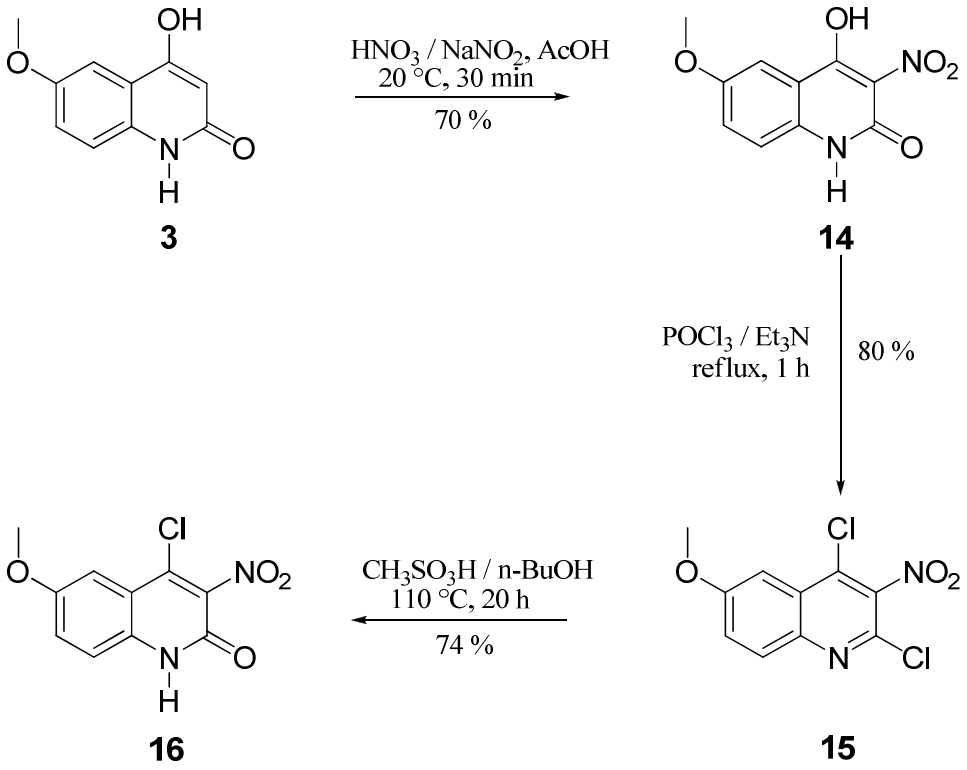
2.11 Synthesis of 2,4-dichloro-6-methoxy-3-nitroquinoline (15)

As the direct sulfinylation in the 3-unsubstituted carbostyril **3** had a low selectivity and 4-methylbenzenesulfinyl chloride **(6)** which we needed as a reagent was not easy to store over a long period we decided to introduce p-toluenesulfinate in 4-chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)**. See chapter **2.3** and **2.20**. In order to prepare 4-Chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)** we obtained first 2,4-dichloro-6-methoxy-3-nitroquinoline **(15)** because a selective mono-chlorination was not possible.

2,4-Dichloro-6-methoxy-3-nitroquinoline **(15)** was prepared from 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)** in phosphoryl chloride. The chlorination of **14** was performed as described in chapter **2.6** but we used in this case additional triethylamine as basic catalyst. It was shown in our group that additional triethylamine could accelerate the exchange of the hydroxy group against a chloro group. Triethylamine destroyed the hydrogen bonds between the 3-nitro and the 4-hydroxy group of compound **14** which retarded or prevented the attack of phosphoryl chloride [21, 22]. A similar hindrance was found for the synthesis of *N*-(4-chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(20)**. By the influence of the nitro group in position 3, a selective mono-chlorination in position 4 of compound **14** was not possible. For the 3-unsubstituted analog of compound **14** a selective mono-chlorination was not possible as well, as already described in chapter **2.6**.

The structural elucidation of compound **15** was based on spectral analyses. There was no lactam carbonyl stretching in the region of 1650 cm^{-1} and there was no characteristic broad band for the N-H stretching in the infrared spectrum. The mass spectrum showed with APCI positive mode a base peak at 255 m / z what indicate that one chloro group was substituted by the hydroxy group during the measurement.

Scheme 8



2.12 Synthesis of 4-chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one (16)

In order to introduce p-toluenesulfinate we needed 4-chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)** as starting material. Compared to the direct sulfinylation we have the disadvantage of two additional reaction steps but we hoped to increase the selectivity. See chapter **2.3** and **2.20**.

4-Chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)** was prepared from 2,4-dichloro-6-methoxy-3-nitroquinoline **(15)** and 70 % methanesulfonic acid. The regioselective hydrolysis was tried to be performed like described in **2.7**. By the electron withdrawing influence of the nitro group in position 3 a regioselective hydrolysis was difficult. We always got a mixture of two compounds. We isolated **14** which was identified by ^{1}H-NMR and the expected compound **16**. The reaction was performed in different solvents under different reaction conditions. Best conditions for the regioselective hydrolysis were found in n-butanol at 110 °C for 20 hours. In this case the reaction did not proceed to completion. The mixture of compounds **14**, **16** and the educt **15** was separated by dry-flash-column chromatography

using ethyl acetate to obtain 4-chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)** in a yield of 74 %.

solvent	temperature (°C)	time (h)	mixing ratio 14 : 15 :16
n-BuOH	80	60	1.0 : 0.0 : 0.4
n-BuOH	100	48	1.0 : 0.0 : 0.4
n-BuOH	110	20	0.1 : 0.1 : 1.0
Ethanol	78	96	no reaction occurred
DMF	80	96	1.0 : 0.0 : 0.1

Table 2: reaction conditions for regioselective hydrolysis of **15** to **16**

Compound **16** was characterized by ^{1}H-NMR. Comparison of the ^{1}H-NMR data of compound **16** and **15** showed that a signal for the N-H proton was observed in **16** which was not present in compound **15**.

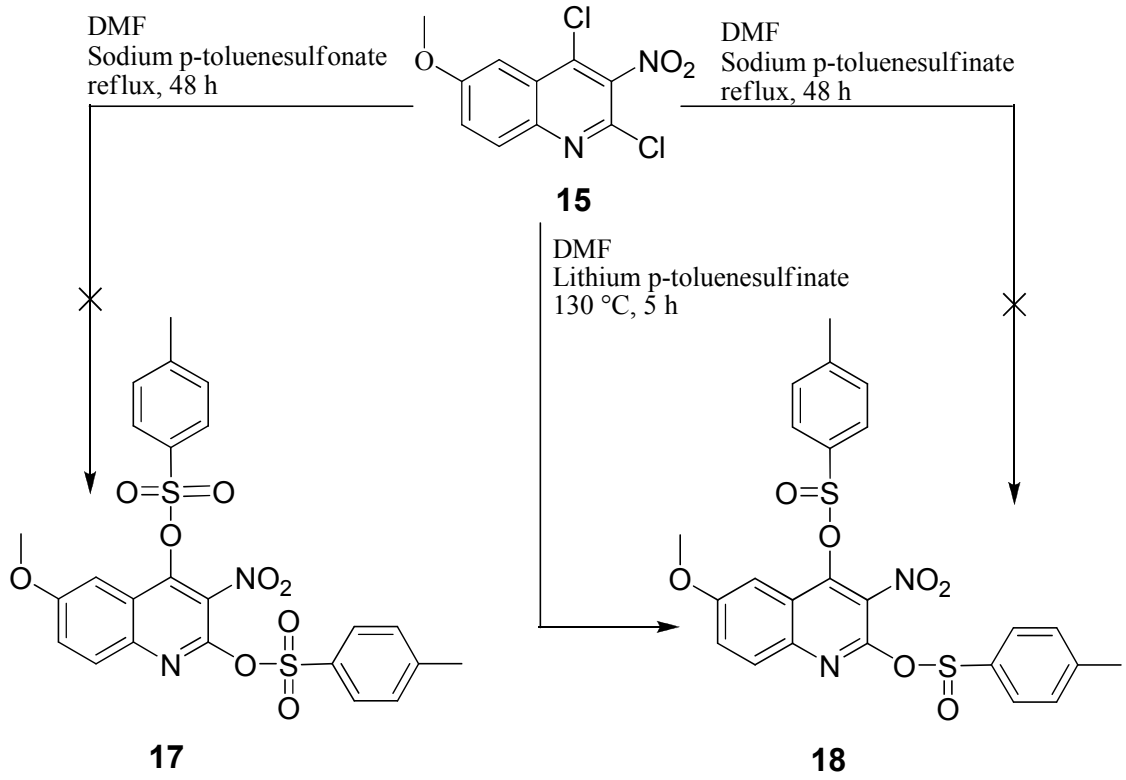
2.13 Attempts of the introduction of p-toluenesulfonate and p-toluenesulfinate in position 2 and 4 of compound 15 by using the appropriate sodium salt

Quinoline-tosylate **10** was found as a new category of fluorescent compounds. Therefore we decided to synthesize similar compounds. See chapter **2.5**.

Attempts to synthesize 6-methoxy-3-nitroquinoline-2,4-diyl bis(4-methylbenzenesulfonate)- **(17)** and 6-methoxy-3-nitroquinoiline-2,4-diyl bis(4-methylbenzenesulfinate) **(18)** by using the appropriate sodium salt failed. The reaction process was monitored by TLC. After no reaction could be detected, we increased the temperature, added the appropriate salt in excess and added 15-crown-5 to the reaction mixture to force the reaction. All these attempts failed. The purified and isolated product could be identified as starting material **15** by ^{1}H-NMR.

Attempts to introduce p-toluenesulfonate and p-toluenesulfinate in position 2 and 4 of compound **15** by using the appropriate sodium salt are comparable to the synthesis already described in chapter **2.8**. Therefore we can use the same explanations why it was not working.

Scheme 9



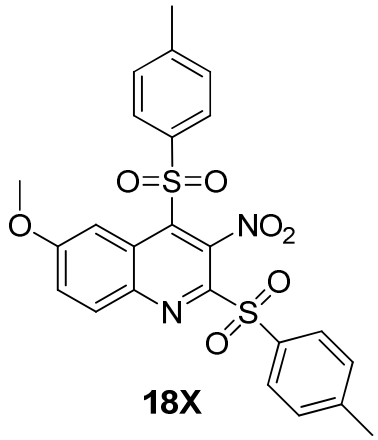
2.14 Introduction of p-toluenesulfinate in position 2 and 4 of compound 15

Quinoline-tosylate **10** was found as a new category of fluorescent compounds. Therefore we decided to synthesize similar compounds. See chapter **2.5**. As our attempt to introduce p-toluenesulfinate failed by using the appropriate sodium salt we used the analog lithium salt. See chapter **2.13**.

The introduction of p-toluenesulfinate was performed in dry DMF at 130 °C for 5 hours with lithium p-toluenesulfinate. We always got a mixture of 4 fluorescent compounds. The strong fluorescence of two of these compounds was even obvious by checking the TLC. All our separation attempts with chromatographic methods failed, because the R*f* values were to close.

2.14.1 Alternative structure for compound 18

Compound **18** could also have the following isomeric structure **18X**.



18X

See chapter **2.9.1**.

