# Comparative Studies of Different Quinolinone Derivatives as Donors in Fluorescence-Resonance-Energy Transfer (FRET) – Systems in Combination with a (Bathophenanthroline)ruthenium(II) Complex as Acceptor

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We describe the preparation as well as a detailed photophysical study of Fmoc-amino acid building blocks carrying different carbostyril (=quinolin-2(1*H*)-one) heterocycles as donors in a FRET (fluorescence-resonance-energy transfer) system in combination with a [$Ru^{II}$(bathophenanthroline)] complex (bathophenanthroline = 4,7-diphenyl-1,10-phenanthroline). The efforts resulted in a clear preference for building block **16** due to its ease of synthesis (*Scheme 2*), its chemical robustness, and the FRET efficiency when incorporated into peptides.

**Introduction.** – Luminescent chromophores as entities of fluorescence-resonance-energy transfer (FRET) systems are important tools to study supramolecular interactions with a special emphasis in the realm of biomolecules like DNA, RNA, and proteins [1]. FRET Systems allow to monitor distance-dependent interactions on the molecular level, and in a real-time mode. Therefore, they are especially suited for the characterization of biochemical events both *in vitro* and *in vivo*. Meanwhile, a myriad of applications has been reported. It involves binding of ligands to their pertinent protein receptors [2], DNA–protein complexation [3], and RNA-folding and catalysis [4]. Other applications are enzyme assays based, *e.g.*, on the *Förster* resonance-energy-transfer principle [5] and monitoring of polymerase chain reactions (PCR) [6]. The FRET technology is based on the nonemissive transfer of energy between a donor (D) and an acceptor (A) fluorophore. It decreases with $r^{-6}$, $r$ being the distance between the donor and the acceptor [7]. A prerequisite for an efficient transfer is an intensive overlap between the emission of the donor with the absorption of the acceptor as well as the correct orientation of their dipole transition moments relative to each other. A plethora of different donor–acceptor pairs have been reported up to date, but despite the multitude of available systems, sensitivity – especially in the presence of background luminescence from matrix constituents – still remains an issue. A further concern is robustness of the applied dyes as well as the possibility to employ them in a modular way as broadly as possible *via* stable covalent bonds and without interference of the spectral properties of the labelled molecules.

One way to solve the sensitivity issue are FRET systems of which one partner possesses a long excited-state decay time and hence, allows for measurements in a time-resolved mode reducing background luminescence. The most prominent candidates for this purpose are lanthanide ions with lifetimes up to milliseconds. Usually, they are employed as caged chelates [8], but leakage of Eu can still be a problem. Furthermore, the very long decay time might be a disadvantage in monitoring events which occur in the μs range.

Our focus has therefore been on $Ru^{II}$ charge-transfer (CT) complexes – and especially [$Ru^{II}$(bathophenanthroline)] complex **1** [9] (*Fig. 1*) – as alternative acceptor units due to their thermodynamic stability, chemical inertness, straightforward synthetic accessibility and relatively long-lived excited states ($\tau_{em} = 1-5$ μs) allowing for time-resolved measurements [10][11] (bathophenanthroline = 4,7-diphenyl-1,10-phenanthroline).

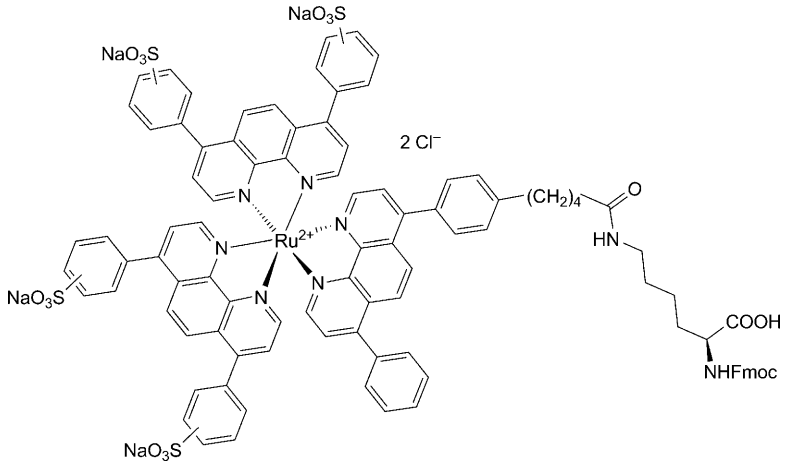


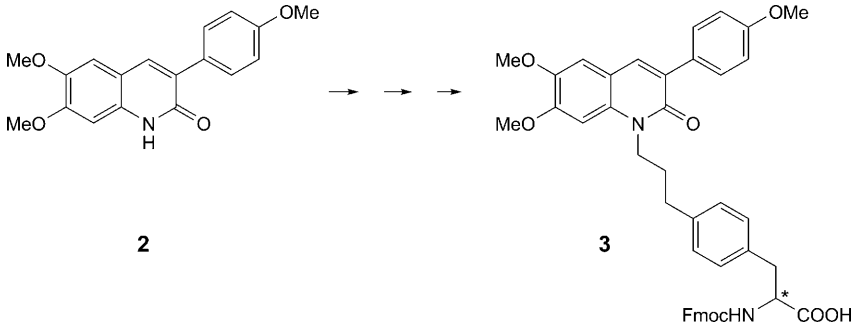

Fig. 1. *[$Ru^{II}$(bathophenanthroline)] complex* **1** *as amino acid building block*