2.15 Synthesis of *N*-(4-hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (19)

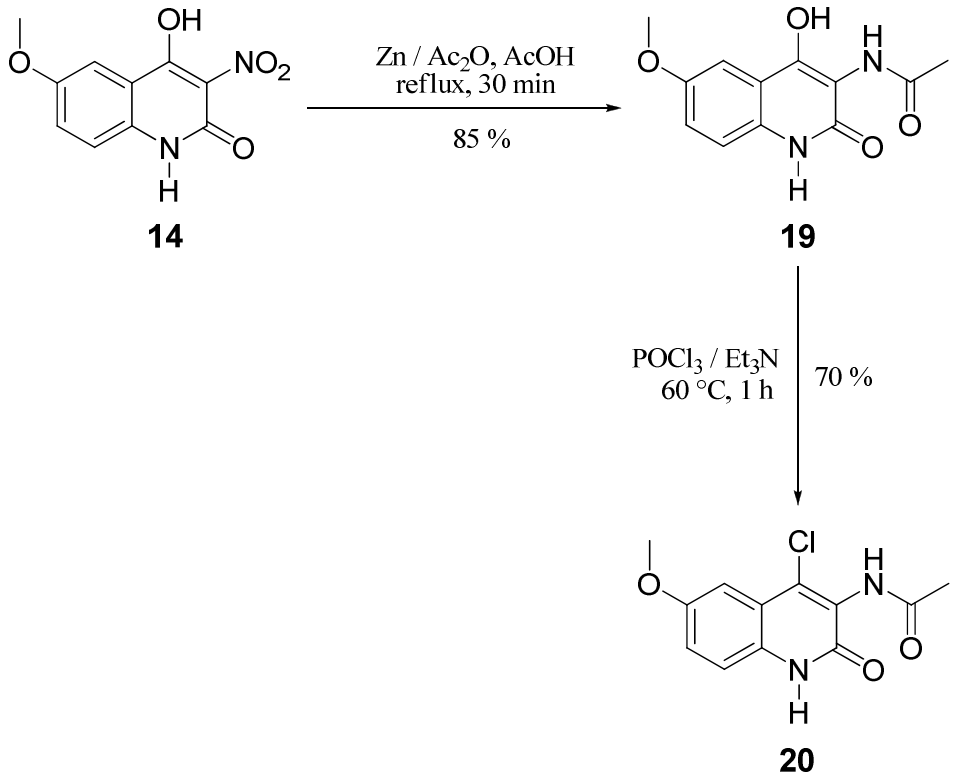
In order to show the influence of position 3 on the fluorescence we prepared a 3-donor substituted carbostyril. Therefore 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)** was reduced to acetylaminoquinolone **19**.

N-(4-Hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(19)** was prepared from 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)** in the presence of zinc-dust, acetic acid and acetic anhydride. Acetic anhydride was added only after the nitro group in position 3 of compound **14** was reduced to the amine. We found out that the method described in ref. [13] was not working. In the method described in ref. [13] at first 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)**, acetic acid and acetic anhydride was brought to reflux and then zinc-dust was added. We always got a mixture of at least three compounds. All our separation attempts with chromatographic methods failed.

Compound **19** was characterized by ^{1}H-NMR. Comparison of the ^{1}H-NMR data of compound **19** and **14** showed that we found two more signals for compound **19** which came from the acetamido group. We found an additional signal at 2.24 ppm from the methyl protons and an additional signal at 9.73 ppm from the NH-amide. The signal for the NH-lactam of compound

19 shifted upfield due to the donor character of the acetamido group. It appeared at 11.74 ppm compared to 11.89 ppm for compound **14**.

Scheme 10



2.16 Synthesis of *N*-(4-chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (20)

As the direct sulfinylation in the 3-unsubstituted carbostyril **3** had a low selectivity and 4-methylbenzenesulfinyl chloride **(6)** which we needed as a reagent was not easy to store over a long period we decided to introduce p-toluenesulfinate in *N*-(4-chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(20)**. See chapter **2.3**.

N-(4-Chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(20)** was prepared from *N*-(4-hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(19)** in phosphoryl chloride in the presence of triethylamine. The use of triethylamine was already explained in chapter **2.11**. By the donor influence of the acetamido group in position 3 of compound **19** a selective mono-chlorination compared to **2.6** and **2.11** was possible. Only the hydroxy group

in position 4 of compound **19** was chlorinated to give directly compound **20** in a yield of 70 %.

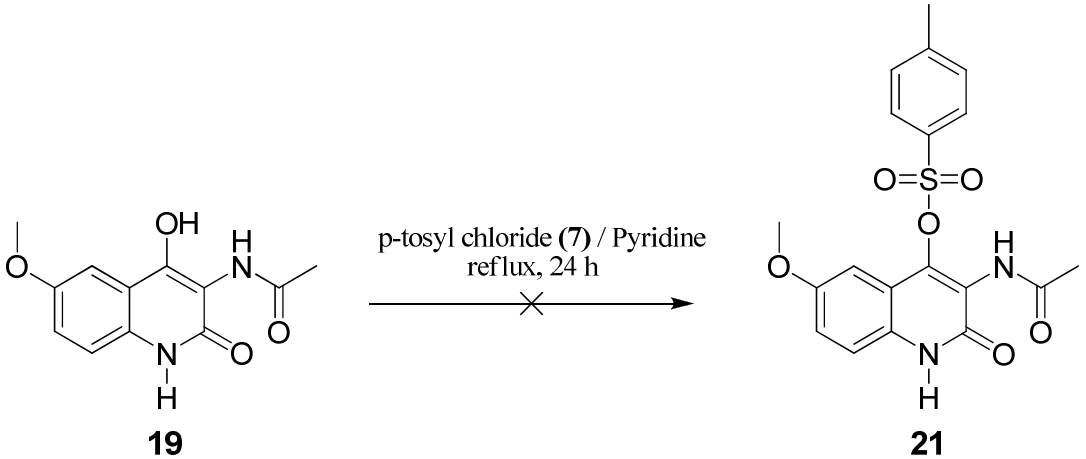
Compound **20** was characterized by ^{1}H-NMR. Comparison of the ^{1}H-NMR data of compound **19** and **20** showed that the signal for the N-H proton of compound **20** was shifted downfield. It appeared at 12.18 ppm compared to 11.74 ppm for compound **19** due to the influence of the chloro group.

2.17 Attempt of the introduction of p-toluenesulfonate in position 4 of compound (19)

As the direct sulfonation in the 3-unsubstituted carbostyril **3** had a sufficient selectivity we decided to introduce p-toluenesulfonate directly in acetylaminoquinolone **19**. See chapter **2.4**.

Attempts to synthesize 3-acetamido-6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzensulfonate **(21)** from *N*-(4-hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (**19)** and p-tosyl chloride **(7)** in dry pyridine failed. The reaction process was monitored by TLC. After no reaction could be detected, we increased the temperature, added p-tosyl chloride **(7)** and 4-(*N,N*-di-methyl)aminopyridine in excess to the reaction mixture to force the reaction. All our attempts failed. The purified and isolated product could be identified as starting material **19** by ^{1}H-NMR.

Scheme 11



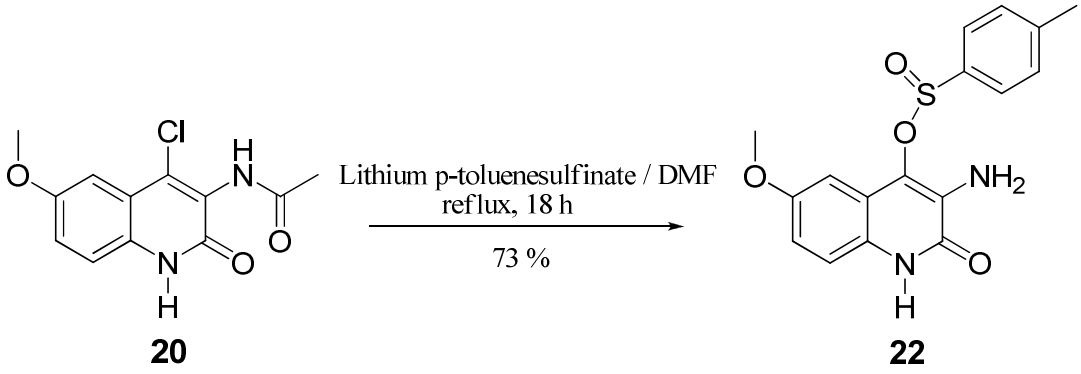
19 **21**

2.18 Synthesis of 3-amino-6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate (22)

Aim of the following synthesis was to get a 3-donor substituted sulfinate. Therefore p-toluenesulfinate was introduced in acetylaminoquinolone **20**.

3-Amino-6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate **(22)** was prepared from *N*-(4-chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(20)** and lithium p-toluenesulfinate in dry DMF. Surprisingly the acetamido group was reduced to the amine. We obtained the compound in a yield of 73 %. This compound showed excellent fluorescence properties. The strong fluorescence was even obvious by checking the TLC.

Scheme 12



20 **22**

The structural elucidation of compound **22** was based on spectral analyses. We found two sharp stretching bands at 3475 cm^{-1} and at 3360 cm^{-1} in the infrared spectrum which is typical for a primary amine. The strong lactam carbonyl stretching was observed in the infrared spectrum at 1670 cm^{-1}.

The ^{1}H-NMR spectrum of **22** showed two singlets at 2.33 ppm, and 3.66 ppm. They could be dedicated to the methyl protons and to the methoxy protons. Furthermore we found AA',BB'-pattern at 7.82 ppm (J = 8.4 Hz) and at 7.38 ppm (J = 8.1 Hz) which is typical for the para-substituted patterns of a benzene ring. H_A and $H_{A'}$ was shifted downfield at 7.82 ppm due to the influence of SO_2. We also found a signal for the NH-lactam at 12.27 ppm.

The ^{13}C-NMR spectrum showed 15 different peaks as expected. The peaks for the $C_{BB'}$ at 126.5 ppm and for the $C_{AA'}$ at 130.5 had twice the intensity as the other 13 peaks. The peak

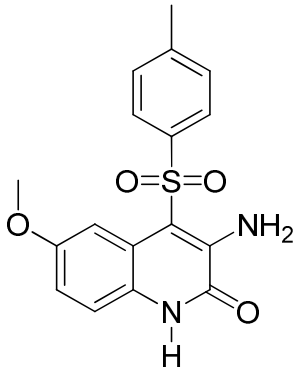
for the lactam carbon appeared at 156.2 ppm. The peak for the 3-O appeared at 106.2 ppm and the peak for the 6-O at appeared at 155.1 ppm.

The mass spectrum showed with API-ES negative mode a base peak with 100 %.

The findings were confirmed by elemental analyses.

2.18.1 Alternative structure for compound 22

Compound **22** could also have the following isomeric structure **22X**.



22X

See chapter **2.9.1**.

In this chapter we will just add deviations and amendments to **2.9.1**.

We found two characteristic bands in the IR spectra at 1278 cm^{-1} and at 1134 cm^{-1} which indicate sulfone [15].

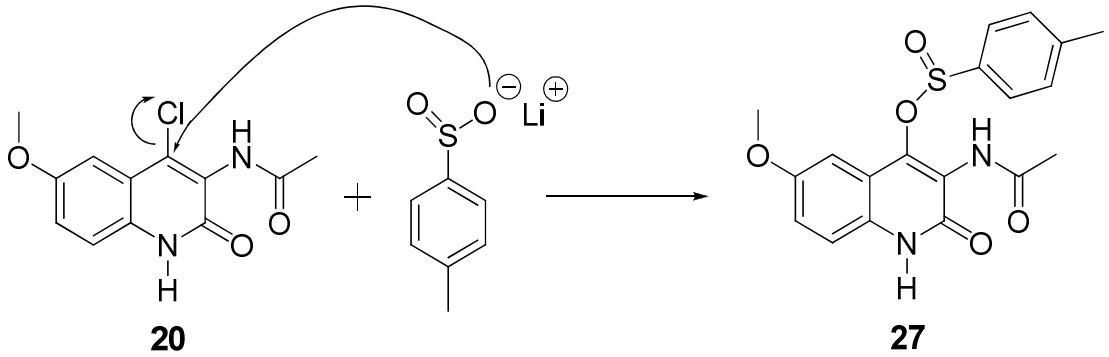
Compared to the compound described in chapter **2.9**, the compound described in chapter **2.16** still showed excellent fluorescence properties, but with lower extinction and emission wavelength.

A structure clarification will bring the X-ray crystallography which is in process.

2.18.2 Proposed mechanism for the formation of compound 22 or 22X

In the first step the sulfinated compound **27** was obtained by nucleophilic substitution with lithium p-toluenesulfinate. By this proposed mechanism, we assume that the substitution took place on the oxygen

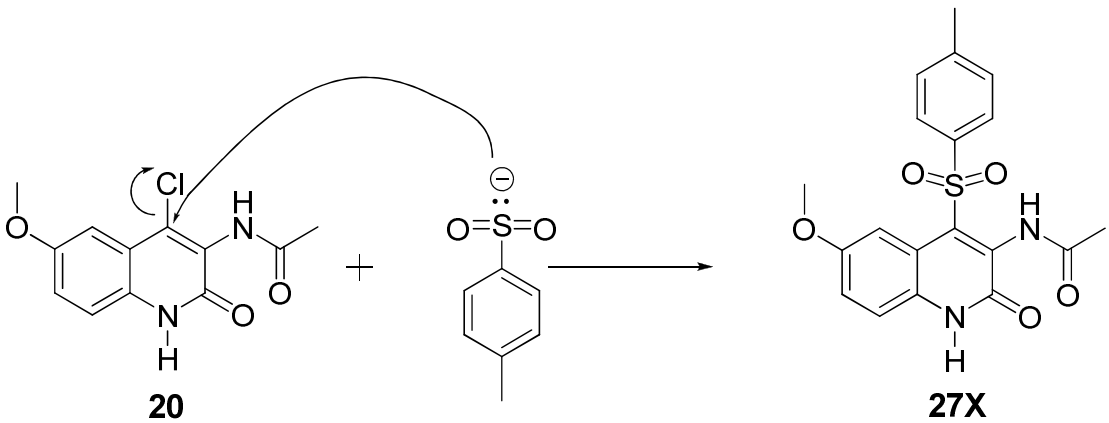
Scheme 13



20 **27**

In the other case the substitution took place on the sulphur as described in **Scheme 14**.

Scheme 14



20 **27X**

In the next steps the acetamido group of **27** or **27X** was reduced to the amine.

2.19 Attempts of the introduction of p-toluenesulfonate and p-toluenesulfinate in position 4 of compound 16 by using the appropriate sodium salt

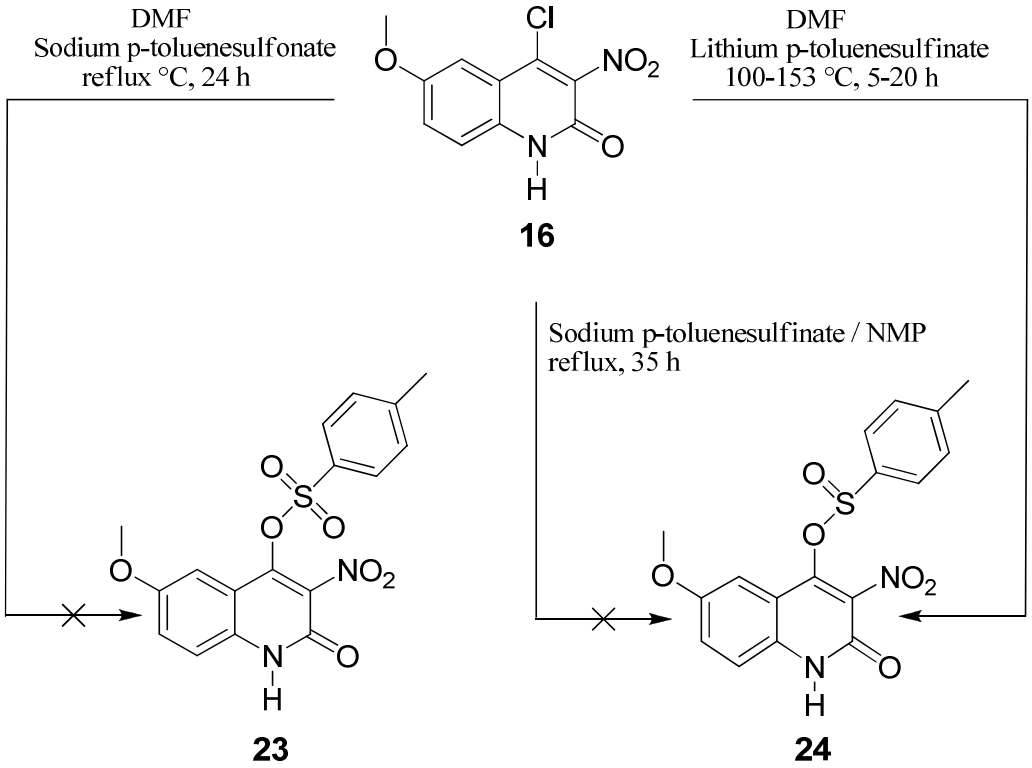
Aim of the following two syntheses was to get a 3-acceptor substituted sulfinate and sulfonate by using the appropriate sodium salt. Furthermore we wanted to show if the nitro group influence the reactivity of sodium p-toluenesulfonate and sodium p-toluenesulfinate.

Attempts to synthesize 6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfonate **(23)** and 6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate **(24)** by using the appropriate sodium salt according to ref. [6] failed. The reaction process was monitored by TLC. After no reaction could be detected, we increased the temperature, added the appropriate salt in excess and added 15-crown-5 to the reaction mixture to force the reaction. All these attempts failed. The purified and isolated product could be identified as starting material **16** by ^{1}H-NMR.

The introduction of p-toluenesulfinate by using the appropriate sodium salt was also performed in dry NMP instead of dry DMF to increase the reaction temperature. This attempt failed too. Still no reaction occurred.

Attempts to introduce p-toluenesulfonate and p-toluenesulfinate in position 4 of compound **16** by using the appropriate sodium salt are comparable to the synthesis already described chapter **2.8**. Therefore we can use the same explanations why it was not working.

Scheme 15



2.20 Introduction of p-toluenesulfinate in position 4 of compound 16

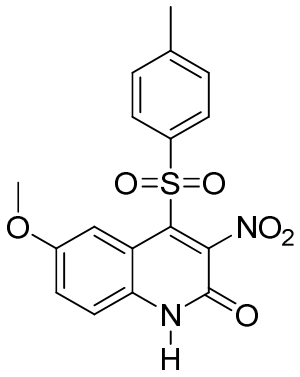
Aim of the following synthesis was to get a 3-acceptor substituted sulfinate. Therefore p-toluenesulfinate was introduced in 4-chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)**.

The introduction of p-toluenesulfinate was performed in dry DMF under reflux for 5 hours with lithium p-toluenesulfinate. We always got a mixture of 3 fluorescent compounds. The strong fluorescence of two of these compounds was even obvious by checking the TLC.
All our separation attempts with chromatographic methods failed because the R_f values were to close.

The introduction of p-toluenesulfinate by using the appropriate lithium salt was also performed in DMF at 100 °C for 20 hours in order to avoid byproducts. We still got 3 fluorescent compounds which could not be separated by dry-flash-column chromatography. It was not possible to perform the reaction at lower temperature because of the solubility of lithium p-toluenesulfinate.

2.20.1 Alternative structure for compound 24

Compound **24** could also have the following isomeric structure **24X**.



24X

See chapter **2.9.1**.

2.21 Synthesis of 1-(6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)pyridinium chloride (25)

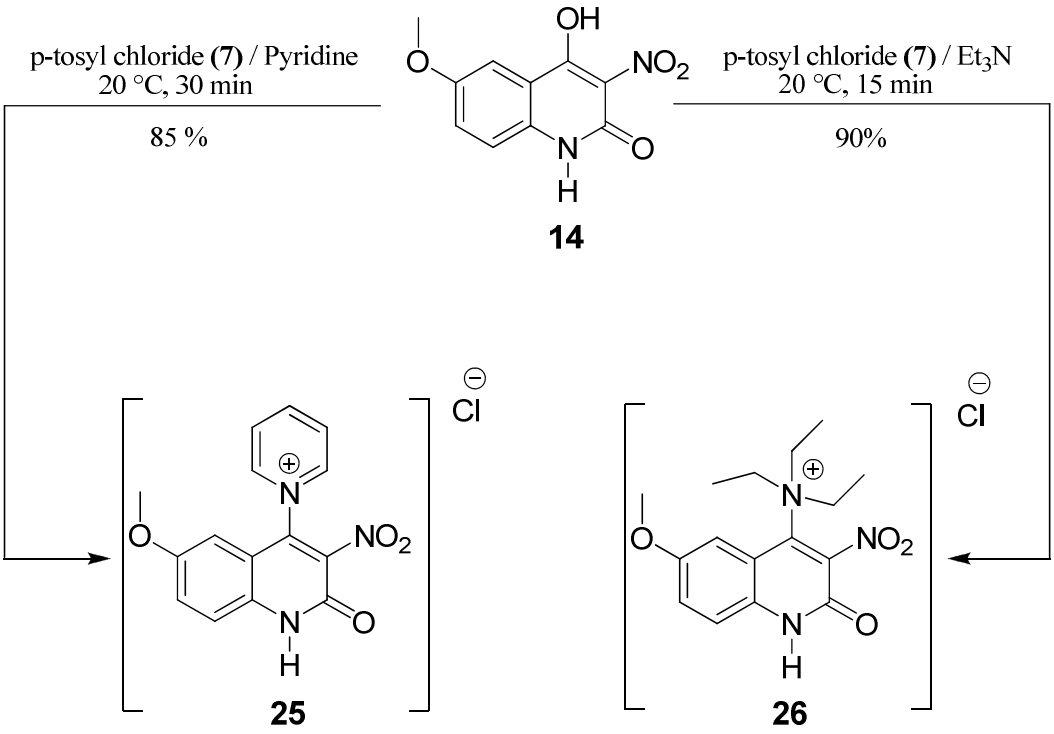
As the direct sulfonation in the 3-unsubstituted carbostyril **3** had a sufficient selectivity we decided to introduce p-toluenesulfonate directly into 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)**. See chapter **2.4**. Surprisingly we got a pyridinium chloride instead of a sulfonate.

1-(6-Methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)pyridinium chloride **(25)** was prepared from 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)** and p-tosyl chloride **(7)** in dry pyridine. The reaction was also performed without 4-(*N,N*-di-methyl)aminopyridine as a basic catalyst. There was no difference in the reaction time.
This is a fast and efficient reaction to introduce nucleophiles. The strong reactivity of compound **14** compared to compound **3** is caused by the nitro group in position 3. To prove our postulate of a fast and efficient reaction to introduce nucleophiles, the reaction was also performed with triethylamine as described in chapter **2.22**.

The structural elucidation of compound **25** was based on spectral analyses. The strong lactam carbonyl stretching was observed in the infrared spectrum at 1669 cm^{-1}.
The ^{1}H-NMR spectrum of **25** showed a singlet at 3.70 ppm for the methoxy protons and a singlet at 13.66 ppm for the NH proton. Furthermore we found AA',BB',C-pattern at 9.50 ppm (J = 5.4 Hz), at 8.57 ppm (J = 7.5 Hz) and at 9.06 ppm (J = 8.1 Hz). The doublet at 9.50 ppm and the triplet at 8.57 ppm had twice the intensity compared to the triplet at 9.06 ppm which is typical for the patterns of pyridine.
The mass spectrum showed with API-ES positive mode a base peak with 100 %.

The findings could not been verified by elemental analyses. Compound **25** is a salt and therefore not easy to dry what influence the elemental analyses.

Scheme 16



2.22 Synthesis of N,N,N-triethyl-6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-aminium chloride (26)

To prove the postulate decribed in chapter **2.21** the reaction was also performed with triethylamine.

N,N,N-Triethyl-6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-aminium salt **(26)** was prepared from 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)** and p-tosyl chloride **(7)** in dry triethylamine. We got compound **26** in 15 minutes at room temperature in a yield of 90 % what proved the postulate from chapter **2.21** of a fast and efficient reaction to introduce nucleophiles.