As suitable donor chromophore, we had identified carbostyril (= quinolin-2(1*H*)-one) derivative **2** [12] which we turned into the amino acid building block **3** (*Scheme 1*) directly applicable to solid-phase peptide synthesis. The FRET system composed of the quinolinone donor and the [$Ru^{II}$(bathophenanthroline)] complex as acceptor was then verified in peptides. It turned out to be very robust and revealed an intensive FRET measurable with high sensitivity in a time-resolved mode [13] (for application of the system in DNA, see [14]).

Although efficient, the system still entailed a number of disadvantages: The synthetic route from **2** to **3** was not straightforward and yielded furthermore **3** as a racemate. An enantiomerically pure building block would be more compatible with defined peptide structures. In addition, we were aiming at an even more intense FRET.

**Results and Discussion.** – Recently, *Uray*, *Stadlbauer*, and co-workers published their efforts on improving the photophysical properties of carbostyrils by a systematic investigation of substituent effects which converged in a push-pull model with two

Scheme 1. *Quinolin-2(1H)-one* **2** *and the Pertinent Amino Acid Derivative* **3**

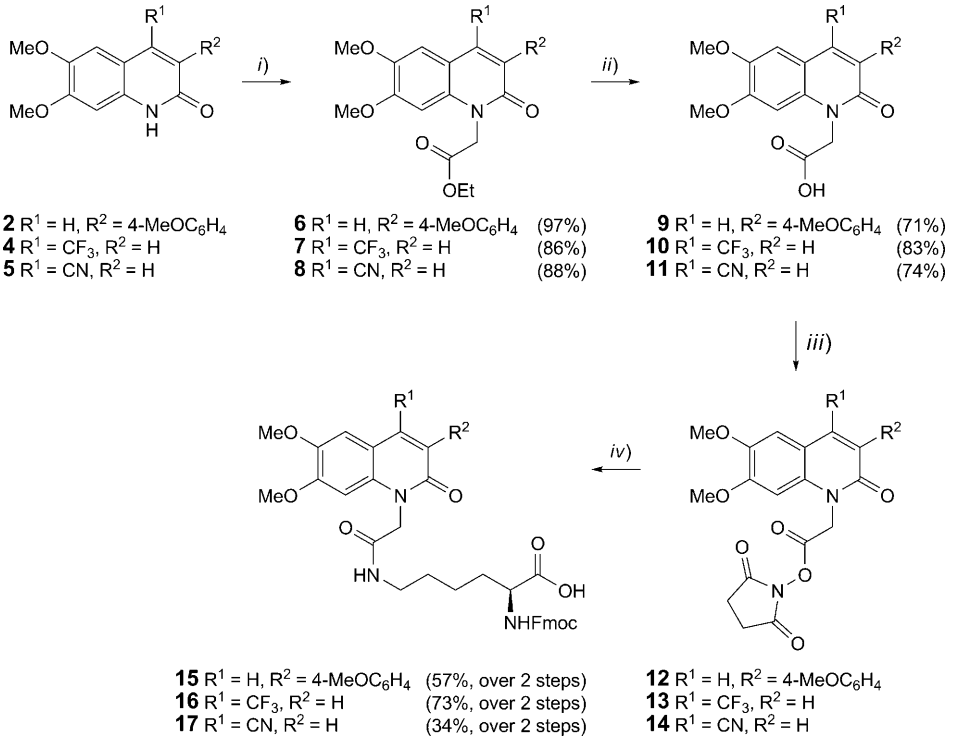



electron-donating groups at position 6 and 7 and an electron-withdrawing group at the 4-position. Their first attempts culminated in compound **4** carrying a $CF_3$ group at C(4) [15]. Alkylation at N(1) had no influence on the photophysical properties, and derivatives of **4** showed an absorption maximum $\lambda_{max}$ at 359–361 nm in $H_2O$ and extinction coefficients around 10000. Quantum yields up to 0.50 and a *Stokes* shift of 70 nm were observed. Last year, the group reported on carbostyril **5** and derivatives of it [16]. The compound revealed a broad double maximum between 385 and 410 nm independent of the pH value (pH 1–11). Furthermore, derivatives of **5** fluoresce in $H_2O$ and polar and apolar solvents in a narrow 430–440 nm window and with a quantum yield around 0.5. These data prompted us to perform a comparative study concerning the suitability of the above mentioned chromophores **2**, **4**, and **5** (*Scheme 2*) as donors in FRET systems in combination with [$Ru^{II}$(bathophenanthroline)] complexes as acceptors.

After having synthesized the parent carbostyrils **2**, **4**, and **5**, they were turned into their pertinent Fmoc–Lys–OH building blocks according to *Scheme 2* and following essentially the procedures published by *Uray*, *Stadlbauer*, and co-workers [15][16]. In the alkylation reaction with $BrCH_2COOEt$, we used lithium diisopropylamide (LDA) for the deprotonation instead of $NaOH/CH_2Cl_2$ or $K_2CO_3$/MeCN. This led to a quantitative deprotonation under mild conditions and avoided at the same time the formation of the *O*-alkylated product. The intermediates **6**–**8** were thus obtained in yields of 97, 86, and 88%, respectively. After saponification with NaOH (→ **9**–**11**), the carboxyl functions were activated by reaction with *N*-hydroxysuccinimide in the presence of *N*,*N*′-diisopropylcarbodiimide (→ **12**–**14**). The activated esters were then treated without further purification with Fmoc–Lys–OH using an aqueous DMF solution and *Titrisol*® buffer (pH 7) to yield the desired amino acid building blocks **15**–**17** in pure form. Compared to our previous synthesis route for the amino acid building block **3** in racemic form and comprising 7 steps, the preparation according to *Scheme 2* is rather straightforward involving just two chromatographic steps and yielding the enantiomerically pure compounds.

During preliminary experiments, we learned that building blocks **16** and **17** were completely stable towards 95% $CF_3COOH$ solution, whereas in **15**, the amide bond was partially cleaved under these conditions. Hence, the latter is not compatible with peptide synthesis and was for this reason in this study replaced by building block **3**.

Scheme 2. *Synthesis of the Amino Acid Building Blocks* **15**–**17**




**2** $R^1$ = H, $R^2$ = 4-$MeOC_6H_4$
**4** $R^1$ = $CF_3$, $R^2$ = H
**5** $R^1$ = CN, $R^2$ = H

**6** $R^1$ = H, $R^2$ = 4-$MeOC_6H_4$ (97%)
**7** $R^1$ = $CF_3$, $R^2$ = H (86%)
**8** $R^1$ = CN, $R^2$ = H (88%)

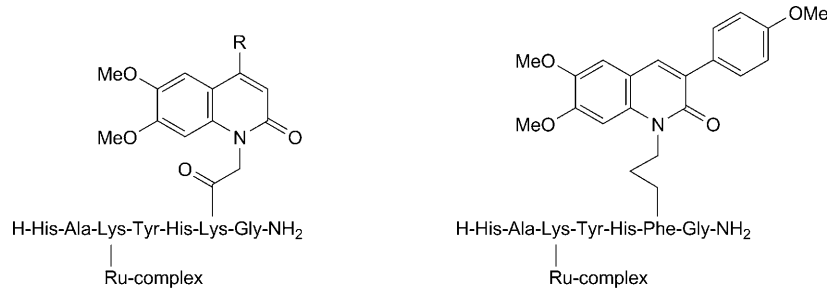
**9** $R^1$ = H, $R^2$ = 4-$MeOC_6H_4$ (71%)
**10** $R^1$ = $CF_3$, $R^2$ = H (83%)
**11** $R^1$ = CN, $R^2$ = H (74%)

**15** $R^1$ = H, $R^2$ = 4-$MeOC_6H_4$ (57%, over 2 steps)
**16** $R^1$ = $CF_3$, $R^2$ = H (73%, over 2 steps)
**17** $R^1$ = CN, $R^2$ = H (34%, over 2 steps)

**12** $R^1$ = H, $R^2$ = 4-$MeOC_6H_4$
**13** $R^1$ = $CF_3$, $R^2$ = H
**14** $R^1$ = CN, $R^2$ = H

*i*) LDA, THF, 0°, 1 h; $BrCH_2COOEt$, 0°, 30 min; r.t., 16 h. *ii*) NaOH, EtOH/$H_2O$ 9 : 1, reflux, 16 h. *iii*) *N*-Hydroxysuccinimide, *N*,*N*′-diisopropylcarbodiimide, THF, 0°, 15 h. *iv*) Fmoc–Lys–OH, *Titrisol*® buffer (pH 7.0), DMF/$H_2O$ 9 : 1, r.t., 20 h.

For the evaluation of the different donor chromophores in the FRET system with the [$Ru^{II}$(bathophenanthroline)] complex, we synthesized peptides **18**–**20** (*Fig. 2*) with standard Fmoc-building blocks and introducing the Ru-complex-modified L-lysine




**18** R = $CF_3$
**19** R = CN

**20**

Fig. 2. *FRET Peptides* **18**–**20**

**1** in the third position. The carbostyril entities were inserted by using an excess of 1.2 equiv. and TBTU as coupling reagent [17], whereupon complete coupling was achieved. After deprotection of the side chains and removal from the support, the peptides were obtained in pure form after semiprep. HPLC.