The structural elucidation of compound **26** was based on spectral analyses. The strong lactam carbonyl stretching was observed in the infrared spectrum at 1664 cm^{-1}.
The ^{1}H-NMR spectrum of **25** showed a triplet at 1.19 ppm and a multiplet between 3.04-3.10 ppm. The ratio between the multipet and the triplet was 2:3, what is typical for triethylamine.

The mass spectrum showed with API-ES negative mode a base peak at 235 m / z what indicate that triethylammonium was substituted by the hydroxy group during the measurement.

The findings could not been verified by elemental analyses. Compound **26** is a salt and therefore not easy to dry what influence the elemental analyses.

3. Fluorescence properties of the new carbostyrils

3.1 Introduction[24-26]

Fluorescence is the emission of light by a substance in an excited state. We can divide the fluorescence process in three-stages.

1. Excitation: The fluorophore absorbs a photon and creates an excited electronic singlet state.
2. Excited-state Lifetime: The fluorophore undergoes during this time conformational changes and lots of different interactions with his environment are possible.
3. Fluorescence emission: The fluorophore emits a photon and return to his ground state.

In most cases the emitted photon has less energy than the absorbed one. This is caused by vibronic relaxation during the excited-state lifetime. The difference between the positions of the band maxima of the absorption and emission spectra is called Stokes shift. The Stokes shift allows us to detect emission photons isolated from the excitation photons. Therefore it is fundamental for the sensitivity.

Through interactions with the environment during the excited-state lifetime not all excited molecules return to the ground state by fluorescence emission. The quantum yield is the ratio between emitted to absorbed photons. The quantum yield gives the efficiency of the fluorescence process.

$$\Phi = \frac{Number\ of\ Photons\ emitted}{Number\ of\ Photons\ absorbed}$$

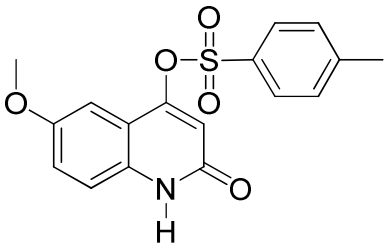
We are looking for compounds which absorb light of less energy in order that they can be excited by diodes. Furthermore they should have a large Stokes shift and a high quantum yield in order to make it easier to measure them.

3.2 Results and Discussion

About 140 structures were already synthesized and calculated. Recently we have shown that carbostyrils can be improved in terms of absorption, emission and quantum yield using the push pull concept [27] by a systematic investigation of substituents. Two donor substituents were used at position 6 and 7, and an acceptor substituent at position 4. The influence of positions 4, 6 and 7 on the fluorescence is therefore already studied intensively. Aim of this work was to show the influence of position 3 on the fluorescence and to investigate new electron withdrawing acceptors on position 4.

3.2.1 Summary of photophysical properties of the new carbostyrils

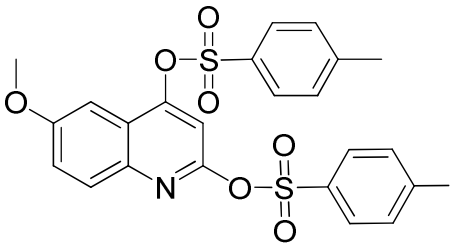
6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzensulfonate **(9)**



solvent	Ethanol
excitation coefficient	5290
λ_{max} / UV-Vis (nm)	357
λ_{max} / em. (nm)	425
Φ	0.023

Table 3: Photophysical properties of compound **9**

6-Methoxyquinoline-2,4-diyl bis(4-methylbenzenesulfonate) **(10)**

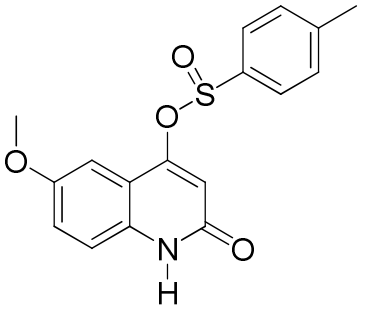


solvent	Ethanol
excitation coefficient	5890
λ_{max} / UV-Vis (nm)	350
λ_{max} / em. (nm)	420
Φ	0.021

Table 4: Photophysical properties of compound **10**

6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate **(8)**

or 6-methoxy-4-[(4-methylphenyl)sulfonyl]quinolin-2(1H)-one **(8X)**

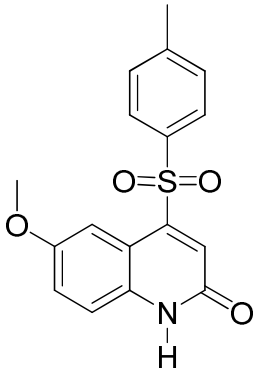


8

solvent	DMSO
excitation coefficient	7750
λ_{max} / UV-Vis (nm)	385
λ_{max} / em. (nm)	490
Φ	0.180

Table 5: Photophysical properties of compound **8** or **8X**

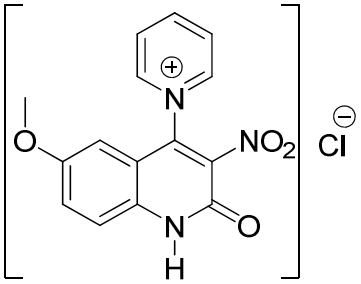
Compound **8** could also have the following isomeric structure **8X**.



8X

See chapter **2.9.1**

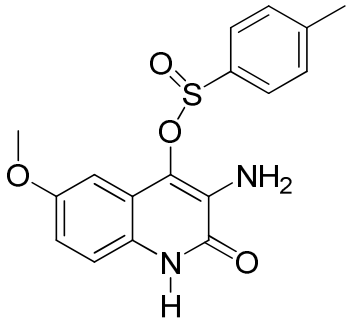
1-(6-Methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)pyridinium chloride **(25)**



Solvent	Ethanol
excitation coefficient	3150
λ_{max} / UV-Vis (nm)	384
λ_{max} / em. (nm)	460
Φ	0.001

Table 6: Photophysical properties of compound **25**

3-Amino-6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate **(22)**
or 3-amino-6-methoxy-4-[(4-methylphenyl)sulfonyl]quinolin-2(1H)-one **(22X)**

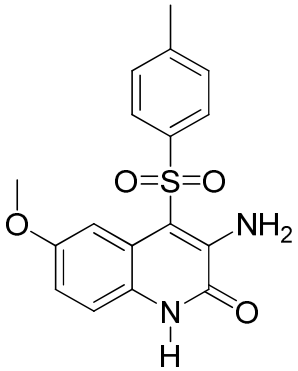


22

Solvent	Ethanol
excitation coefficient	14500
λ_{max} / UV-Vis (nm)	356
λ_{max} / em. (nm)	455
Φ	0.100

Table 7: Photophysical properties of compound **22** or **22X**

Compound **22** could also have the following isomeric structure **22X**.



22X

See chapter **2.9.1**.

3.2.2 Comparison of λ_{max} / UV-Vis in different solvents

Compound	**λ_{max} / UV-Vis (nm) DMSO**	**λ_{max} / UV-Vis (nm) Ethanol**
9	357	357
10	350	356
22 or 22X	354	356
25	384	390

Table 8: Comparison of λ_{max} / UV-Vis in different solvents

All compounds showed similar absorption spectra in DMSO and Ethanol, which means that the spectra showed no solvent dependence in reference to absorption.

3.2.3 Electronic spectra

The following excitation and emission spectra are all normalized.

Figure 5 shows excitation and emission spectra of the mono tosylated carbostyril **9** vs. quinoline-tosylate **10** in ethanol. They show similar properties in reference to all photophysical properties. The UV-Vis spectra shows absorption maximum at 357 nm for compound **9** and at 350 nm for compound **10**. The excitation coefficient is also similar with ε = 5290 for compound **9** and ε = 5890 for compound **10**. They have both a Stokes shift of 60 nm and a weak quantum yield with about 2 % (**Table 3** and **4**). 6-Methoxyquinoline-2,4-diyl bis(4-methylbenzenesulfonate) **(10)** is a new kind of fluorescence compound which is not described yet, comparable to the mono tosylated carbostyril **9**.

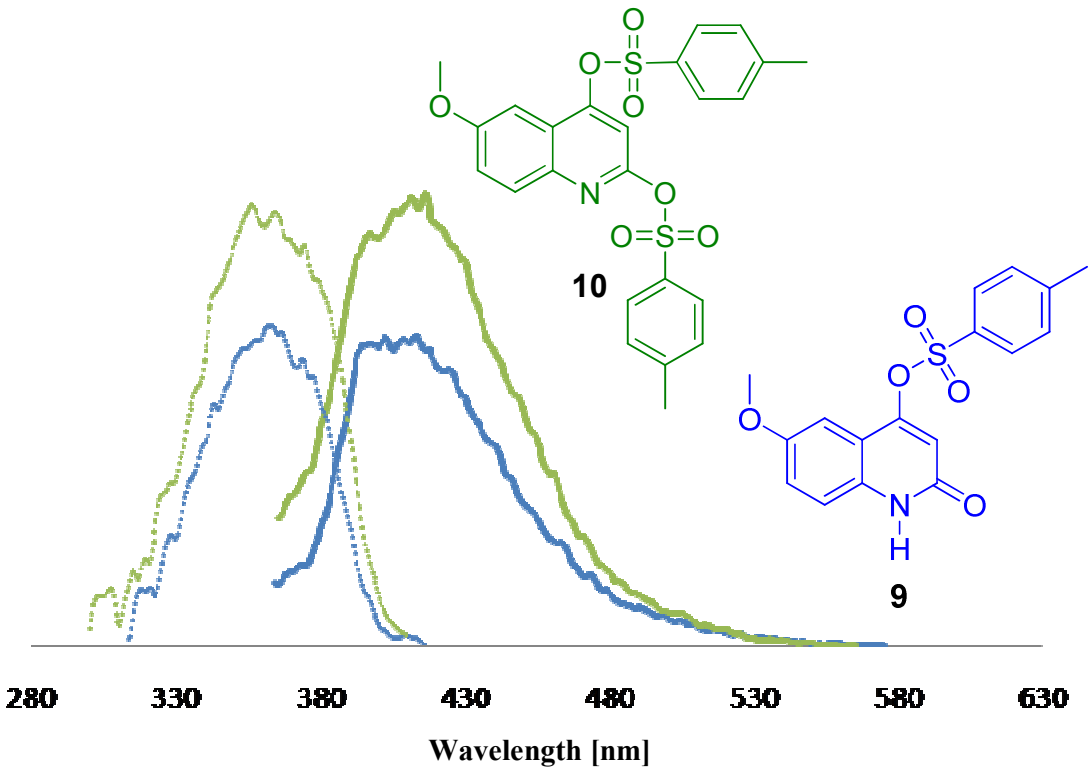


Figure 5: Excitation (dotted line) and emission (continuous line) spectra for carbostyril **9** (blue curves) and quinoline **10** (green curves)

Figure 6 shows excitation and emission spectra of tosylate **9** vs. sulfinate **8** or sulfone **8X**. Compared to tosylate **9** the electronic spectra of sulfinate **8** or sulfone **8X** show higher

absorption and emission wavelength. The absorption maximum for **8** or **8X** shifted up to 385 nm and is therefore 35 nm higher. The Stokes shift is 105 nm so 45 nm larger as for tosylate **9**. Also the quantum yield is with Φ = 0.180 10 times more. Sulfinate **8** or sulfone **8X** show better photophysical properties compared to tosylate **9** in all aspects (**Table 3** and **5**).

The emission wavelength of sulfinate **8** or sulfon **8X** is already in the green-yellow part of the spectra. There are not many compounds emitting in this part of the spectra. Sulfinate **8** or sulfone **8X** is at the moment the best 3-unsubstituted carbostyril in terms of absorption, emission maxima and the Stokes shift.