*Photophysical Investigations. Fig. 3* shows the absorption spectra of building blocks **3** and **15**–**17**. Only the absorption of the CN-substituted building block **17** is significantly shifted to longer wavelengths. The data obtained for building blocks **16** and **17** agree well with the ones reported for the pure dyes by *Uray*, *Stadlbauer*, and co-workers [15][16]. Since these dyes are intended to be used as fluorescence donors, their fluorescence properties are important. The location of the fluorescence maximum is very similar and is found in the spectral range of $430\ \text{nm} < \lambda_{em} < 445\ \text{nm}$ (*Table*). The fluorescence quantum efficiencies $\Phi_{em}$ are around 0.18 for compounds **3** and **15** but significantly higher for compound **16** and **17** for which an efficiency $\Phi_{em}$ of roughly 0.5 was determined (*Table*). The fluorescence decay curves found for **3** and **15**–**17** follow first-order kinetics. The obtained fluorescence decay times $\tau$ correspond to the observed trends found for $\Phi_{em}$: For **3** and **15**, short decay times $\tau$ of *ca.* 600 ps were determined, while for **16** and **17**, $\tau$ of *ca.* 3.5 ns were found (*Table*).

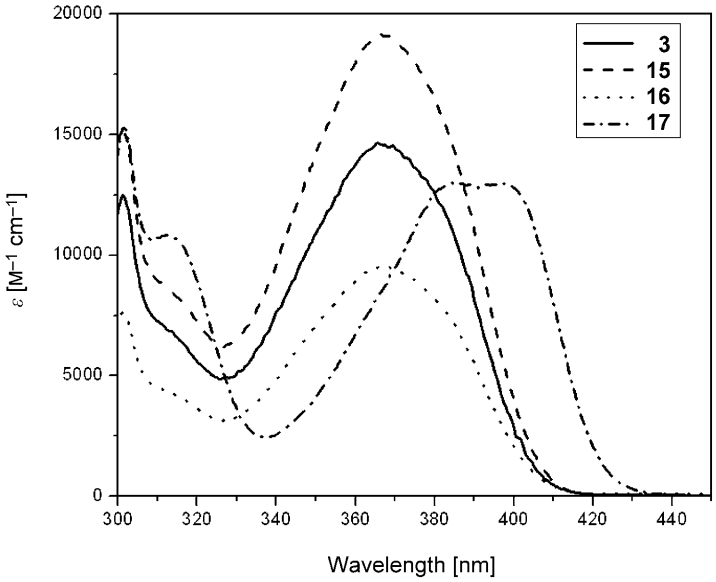



Fig. 3. *Absorption spectra of building blocks* **3**, **15**, **16**, *and* **17**

Based on the absorption and fluorescence spectra of the building-block dyes **3** and **15**–**17**, first the critical *Förster* distance $R_0$ was calculated with *Eqns. 1* and *2*. Here, $R_0$ is the distance, at which the energy transfer occurs with a transfer efficiency $E$ of 50%. As a rule of thumb, a specific D/A pair can be used for distance determinations within $R_0/2 < R < 2R_0$.

Table. *Photophysical Properties of the Building Blocks* **3**, **15**, **16**, *and* **17**

| | $\lambda_{abs}$ [nm] | $\varepsilon_A$ ($\lambda$) [l mol$^{-1}$ cm$^{-1}$] | $\lambda_{exc}$ [nm] | $\lambda_{em}$ [nm] | $\tau$ [ns] | $\Phi_{em}$ | $R_0$[a]) [Å] |
|---|---|---|---|---|---|---|---|
| **3** | 367 | 14500 | 367 | 433 | 0.6 | 0.17 | 37 |
| **15** | 366 | 19000 | 367 | 435 | 0.7 | 0.19 | 38 |
| **16** | 368 | 9500 | 368 | 440 | 3.5 | 0.50 | 45 |
| **17** | 398 | 13000 | 380 | 442 | 3.7 | 0.47 | 45 |

[a]) Based on *Eqn. 1* calculated critical *Förster* distance $R_0$ with **1** as acceptor.

$$R_0^6 = \frac{(9000 \ln 10)\kappa^2 \Phi_D}{128\pi^5 n^4 N_A} J(\lambda) \quad (1)$$

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (2)$$

In *Eqn. 1*, $\kappa^2$ is the orientation factor describing the relative orientation of the transition dipol moments of D and A, $\Phi_D$ is the fluorescence quantum efficiency of the donor, $n$ represents the refractive index of the solvent, and $N_A$ is the *Avogadro* constant. $J(\lambda)$ is the spectral-overlap integral calculated from the area-normalized fluorescence spectrum $F_D(\lambda)$ of the donor and the absorption spectrum $\varepsilon_A(\lambda)$ of the acceptor (see *Eqn. 2*). The values of $R_0$ for the investigated compounds are summarized in the *Table*. At this point, **16** and **17** seemed to be the preferred donors for the envisaged FRET system because of the largest $R_0$ and a quantum yield of *ca.* 0.5.

By incorporation of the FRET chromophores into (bio)macromolecules, a further effect might come into play which may influence the effective transfer efficiency of the FRET pair. Such an effect could be the limitation in the rotational freedom of the dye molecule because of specific interactions with the (bio)macromolecule or of the dyes themselves. To compare these building blocks as potential FRET donors (D) in combination with the [Ru$^{II}$(bathophenanthroline)] complex as FRET acceptor (A), FRET peptides **18**–**20** were further characterized with respect to their spectroscopic properties.

The absorption spectra of the FRET peptides **18**–**20** show the typical double peak of the Ru$^{II}$-complex absorption at $\lambda$ 445 and 467 nm as well as a less resolved peak around $\lambda$ 367 nm of the donor entity. These absorption spectra are equal to the sum of the absorption found for the D and A building blocks, respectively. Accordingly, it can be concluded that no direct interaction of the dyes in their electronic ground states is present.

*Fig. 4* shows the fluoresence emission spectra of **18**–**20** excited at $\lambda_{ex}$ 360 nm. The excitation wavelength of $\lambda_{ex}$ 360 nm was chosen to avoid direct excitation of the Ru$^{II}$ complex, which has an absorption minimum around 360 nm. For a better overview, the spectra were normalized to the donor emission maximum. In addition to the emission around $\lambda_{em}$ 440 nm of the donor entities, a second emission feature at $\lambda_{em}$ 620 nm was observed, which belongs to the luminescence of the Ru$^{II}$-complex acceptor.

To determine the FRET efficiencies $E$ of the dye-labeled peptides **18**–**20**, *Eqn. 3* was used. This ratiometric method introduced by *Clegg* and co-workers allows the

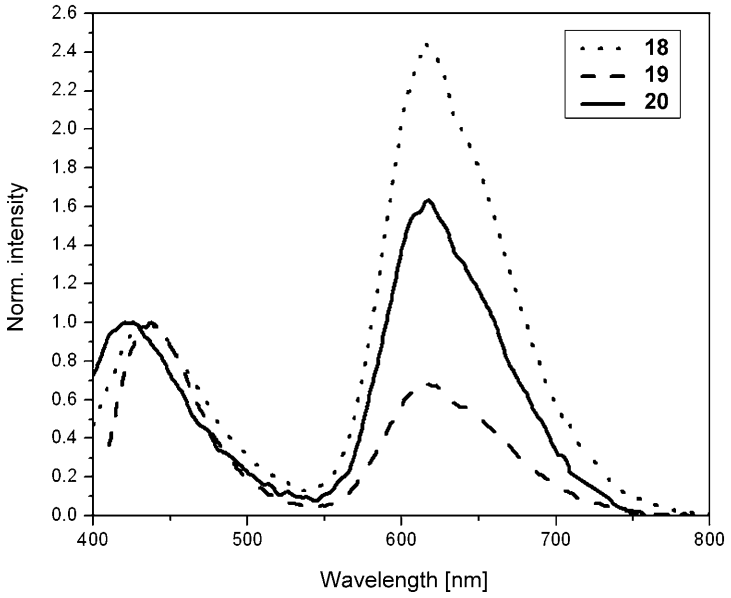



Fig. 4. *Fluorescence emission spectra of FRET peptides* **18**–**20** *at 620 nm after excitation at 360 nm* (normalized to donor emission maximum, not corrected for the quantum yield)

direct determination of the FRET efficiency of DA pairs labeled to (bio)macromolecules by only two separate measurements [18][19]. From the measurement of the fluorescence of the samples exciting *i*) the donor and *ii*) the acceptor, $E$ can be determined without further need for reference samples labeled only with D or A, respectively.

$$\frac{F^{A}_{FRET}(\lambda_1)}{F^{A}_{direct}(\lambda_2)} = \left[E\frac{\varepsilon^{D}(\lambda_1)}{\varepsilon^{A}(\lambda_2)} + \frac{\varepsilon^{A}(\lambda_1)}{\varepsilon^{A}(\lambda_2)}\right]\frac{\Phi^{A}}{\Phi^{D}} \tag{3}$$

In *Eqn. 3*, $F_{FRET}$ is the fluorescence intensity of the acceptor excited *via* FRET ($\lambda_{ex}$ 360 nm corresponding to optimal excitation of the donor) and $F_{direct}$ for direct exitation of the acceptor at $\lambda_{ex}$ 450 nm, $E$ is the energy-transfer efficiency, $\varepsilon$ the molar extinction coefficient at the according wavelength $\lambda$, and $\Phi$ is the fluorescence quantum yield. The resulting FRET efficiencies are 0.28 for **20**, 0.78 for **18**, and 0.30 for **19**. Based on the photophysical data presented, the $CF_3$-substituted derivative **16** shows the best donor properties for the FRET pair with the [$Ru^{II}$(bathophenanthroline)] complex.