We conclude that the sulfinate/sulfone-group in position 4 of compound **8** or **8X** is a much stronger electron withdrawing group than the tosylate group. This aspect indicates the generation of compound **8X**, because sulfones are better withdrawing groups than sulfinates.

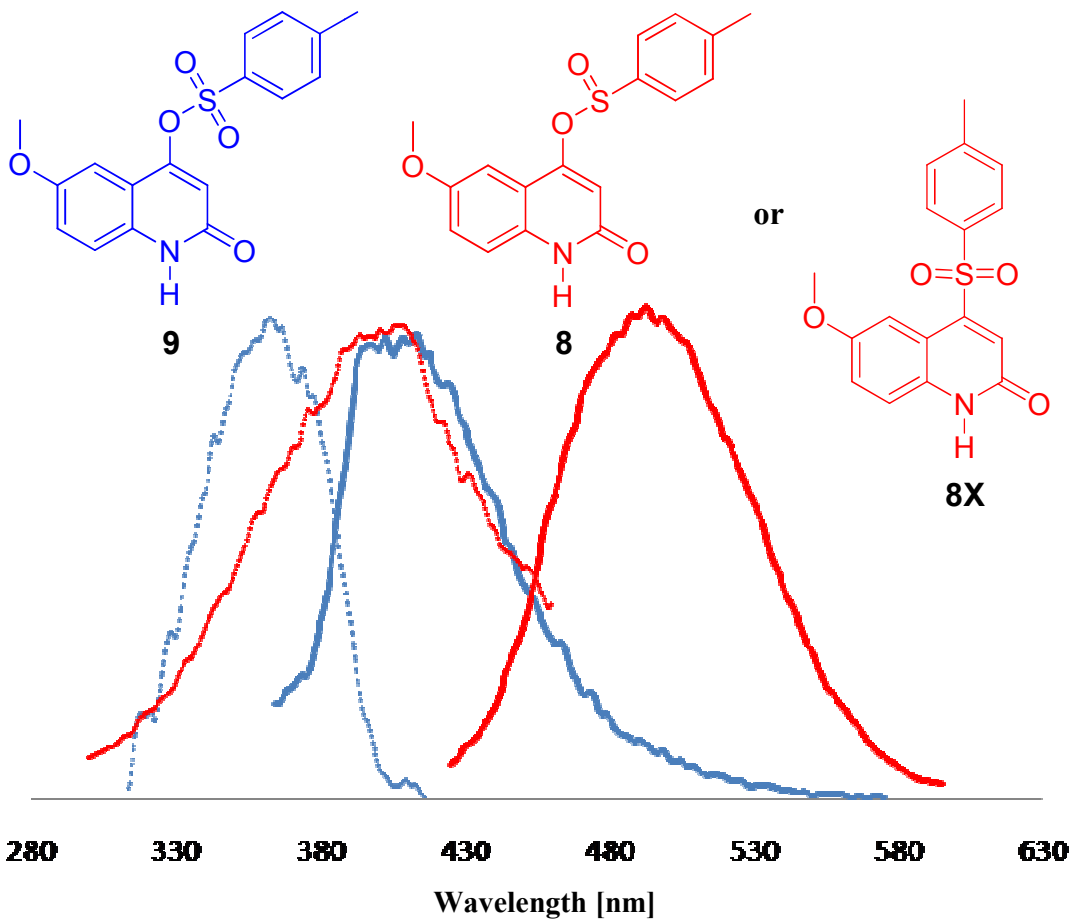


Figure 6: Excitation (dotted line) and emission (continuous line) spectra for carbostyril **9** (blue curves) and carbostyril **8** or **8X** (red curves)

Figure 7 shows excitation and emission spectra of 3-unsubstituted carbostyril **8** or **8X** vs. amino-substituted carbostyril **22** or **22X**. If we compare the spectra of the 3-unsubstituted carbosyril **8** or **8X** with those of the amino-substituted carbostyril **22** or **22X** we see that the amino-substituted has a lower extinction and emission wavelength. The absorption maximum for **22** or **22X** shifted down to 356 nm and is therefore 30 nm lower. The Stokes shift is with 99 nm still large.

A donor in position 3 seems to lower the extinction and emission wavelength but without an influence on the Stokes shift.

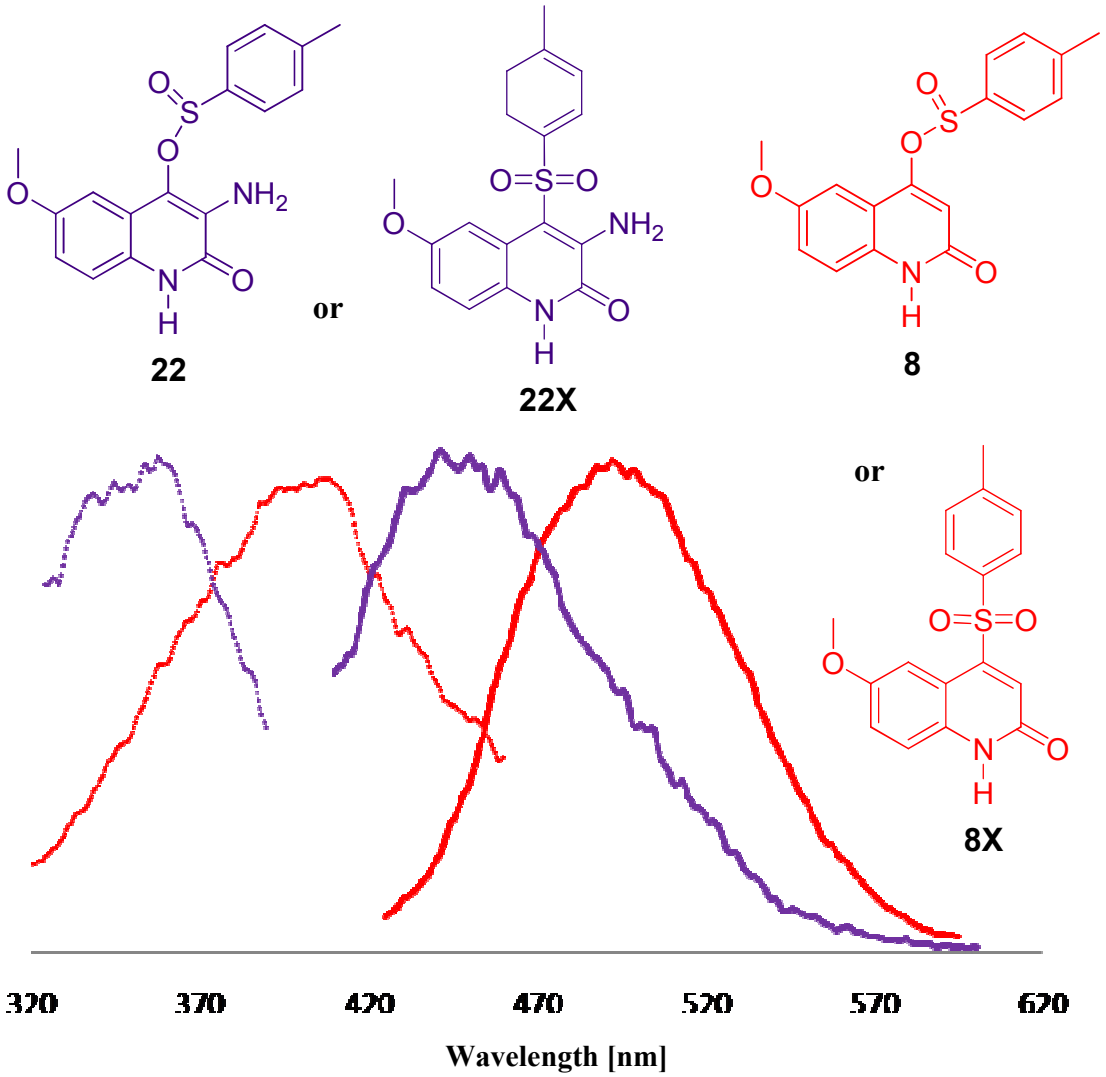


Figure 7: Excitation (dotted line) and emission (continuous line) spectra for carbostyril **22** or **22X** (purple curves) and carbostyril **8** or **8X** (red curves)

Figure 8 shows the structure of already known carbostyrils **27**, **28** and **29** which we needed for a complete consideration.

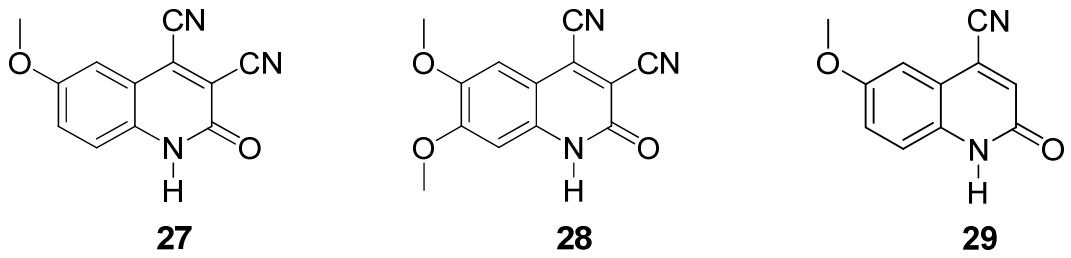


Figure 8: structure of already known carbostyrils **27**, **28** and **29**

Comparison of the photophysical properties of compound **29** and **27** showed that a second cyano group in position 3 increased the absorption and emission wavelength dramatically.
Comparison of the photophysical properties of compound **27** and **28** showed that a second methoxy group in position 7 shifted absorption and emission to shorter wavelength, but increased the quantum yield significantly. An additional methoxy group in position 7 is needed to improve the fluorescence quantum yield.[13]

3.2.4 Conclusion

Quinoline-tosylate **10** is a new kind of fluorescence compound which is not described yet, with similar absorption and emission wavelength like carbostyrils. We found out that the substitution of the chloro group by using lithium p-toluenesulfinate in DMF gave fluorescent compounds with very good photophysical properties. The resulting sulfinate or sulfone is at the moment the best known electron withdrawing group to improve the fluorescence, even better than the cyano group [13]. Therefore compound **8** or **8X** is at the moment the best 3-unsubstituted carbostyril in terms of absorption, emission maxima and the Stokes shift. The 3-donor substituted derivate of **8** or **8X** had lower absorption and emission wavelength. Comparison of compound **27** and **29** showed that a second cyano group in position 3 increased the absorption and emission wavelength dramatically. One can conclude that generally an acceptor in position 3 increases the absorption and emission wavelength and a donor makes the opposite.

At the moment compound **27** shows best fluorescence properties in terms of absorption and emission wavelength. To get a compound with even better fluorescence properties we should synthesize next the 3,4-sulfinated or 3,4-sulfonated analog.

3.3 Measurement of the fluorescence and photophysical properties of the new carbostyrils

3.3.1 Preparation of the stock solution

We prepared for each sample 1 x 10^{-2} M stock solution in DMSO. Therefore we dissolved 1-2 mg of each compound in the appropriate volume DMSO. The purity of the compound was confirmed by elemental analysis.

3.3.2 UV / Vis spectra

UV / Vis spectra were recorded with a Shimadzu UV-2101 PC UV / Vis scanning spectrophotometer. The concentration of the samples was 1 x 10^{-4} M. Therefore we added 990 µL of the corresponding solvent to 10 µL of each stock solution. We used 1 cm PS cells from Brand.

3.3.3 Fluorescence spectra

Fluorescence spectra (emission and excitation) were recorded with a Perkin-Elmer LS50B luminescence spectrometer. The concentration of the samples was 1 x 10^{-5} or 1 x 10^{-6} M. Therefore the UV / Vis solution was diluted 10 or 100 times with the corresponding solvent. We used 1 cm PS cells from Brand. Generally slit width was 5 nm. For strong fluorescence values we used 3 nm slit width.