**Conclusions.** – For the evaluation of different carbostyril chromophores, *i.e.*, **2**, **4**, and **5**, as donors in a FRET system with a [$Ru^{II}$(bathophenanthroline)] complex as acceptor, the carbostyrils were turned into Fmoc-amino acid building blocks **15**–**17** directly applicable to solid-phase peptide synthesis and avoiding postsynthetic labelling. The photophysical properties of the buildings blocks **3** and **15**–**17** did not differ significantly from those of the parent heterocycles but indicated the superiority of **16**

and **17** as far as *Förster* radii and quantum yields were concerned. Comparative studies of FRET efficiencies after incorporation of the donor building blocks into a reference peptide revealed the highest FRET efficiency for building block **16**. Together with its straightforward synthesis and its chemical robustness, it turned out to be the building block of choice for the FRET system in combination with [$Ru^{II}$(bathophenanthroline)] complex **1**. This building block will, therefore, be used in the aspired robust and highly sensitive three-color FRET system we are currently pursuing.

## Experimenal Part

*General*. All reagents were purchased from commercial sources, with the exception of compounds **1**, **2**, and **6** which were synthesized according to established procedures [13b][16]. The molarity of BuLi was determined by titration with salicylaldehyde phenylhydrazone [20]. Amine-free DMF (*Roth*) was employed throughout peptide syntheses. HPLC: *Agilent-1100* system; *Nucleosil-100-5-C18-PPN* column (*Machery-Nagel*). Column chromatography (CC): silica gel *60* ($SiO_2$; *Merck*). NMR Spectra: *Brucker AM400*; at 400 ($^1H$) and 100.6 MHz ($^{13}C$); chemical shifts $\delta$ in ppm rel. to the respective solvent signals, *J* in Hz. MS: *Finnigan MAT-8200* (EI), *Thermo LCQ Advantage* (ESI); *Thermo TCQ 7000* (APCI = atmospheric-pressure chemical ionization); in *m/z* (rel. %).

All photophysical measurements were carried out in DMSO (spectroscopic grade) without further purification. UV/VIS Spectra: *Lambda-750* UV/VIS spectrometer (*Perkin-Elmer*). Steady-state fluorescence measurements: *Fluoromax3* spectrometer (*Jobin Yvon*). A *FL920* fluorescence spectrometer (*Edinburgh Instruments*) in time-correlated single-photon-counting (TCSPC) mode was used for the measuring of the fluorescence decay times. For the excitation, the frequency-doubled output (second harmonic generation, SHG) of a titanium–sapphire laser ($\lambda$ 720 nm, $\lambda_{SHG}$ 360 nm) was focused into the sample. The resulting luminescence was detected in a right-angle configuration to the incoming beam. For the detection, a multichannel plate (*Europhoton*) was used. In the data analysis, the resulting decay curves were deconvoluted with a least-square fitting procedure based on the *Levenberg–Marquardt* algorithm. The fluorescence quantum yields were estimated with a photo-luminescence (PL) quantum-yield measurement system *C9920-02* (*Hamamatsu*, Japan).

*Ethyl 6,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxoquinoline-1(2*H*)-acetate* (**6**). To a soln. of $^iPr_2NH$ (1.35 ml, 9.64 mmol, 3.0 equiv.) in anh. THF (10 ml) was added at $-78°$ dropwise 1.18M BuLi in hexane (6.80 ml, 8.02 mmol, 2.5 equiv.). The mixture was stirred for 1 h at $-78°$ and then allowed to warm to 0°. The soln. was added dropwise to a soln. of quinolin-2(1*H*)-one **2** (1.00 g, 3.21 mmol, 1.0 equiv.) in anh. THF (15 ml). The resulting mixture was stirred at 0° for 1 h, before $BrCH_2COOEt$ (1.77 ml, 16.04 mmol, 5.0 equiv.) was added. The mixture was stirred for 30 min at 0°, and at r.t. for additional 15 h. The reaction was quenched by the addition of an aq. $NH_4Cl$ soln. (30 ml). The aq. phase was extracted with $CH_2Cl_2$ (4 × 100 ml), the combined org. phase dried ($MgSO_4$) and concentrated, and the residue purified by CC ($SiO_2$, $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 98:2 → 95:5): **6** (1.24 g, 97%; [21]: 97%). Pale yellow solid. M.p. 181° ([21]; 182–183°). $^1$H-NMR ($CDCl_3$): 1.26 (*t*, *J* = 7.0, *Me*$CH_2$); 3.84 (*s*, MeO); 3.94 (*s*, MeO); 3.95 (*s*, MeO); 4.25 (*q*, *J* = 7.1, MeC*H*$_2$); 5.11 (*s*, $CH_2N$); 6.56 (*s*, H–C(8)); 6.95 (*dt*, *J* = 9.1, 2.4, H–C(3′,5′)); 7.02 (*s*, H–C(5)); 7.69 (*d*, *J* = 8.9, H–C(2′,6′)); 7.73 (*s*, H–C(4)). EI-MS: 397 (100, $M^+$). Spectroscopic data in accordance with [21].

*6,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxoquinoline-1(2*H*)-acetic Acid* (**9**). A suspension of **6** (1.40 g, 3.52 mmol, 1.0 equiv.), NaOH (0.86 g, 22 mmol, 6.1 equiv.), $H_2O$ (5.5 ml), and EtOH (36 ml) was heated under reflux for 7 h (→ clear soln.). The solvent was evaporated, $H_2O$ (80 ml) added, and the pH adjusted to 1–2 with conc. HCl soln. The resulting precipitate was filtered, washed with ice water (80 ml), and dried by co-evaporation with MeCN: **9** (921 mg, 71%; [21]: 93%). Pale yellow solid. M.p. 203° ([21]: 205–206°). $^1$H-NMR ($(D_6)$DMSO): 3.79 (*s*, MeO); 3.82 (*s*, MeO); 3.89 (*s*, MeO); 5.10 (*s*, $CH_2N$); 6.92 (*s*, H–C(8)); 6.98 (*d*, *J* = 8.0, H–C(3′,5′)); 7.34 (*s*, H–C(5)); 7.69 (*d*, *J* = 8.2, H–C(2′,6′)); 8.00 (*s*, H–C(4)). APCI-MS: 370 (100, $[M+H]^+$). Spectroscopic data in accordance with [21].

*(2*S*)-6-{2-[6,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]acetamido}-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic Acid* (= $N^6$-*{2-[6,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-*

*quinolin-1(2*H*)-yl]acetyl}*-N$^2$-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]*-L-*lysine*; **15**). To a suspension of **9** (369 mg, 1.00 mmol, 1.0 equiv.) in anh. THF (20 ml) was added slowly at 0° *N*-hydroxysuccinimide (115 mg, 1.00 mmol, 1.0 equiv.) and *N,N′*-diisopropylcarbodiimide (88.1 µl, 1.00 mmol, 1.0 equiv.). The mixture was stirred for 15 h at 0°. The solvent was evaporated and the residue redissolved in DMF/$H_2O$ 9:1 (30 ml) and *Titrisol*® buffer (pH 7.0; 7.5 ml). To this soln., Fmoc–Lys–OH (479 mg, 1.30 mmol, 1.3 equiv.) was added, and the mixture was stirred at r.t. for 20 h. The solvent was evaporated, the residue suspended in $H_2O$ (100 ml), and the pH adjusted to 1–2 with conc. HCl soln. The resulting precipitate was filtered and washed with ice water (100 ml). The residue was purified by CC ($SiO_2$, $CH_2Cl_2$ → $CH_2Cl_2$/MeOH/AcOH 95:5:0.1): **15** (410 mg, 57% over two steps). White solid. $^1$H-NMR ((D$_6$)DMSO): 1.29–1.48 (*m*, NHCHC*H*$_2$C*H*$_2$); 1.55–1.76 (*m*, CONHCH$_2$C*H*$_2$); 3.07–3.14 (*m*, CONHC*H*$_2$); 3.79 (*s*, MeO); 3.81 (*s*, MeO); 3.84 (*s*, MeO); 3.89–3.93 ($m_c$, H–C(9) of Fmoc); 4.21 (*t*, *J* = 6.96, C*H*CO$_2$H); 4.27–4.31 (*m*, CH$_2$ of Fmoc); 5.00 (*s*, NCH$_2$CON); 6.78 (*s*, H–C(8) of quin.); 6.98 (*dt*, *J* = 9.8, 2.6, H–C(3,5) of 4-MeOC$_6$H$_4$); 7.31–7.34 (*m*, H–C(4,5) of Fmoc); 7.40 (*dd*, *J* = 7.5, 7.4, H–C(2,7) of Fmoc); 7.60 (*d*, *J* = 8.1, N*H*CHCO$_2$H); 7.68–7.74 (*m*, H–C(3,6) of Fmoc, H–C(2,6) of 4-MeOC$_6$H$_4$); 7.88 (*d*, *J* = 7.5, H–C(1,8) of Fmoc); 7.98 (*s*, H–C(4) of quin.); 8.24 (*t*, *J* = 5.6, CON*H*CH$_2$); 12.61 (br. *s*, CO$_2$H). $^{13}$C-NMR ((D$_6$)DMSO): 13.9; 21.0; 22.1; 23.0; 28.3; 28.7; 30.4; 31.2; 45.4; 46.6; 53.7; 55.1; 55.8; 65.6; 97.8; 110.0; 113.3; 113.5; 120.1; 125.2; 127.0; 127.2; 127.6; 129.3; 129.8; 134.6; 135.8; 140.7; 143.8; 144.7; 151.5; 156.1; 158.7; 160.4; 166.9; 172.0; 173.9. APCI-MS: 718 (100, [*M* – H]$^-$). Anal. calc. for $C_{41}H_{41}N_3O_9 \cdot 0.5\ H_2O$ (728.79): C 67.63, H 6.33, N 5.57; found: C 67.57, H 5.98, N 5.77.