To calculate the quantum yields the area (integration) of the uncorrected emission spectra was compared to the area of the known quantum yield of 6,7-dimethoxy-4-trifluoromethylcarbostyril in DMSO (λ_{exc} = 368 nm, λ_{em} = 438 nm, Φ = 0.41) [23]. The

emission and excitation maxima of the reference should be close to the investigated substances.

For the calculation we used the following equation:

$$\Phi_S = \Phi_{ref} \times \frac{A_S}{A_{ref}} \times \frac{\varepsilon_{ref} \times c_{ref}}{\varepsilon_S \times c_S}$$

Meaning of the letters:

Subscript *S*: data of the sample
Subscript *ref*: data of the reference
c: molar concentration (mol / L)
A: absorbance data at UV / Vis maxima
ε: excitation coefficient

If the samples were not measured in the same solvent like the reference, the quantum yield was corrected by the following factor.

$$f = \left(\frac{n_{ref}}{n_{new}}\right)^2$$

n: refractive index

standard in:	**refractive index**	***f*** **EtOH standard**	***f*** **H_2O standard**	***f*** **DMSO standard**
EtOH	1.361	1.000	0.959	1.194
H_2O	1.333	1.042	1.000	1.244
DMSO	1.487	0.838	0.804	1.000

Table 9: correction factors for quantum yield calculations

4. Experimental Part

4.1 General Remarks

4.1.1 Melting points:

Melting points were determined in open capillary tubes with a Stuart SMP3 apparatus.

4.1.2 NMR spectra

NMR spectra were recorded with a Bruker Avance III NMR spectrometer (300 MHz ^{1}H, 75 MHz ^{13}C) and the deuterated solvents were used as internal standard. The δ values are reported in parts per million (ppm) and the coupling constants J in Hertz (Hz).

Definition: s = singlet

d = doublet

dd = doublet of doublets

t = triplet

q = quadruplet

m = multiplet

4.1.3 Infrared spectra

Infrared spectra were recorded with a Bruker Alpha-P with attenuated total reflection (ATR) measurement and are reported in terms of frequency of absorption (ν, cm^{-1}).

Definition: s = strong

m = medium

w = weak

br = broad

sh = shoulder

4.1.4 Elemental analyses

Elemental analyses were performed at the University of Vienna (Microanalytical laboratory).

4.1.5 Mass spectra

Mass spectra were recorded with a HP 1100 LC / MSD (positive or negative APCI ion source, 50-200 V, nitrogen or positive or negative ESI ion source, 50-200 V, nitrogen). The data are reported as m / z.

4.1.6 Thin-layer chromatography

Thin-layer chromatography was carried out on 0.2-mm silica gel plates (F-254 Merck). They were detected by UV light (254 and 360 nm) and are expressed in reference frontal (R_f).

4.1.7 Column Chromatography

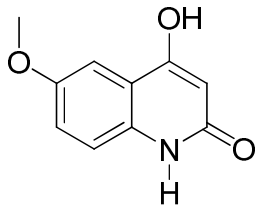
Column chromatography was carried out on silica gel 60 H with the dry flash column chromatography technique [ex-1].

4.1.8 Solvents

Common commercially available solvents were used without further purification or prepared as shown in the experimental part.
DMF was dried by using molecular sieve 4 Å (dynamic dehydration).
Pyridine was dried by using molecular sieve 4 Å (dynamic dehydration).

4.2 Working procedures

4.2.1 4-Hydroxy-6-methoxyquinolin-2(1*H*)-one (3)



To phosphoryl chloride (24 mL, 261 mmol, 1.7 eq) in a 1000 mL Erlenmeyer flask, a mixture of p-anisidine (**1:** 18.90 g, 154 mmol, 1.0 eq) and dry malonic acid (**2:** 23.40 g, 225 mmol, 1.5 eq) was added. The mixture was heated to 95 °C under stirring with a spatula, until a vigorous reaction occurred. Then the reaction mixture was heated to 115 °C and stirred for 90 minutes. After cooling to room temperature, ice / water (2000 mL) and sodium hydroxide pellets (80 g) were added. Then the mixture was heated to 60 °C for 20 minutes. The remaining insoluble solid [mainly *N,N'*-bis(4-mehoxyphenyl)malonamide **(4)**] was filtered by suction.

The alkaline filtrate was brought to pH = 1-2 with concentrated hydrochloric acid. The precipitate was filtered by suction and washed with water. To remove p-anisidine the residue was dissolved in methanol (100 mL) for 15 minutes under reflux. After cooling to room temperature the remaining insoluble solid mainly p-anisidine was filtered by suction.

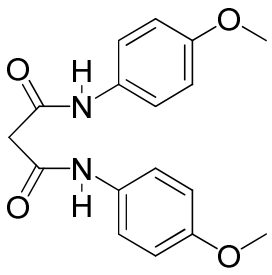
The methanol filtrate was poured onto ice / water (200 mL). The precipitate was filtered by suction and washed with water to afford 4-hydroxy-6-methoxyquinolin-2(1*H*)-one **(3)**. The yield was 20.60 g (70 %), yellow prisms, mp 316-321 °C (methanol), lit. mp 298-328 °C [ex-2, ex-3, ex-13].

R_f = 0.21 (chloroform / ethanol 9:1)

^{1}H-NMR (DMSO-d_6): δ = 3.77 (s, 3 H, CH_3O), 5.74 (s, 1 H, 3-H), 7.12 (dd, J = 9.0 + 2.6 Hz, 1 H, 7-H), 7.19 (d, J = 8.7 Hz, 1 H, 8-H), 7.20 (d, J = 2.7 Hz, 1 H, 5-H), 11.10 (s, 1 H, NH), 11.30 (s, 1 H, OH)

$C_{10}H_9NO_3$ (191.18)

4.2.2 *N,N'*-Bis(4-methoxyphenyl)malonamide (4)

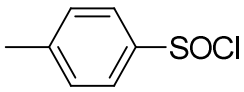


Compound **4** was obtained during the work-up of compound **3**. The remaining in alkaline solution insoluble solid was recrystallized from acetic acid / methanol (1:3) to afford *N,N'*-bis(4-mehoxyphenyl)malonamide **(4).** The yield was 7.34 g (15 %), grey prisms, mp 230-234 °C (acetic acid / methanol 1:3), lit. mp 226-240 °C [ex-2a-c, ex-4, ex-5, ex-6, ex-7, ex-13].
R_f = 0.85 (chloroform / acetone 3:7)

^{1}H-NMR (DMSO-d_6): δ = 3.39 (s, 2 H, CH_2), 3.72 (s, 6 H, 2 OCH_3), 6.87 (d, J = 9.04 Hz, 4 H, Ar-H), 7.50 (d, J = 9.04 Hz, 4 H, Ar-H), 10.02 (s, 1 H, NH)

$C_{17}H_{18}N_2O_4$ (314.34)

4.2.3 4-Methylbenzenesulfinyl chloride (6)



4-Methylbenzenesulfinyl chloride **(6)** was prepared according to ref. [ex-8]. To redistilled thionyl chloride (110 mL, 1.52 mol, 7.24 eq) *p*-toluenesulfinic acid sodium salt hydrate **(5)** (42.2 g, 0.21 mol, 1 eq), was added in portions at room temperature over a period of 15 minutes. A vigorous reaction occurred and the temperature rose to 50 °C. After the first portions of the sulfinate were added, the temperature soon dropped to approximately 0 C°. The reaction mixture was protected by a calcium chloride trying tube and kept at room temperature for 2.5 hours. The excess amount of thionyl chloride was removed by distillation under reduced pressure, followed by three triturations with anhydrous diethyl ether (50, 30 and 30 mL) which were decanted from the inorganic residue. The diethyl ether was removed under reduced pressure from the mixture and the residue gave the sulfinyl chloride as straw-

yellow oil. The yield was 25.5 g (70 %), b.p. 125 °C (5 Torr), lit. b.p. 40-120 °C (0.005-5 Torr) [ex-8, ex-9].

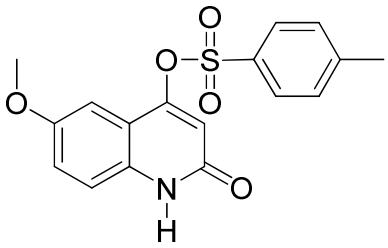
IR (ATR-measurement): 3056 (w), 2923 (w), 1592 (m), 1175 (s)

^{1}H-NMR ($CDCl_3$): δ = 2.48 (s, 3 H, CH_3), 7.40 (d, J = 8.0 Hz, 2 $H_{BB'}$ Ar-H), 7.77 (d, J = 8.3 Hz, 2 $H_{AA'}$ Ar-H)

MS (API-ES neg): m / z (%) = 173 (30, M - 1), 171 (100, M - 3)

C_7H_7ClOS (174.65)

4.2.4 6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzensulfonate (9)



6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzensulfonate **(9)** was prepared according to ref. [ex-10]. To dry pyridine (50 mL) a mixture of 4-hydroxy-6-methoxyquinolin-2(1*H*)-one (**3:** 1.5 g, 7.85 mmol, 1 eq), p-tosyl chloride (**7:** 2.2 g, 11.54 mmol, 1.5 eq) and 4-(*N,N*-dimethyl)aminopyridine (50 mg) was added. The mixture was stirred for 30 hours at 20 °C. Then the mixture was poured into ice / water (100 mL), the solid was filtered by suction and washed with water. The solid was purified in a Soxhlet extractor with cyclohexane to afford compound **9** as remaining insoluble solid and compound **10** dissolved in cyclohexane. The yield was 1.08 g (40 %) of colorless prisms. The melting point was 226-229 °C (dioxane).

R_f = 0.54 (chloroform / ethanol 9:1)

IR (ATR-measurement): 3153 (w), 3002 (m), 2853 (m), 2831(m), 2360 (m), 2342 (m, sh), 1650 (s), 1620 (s, sh), 1495 (s)

^{1}H-NMR (DMSO-d_6): δ = 2.40 (s, 3 H, CH_3), 3.68 (s, 3 H, CH_3O), 6.28 (s, 1 H, 3-H), 6.80 (d, J = 2.4 Hz, 1 H, 5-H), 7.19 (dd, J = 9.1 + 2.6 Hz, 1 H, 7-H), 7.25 (d, J = 9.0 Hz, 1 H, 8-H), 7.48 (d, J = 8.2 Hz, 2 $H_{BB'}$ Ar-H), 7.92 (d, J = 8.3 Hz, 2 $H_{AA'}$ Ar-H), 11.95 (s, 1 H, NH)

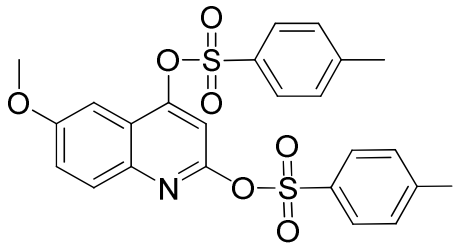
^{13}C-NMR (DMSO-d_6): δ = 21.6 (CH_3), 55.8 (CH_3O), 103.6 (3-C), 112.5 (Ar-C), 115.0 (Ar-C), 117.6 (Ar-C), 122.0 (Ar-C), 128.8 (2 x Ar-$C_{BB'}$), 131.0 (2 x Ar-$C_{AA'}$), 131.6 (Ar-C), 134.0 (Ar-C), 147.1 (Ar-C), 154.5 and 154.8 (C-4 and C-6), 161.5 (lactam-C=O)

MS (APCI- pos): m / z (%) = 347 (20, M + 2), 346 (100, M + 1), 192 (30, M - 153)
MS (APCI- neg): m / z (%) = 345 (20, M), 344 (100, M - 1), 190 (90, M - 155)

$C_{17}H_{15}NO_5S$ (345.37)

Calcd:	C 59.09,	H 4.05,	N 4.03
Found:	C 59.03,	H 3.99,	N 4.02

4.2.5 6-Methoxyquinoline-2,4-diyl bis(4-methylbenzenesulfonate) (10)



Compound **10** was obtained during the work-up of compound **9**. The filtrate of the Soxhlet-extraction was cooled to room temperature and filtered by suction to afford 6-methoxyquinoline-2,4-diyl bis(4-methylbenzenesulfonate) **(10)**. The yield was 0.67 g (17 %) of colorless prisms. The melting point was 130-131 °C (cyclohexane).

R_f = 0.85 (chloroform / aceton 7:3)

IR (ATR-measurement): 3081 (w), 2921 (w), 1920 (w), 1597 (m), 1577 (w)

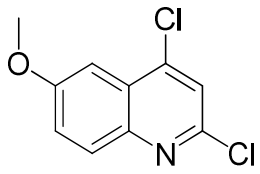
^{1}H-NMR (DMSO-d_6): δ = 2.38 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3), 3.75 (s, 3 H, CH_3O), 6.91 (d, J = 2.8 Hz, 1 H, 5-H), 7.18 (s, 1 H, 3-H), 7.42-7.46 (m, 3 H, Ar-H), 7.50 (d, J = 8.0 Hz, 2 $H_{DD'}$ Ar-H), 7.73 (d, J = 9.2 Hz, 1 H, 8-H), 7.84 (d, J = 8.3 Hz, 2 $H_{AA'}$ Ar-H), 7.93 (d, J = 8.3 Hz, 2 $H_{CC'}$ Ar-H)

MS (APCI- pos): m / z (%) = 500 (100, M + 1)

$C_{24}H_{21}NO_7S_2$ (499.56)

Calcd:	C 57.94,	H 3.94,	N 2.83
Found:	C 57.76,	H 3.93,	N 2.81

4.2.6 2,4-Dichloro-6-methoxyquinoline (11)



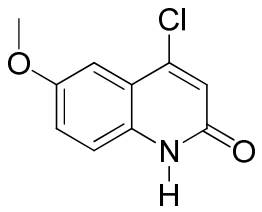
To phosphoryl chloride (50 mL) 4-hydroxy-6-methoxyquinolin-2(1*H*)-one (**3:** 4.20 g, 22 mmol, 1.0 eq), was added. The mixture was heated to reflux under stirring for 8 hours. The excess amount of phosphoryl chloride was removed under reduced pressure. After cooling to room temperature, the residue was poured into ice / water (600 mL) and brought to pH = 4-6 with aqueous sodium hydroxide (5 M). The residue was filtered by suction and washed with water. The remaining solid was recrystallized from ethanol to afford 2,4-dichloro-6-methoxyquinoline **(11)**. The yield was 3.98 g (80 %), brown prisms, mp 172-174 °C (ethanol), lit. mp 130-177 °C [ex-11, ex-13].

R_f = 0.95 (chloroform / aceton 3:7)

^{1}H-NMR (DMSO-d_6): δ = 3.96 (s, 3 H, CH_3O), 7.43 (d, J = 2.8, 1 H, 5-H), 7.55 (dd, J = 9.2 + 2.8 Hz, 1 H, 7-H), 7.93 (s, 1 H, 3-H), 7.94 (d, J = 9.2 Hz, 1 H, 8-H)

$C_{10}H_7Cl_2NO$ (228.07)

4.2.7 4-Chloro-6-methoxyquinolin-2(1*H*)-one (12)



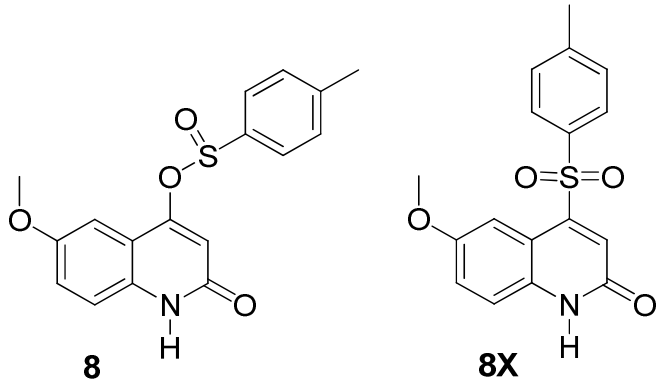
To ethanol (120 mL) 2,4-dichloro-6-methoxyquinoline (**11:** 5.1 g, 22.4 mmol, 1.0 eq) and 70 % methanesulfonic acid (9.6 mL, 148 mmol, 6.6 eq) was added. The mixture was heated under reflux for 40 hours. After cooling to room temperature the mixture was poured onto ice / water (150 mL) and brought to pH = 4-6 with aqueous sodium hydroxide (2 M). The precipitate was filtered by suction and washed with water. The remaining solid was recrystallized from toluene to afford 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12)**. The yield was 4.22 g (88 %), brown prisms, mp 298-302 °C (toluene), lit. mp 265-300 °C [ex-12, ex-13].

R_f = 0.50 (chloroform / aceton 3:7)

^{1}H-NMR (DMSO-d_6): δ = 3.83 (s, 3 H, CH_3O), 6.82 (s, 1 H, 3-H), 7.25-7.36 (m, 3 H, Ar-H), 11.96 (s, 1 H, NH)

$C_{10}H_8ClNO_2$ (209.63)

4.2.8 6-Methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate (8) or 6-methoxy-4-[(4-methylphenyl)sulfonyl]quinolin-2(1H)-one (8X)



8 **8X**

To dry DMF (15 mL) a mixture of 4-chloro-6-methoxyquinolin-2(1*H*)-one **(12:** 350 mg, 1.7 mmol, 1 eq) and lithium p-toluenesulfinate (350 mg, 2.2 mmol, 1.5 eq) was added. The reaction mixture was heated under reflux for 15 hours. The resulting solution was cooled to room temperature and poured into ice / water (50 mL). The precipitate was filtered by suction and washed with water. The residue was dissolved in dioxane (50 mL) for 15 minutes under reflux. After cooling to room temperature the remaining insoluble solid was filtered by suction.

The dioxane filtrate was poured onto ice / water (200 mL). The precipitate was filtered by suction and washed with water to afford compound **8/8X**. The yield was 470 mg (84 %), yellow prisms, mp 257-261 C° (dioxane), lit. mp 255-258 °C [ex-13].

R_f= 0.80 (chloroform / aceton 3:7)

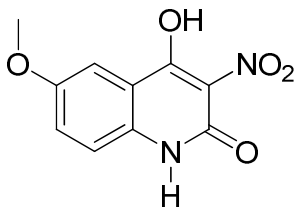
IR (ATR-measurement): 3139 (m), 2996 (m), 2839 (m), 1659 (s), 1623 (m, sh), 1594 (m), 1545 (w)

^{1}H-NMR (DMSO-d_6): δ = 2.38 (s, 3 H, CH_3), 3.73 (s, 3 H, CH_3O), 7.25 (s, 1 H, 3-H), 7.26-7.47 (m, 3 H, Ar-H), 7.48 (d, J = 8.4 Hz, 2 $H_{BB'}$ Ar-H), 7.95 (d, J = 8.2 Hz, 2 $H_{AA'}$ Ar-H), 12.31 (s, 1 H, N-H)

^{13}C-NMR (DMSO-d_6): δ = 21.6 (CH_3), 66.8 (CH_3O), 106.7 (3-C), 118.3 (Ar-C), 121.5 (Ar-C), 125.6 (Ar-C), 128.41 (2 x Ar-$C_{BB'}$), 130.9 (2 x Ar-$C_{AA'}$), 136.2 (Aromatic-C'), 136.3 (Ar-C), 146.1 (Ar-C), 147.5 (Ar-C), 154.6 (C-6), 159.8 (lactam-C=O)

$C_{17}H_{15}NO_4S$ (329.37)

4.2.9 4-Hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one (14)



To 4-hydroxy-6-methoxyquinolin-2(1*H*)-one (**3:** 17.40 g, 90.9 mmol, 1.0 eq) in glacial acetic acid (200 mL), a mixture of sodium nitrite (0.85 g, 12.32 mmol, 0.14 eq) and concentrated

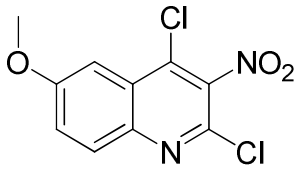
nitric acid (19.7 mL) was added within 2 minutes. The starting material was dissolved immediately followed by precipitation of the product. The reaction mixture was stirred for 45 minutes at 20 °C. Then the mixture was poured onto ice / water (1500 mL), the solid was filtered by suction and washed with water. The remaining solid was recrystallized from methanol, to afford 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)**. The yield was 16.01 g (74 %), orange prisms, mp 250-251 °C (methanol), lit. mp 254-257 °C [ex-13].

R_f = 0.44 (chloroform / ethanol 9:1)

^{1}H-NMR (DMSO-d_6): δ = 3.81 (s, 3 H, CH_3O), 7.28-7.31 (m, 2 H, 7-H and 8-H), 7.49 (d, J = 2.2 Hz, 1 H, 5-H), 11.89 (s, 1 H, NH)

$C_{10}H_8N_2O_5$ (236.19)

4.2.10 2,4-Dichloro-6-methoxy-3-nitroquinoline (15)



To 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one (**14:** 3.63 g, 15.37 mmol) in phosphoryl chloride (18.9 mL), dry triethylamine (2.52 mL) was added within 2 minutes. The reaction mixture was refluxed for 2 hours. The excess amount of phosphoryl chloride was removed under reduced pressure. After cooling to room temperature, the residue was poured onto ice / water (400 mL) and brought to pH = 4-6 with aqueous sodium hydroxide (5 M). The residue was filtered by suction and washed with water. The remaining solid was recrystallized from dioxane to afford 2,4-dichloro-6-methoxy-3-nitroquinoline **(15)**. The yield was 3.34 g (80 %), brown prisms, mp 190-194 °C (dioxane), lit. mp 195-196 °C [ex-13].

R_f = 0.93 (chloroform / aceton 3:7)

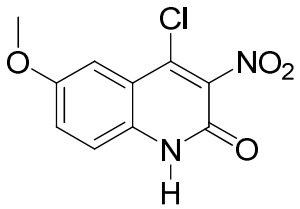
IR (ATR-measurement): 3120 (w), 3077 (w), 2971 (w), 2936 (w), 1680 (m), 1616 (s), 1230 (s), 831 (s)

^{1}H-NMR (DMSO-d_6): δ = 4.01 (s, 3 H, CH_3O), 7.52 (d, J = 2.6 Hz, 1 H, 5-H), 7.73 (dd, J = 9.4 + 2.5 Hz, 1 H, 7-H), 8.08 (d, J = 9.4 Hz, 1 H, 8-H)

MS (APCI- pos): m / z (%) = 365 (30, M + 93), 333 (30, M + 61), 311 (30, M + 39), 255 (100, M - 17), 253 (30, M - 19)
MS (APCI- neg): m / z (%) = 299 (50, M +27), 253 (60, M - 19), 165 (100, M - 107), 163 (40, M - 109)

$C_{10}H_6Cl_2N_2O_3$ (273.07)

4.2.11 4-Chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one (16)



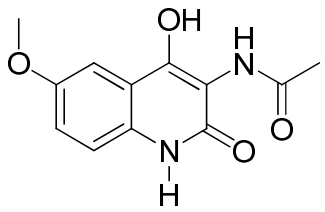
To 2,4-dichloro-6-methoxy-3-nitroquinoline (**15:** 5.10 g, 18.67 mmol) in n-butanol (100 mL), 70 % methanesulfonic acid (15 mL) was added. The reaction mixture was stirred for 20 hours at 110 °C. After cooling to room temperature, the solid [mainly 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one **(14)**] was filtered by suction.
The filtrate was poured onto ice / water (300 mL) and brought to pH = 4-6 with aqueous sodium hydroxide (5 M). The remaining solid was purified by dry-flash-column chromatography using ethyl acetate as eluent, to afford 4-chloro-6-methoxy-3-nitroquinolin-2(1*H*)-one **(16)**. The yield was 3.52 g (74 %), yellow prisms, mp 272-274 °C, lit. mp 274-276 °C [ex-13].

R_f = 0.48 (ethyl acetate)

^{1}H-NMR (DMSO-d_6): δ = 3.87 (s, 3 H, CH_3O), 7.33 (d, J = 2.4 Hz, 1 H, 5-H), 7.44-7.47 (m, 2 H, 7-H and 8-H), 12.97 (s, 1 H, NH)

$C_{10}H_7ClN_2O_4$ (254.63)

4.2.12 *N*-(4-Hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (19)



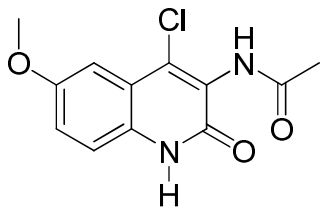
To 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one (**14:** 1.5 g, 6.48 mmol, 1 eq) in acetic acid (50 mL), zinc-dust (2.60 g, 38.91 mmol, 6 eq) was added. The reaction mixture was refluxed for 15 minutes. Then acetic anhydride (33 mL) was added and the mixture refluxed for further 15 minutes. The remaining zinc salt was filtered by suction while hot and the filtrate was taken to dryness under reduced pressure. The remaining residue was dissolved in aqueous sodium hydroxide (100 mL, 1 M), kept for 10 minutes at room temperature to alleviate the filtration. The solution was brought under stirring with concentrated hydrochloric acid to pH = 1-2. The precipitate was filtered by suction and washed with water. The remaining solid was recrystallized from toluene to afford *N*-(4-hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(19)**. The yield was 1.37 g (85 %), beige prisms, mp 304-306 °C (toluene), lit. mp 300-303 °C [ex-13].

R_f = 0.75 (chloroform / aceton 3:7)

^{1}H-NMR (DMSO-d_6): δ = 2.24 (s, 3 H, CH_3), 3.79 (s, 3 H, CH_3O), 7.13 (dd, J = 9.0 + 2.7 Hz, 1 H, 7-H), 7.23 (d, J = 9.0 Hz, 1 H, 8-H), 7.28 (d, J = 2.7 Hz, 1 H, 5-H), 9.73 (s, 1 H, NHAc), 11,74 (s, 1 H, NH)

$C_{12}H_{12}N_2O_4$ (248.23 g/mol)

4.2.13 *N*-(4-Chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (20)



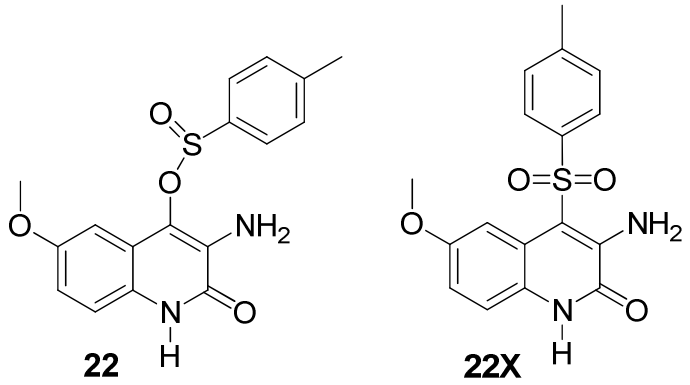
To *N*-(4-hydroxy-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (**19:** 500 mg, 2.01 mmol, 1 eq) in phosphoryl chloride (2.4 mL), dry triethylamine (0.33 mL, 2.37 mmol, 1.18 eq) was added. The reaction mixture was stirred for 1 hour at 60 °C. The excess amount of phosphoryl chloride was removed under reduced pressure. After cooling to room temperature, the residue was poured onto ice / water (50 mL) and brought to pH = 4-6 with aqueous sodium hydroxide (2 M). The residue was filtered by suction and washed with water. The remaining solid was recrystallized from dioxane to afford *N*-(4-chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide **(20)**. The yield was 442 mg (70 %), brown prisms, mp 274-282 °C (dioxane), lit. mp 283-285 °C [ex-13].

R_f = 0.3 (chloroform / aceton 3:7)

^{1}H-NMR (DMSO-d_6): δ = 2.04 (s, 3 H, CH_3), 3.83 (s, 3 H, CH_3O), 7.27-7.35 (m, 3 H, 5-H, 7-H and 8-H), 9.59 (s, 1 H, NHAc), 12.18 (s, 1 H, NH)

$C_{12}H_{11}ClN_2O_3$ (266.68)

4.2.14 3-Amino-6-methoxy-2-oxo-1,2-dihydroquinolin-4-yl 4-methylbenzenesulfinate (22) or 3-amino-6-methoxy-4-[(4-methylphenyl)sulfonyl]quinolin-2(1H)-one (22X)



To dry DMF (11 mL) *N*-(4-chloro-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)acetamide (**20:** 150 mg, 0.56 mmol, 1 eq) and lithium p-toluenesulfinate (140 mg, 0.88 mmol, 1.6 eq) were added. The reaction was heated under reflux for 16 hours. The resulting solution was cooled to room temperature and poured onto 50 mL ice / water. The obtained solid was filtered by suction and washed with water. The remaining solid was purified by dry-flash-column chromatography using chloroform-acetone (8:2) as eluent, to afford compound **22/22X**. The yield was 140 mg (73 %), yellow prisms, mp 245-247 °C (toluene).

R_f = 0.45 (chloroform / aceton 7:3)

IR (ATR-measurement): 3475 (s), 3360 (s), 2837 (s, br), 1671 (s), 1593 (s, sh), 1572 (s)

^{1}H-NMR (DMSO-d_6): δ = 2.33 (s, 3 H, CH_3), 3.66 (s, 3 H, CH_3O), 6.80 (dd, J = 9.0 + 2.4 Hz, 1 H, 7-H), 7.13 (d, J = 9.9 Hz, 1 H, 8-H), 7.38 (d, J = 8.1 Hz, , 2 $H_{BB'}$, Ar-H), 7.44 (d, J = 2.4 Hz, 1 H, 5-H), 7.82 (d, J = 8.4 Hz, 2 $H_{AA'}$, Ar-H), 12.27 (s, 1 H, NH)

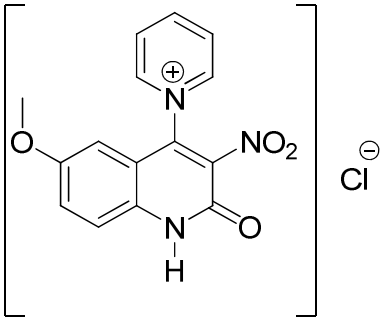
^{13}C-NMR (DMSO-d_6): δ = 21.5 (CH_3), 55.7 (CH_3O), 106.2 (3-O), 106.8 (5-C), 113.1 (7-C), 117.1 (Ar-C), 117. (8-C), 124.9 (Ar-C), 126.5 (2 x Ar-$C_{BB'}$), 130.5 (2 x Ar-$C_{AA'}$), 139.6 (Ar-C), 141.8 (Ar-C), 144.9 (Ar-C), 155.1 (6-O), 156.2 (lactam-C=O)

MS (API-ES pos): m / z (%) = 377 (95, M + 33), 367 (100, M + 23)
MS (API-ES neg): m / z (%) = 452 (35, M + 108), 343 (100, M - 1)

$C_{17}H_{16}N_2O_4S$ (344.38)

Calcd:	C 59.29	H 4.68	N 8.13
Found:	C 59.88	H 4.46	N 7.99

4.2.15 1-(6-Methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)pyridinium chloride (25)



To dry pyridine (35 mL) a mixture of 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one (**14:** 1.0 g, 3.92 mmol, 1 eq), p-tosyl chloride (**7:** 1.2 g, 6.29 mmol, 1.6 eq) and 4-(*N,N*-dimethyl)aminopyridine (55 mg) was added. The reaction mixture was stirred for 30 minutes at 20 °C. The obtained solid was filtered by suction and washed with ice / water. The remaining solid was recrystallized from ethanol to afford 1-(6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-yl)pyridinium salt **(25)**. The yield was 0.98 g (85 %), yellow powder, mp 274-278 °C (ethanol).

R_f = 0.24 (chloroform / methanol 7:3)

IR (ATR-measurement): 3117 (w), 3057 (s), 2967 (m), 2788 (s, br), 2687 (s, br), 1669 (s), 1625 (s), 1569 (m, sh), 1550 (s)

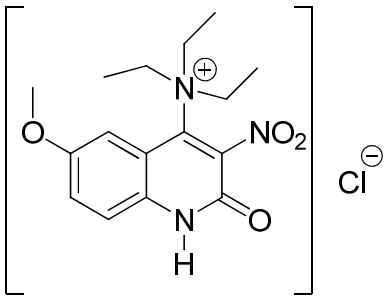
^{1}H-NMR (DMSO-d_6): δ = 3.70 (s, 3 H, CH_3O), 6.49 (d, J = 2.4 Hz, 1 H, 5-H), 7.59 (dd, J = 9.0 + 2.4 Hz, 1 H, 7-H), 7.65 (d, J = 9.3 Hz, 1 H, 8-H), 8.57 (t, J = 7.5 Hz, 2 $H_{BB'}$ Ar-H), 9.06 (t, J = 8.1 Hz, 1 H_C Ar-H), 9.50 (d, J = 5.4 Hz, 2 $H_{AA'}$ Ar-H), 13.66 (s, 1 H, NH)

MS (API-ES pos): m / z (%) = 516 (20, M + 218), 298 (100, M)

$C_{15}H_{12}N_3O_4Cl$ (333.73)

Calcd:	C 53.98,	H 3.62,	N 12.59
Found:	C 51.65,	H 3.87,	N 11.21

4.2.16 N,N,N-Triethyl-6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-aminium chloride (26)



To dry triethylamine (10 mL) a solution of 4-hydroxy-6-methoxy-3-nitroquinolin-2(1*H*)-one (**14:** 300 mg, 1.18 mmol, 1 eq) in 2 mL of dry DMF and p-tosyl chloride (**7:** 350 mg, 1.83 mmol, 1.6 eq) were added. The mixture was stirred for 15 minutes at 20 °C. The obtained solid was filtered by suction and washed with ice / water. The remaining solid was recrystallized from ethanol to afford N,N,N-triethyl-6-methoxy-3-nitro-2-oxo-1,2-dihydroquinolin-4-aminium salt **(26)**. The yield was 340 mg (90 %), yellow powder, mp 173-176 °C (ethanol).

R_f = 0.48 (chloroform / methanol 5:5)

IR (ATR-measurement): 2979 (s), 2944 (s), 2845 (s), 2602 (s), 2530 (s), 2496 (s), 1664 (s), 1609 (s)

^{1}H-NMR (DMSO-d_6): δ = 1.19 (t, J = 7.2 Hz, 9 H, 3 CH_3), 3.04-3.10 (m, 6 H, 3 CH_2), 3.75 (s, 3 H, CH_3O), 7.01- 7.04 (m, 2 H, 7-H and 8-H), 7.40 (d, J = 1.3 Hz, 1 H, 5-H), 10.34 (s, 1 H, NH)

MS (API-ES pos): m / z (%) = 241 (30, M - 79), 239 (100, M - 81)
MS (API-ES neg): m / z (%) = 235 (100, M - 85)

$C_{16}H_{22}N_3O_4Cl$ (355.82)

Calcd:	C 54.01,	H 6.23,	N 11.81
Found:	C 53.80,	H 8.00,	N 10.86

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5.1 References Introduction

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