*Ethyl 6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)quinoline-1(2*H*)-acetate* (**7**). As described for **6**, with $^i$Pr$_2$NH (1.24 ml, 8.79 mmol, 3.0 equiv.), THF (10 ml) 1.18M BuLi in hexane (6.21 ml, 7.33 mmol, 2.5 equiv.), 6,7-dimethoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (**4**; 0.80 g, 2.93 mmol, 1.0 equiv.), THF (15 ml), and BrCH$_2$COOEt (1.62 mmol, 16.7 mmol, 5.0 equiv.). CC ($SiO_2$, $CH_2Cl_2$ → $CH_2Cl_2$/MeOH 98:2) afforded **7** (0.91 g, 86%). Pale yellow solid. M.p. 150° ([15a]: 150–151°). $^1$H-NMR ($CDCl_3$): 1.26 (*t*, *J* = 7.2, MeCH$_2$); 3.94 (*s*, MeO); 3.96 (*s*, MeO); 4.25 (*q*, *J* = 7.1, MeC*H*$_2$); 5.11 (*s*, CH$_2$N); 6.58 (*s*, H–C(3)); 7.01 (*s*, H–C(8)); 7.23 (*s*, H–C(5)). EI-MS: 359 (100, *M*$^+$). Spectroscopic data in accordance with [15a].

*6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)quinoline-1(2*H*)-acetic Acid* (**10**). As described for **9**, with **7** (0.90 g, 2.50 mmol, 1.0 equiv.), NaOH (0.60 g, 15 mmol, 6.0 equiv.), $H_2O$ (3.9 ml), EtOH (35 ml), $H_2O$ (50 ml), and conc. HCl. The resulting precipitate was filtered, washed with ice water (100 ml) and dried by azeotropic co-evaporation with MeCN. The combined aq. phase was extracted with $CH_2Cl_2$ (4 × 100 ml), the combined org. phase dried ($MgSO_4$) and concentrated, and the residue combined with the precipitate to yield **10** (688 mg, 83%: [15a]: 81%). Pale yellow solid. M.p. 282° ([15a]: 284–285°). $^1$H-NMR ((D$_6$)DMSO): 3.84 (*s*, MeO); 3.93 (*s*, MeO); 5.13 (*s*, CH$_2$N); 6.97 (*s*, H–C(3)); 7.04 (*s*, H–C(8)); 7.10 (*s*, H–C(5)); 13.16 (br. *s*, CO$_2$H). APCI-MS: 330 (100, [*M* – H]$^-$). Spectroscopic data in accordance with [15a].

*(2*S*)-6-{2-[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)quinolin-1(2*H*)-yl]acetamido}-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic Acid* (= N$^6$-*{2-[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)quinolin-1(2*H*)-yl]acetyl}*-N$^2$-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]*-L-*lysine*; **16**). As described for **15**, with **16** (500 mg, 73% over two steps). White solid. $^1$H-NMR ((D$_6$)DMSO): 1.22–1.46 (*m*, NHCHC*H*$_2$C*H*$_2$); 1.55–1.75 (*m*, CONHCH$_2$C*H*$_2$); 3.06–3.12 (*m*, CONHC*H*$_2$); 3.81 (*s*, MeO); 3.88 (*s*, MeO); 3.89–3.92 (*m*, H–C(9) of Fmoc); 4.21 (*t*, *J* = 6.95, C*H*CO$_2$H); 4.26–4.31 (*m*, CH$_2$ of Fmoc); 4.99 (*s*, CH$_2$ of quin.); 6.87 (*s*, H–C(5) of quin.); 6.94 (*s*, H–C(8) of quin.); 7.07 (*s*, H–C(3) of quin.); 7.30 (*d*, *J* = 7.1, H–C(4) of Fmoc); 7.32 (*d*, *J* = 7.1, H–C(5) of Fmoc); 7.39 (*dd*, *J* = 7.5, 7.4, H–C(2,7) of Fmoc); 7.59 (*d*, *J* = 8.0, N*H*CHCO$_2$H); 7.72 (*dd*, *J* = 7.5, 7.1, H–C(3,6) of Fmoc); 7.87 (*d*, *J* = 7.6, H–C(1,8) of Fmoc); 8.28 (*t*, *J* = 5.5, CON*H*CH$_2$); 12.61 (br. *s*, CO$_2$H). $^{13}$C-NMR ((D$_6$)DMSO): 13.9; 22.1; 23.0; 23.3; 28.6; 30.4; 31.2; 45.3; 46.6; 53.7; 55.8; 65.6; 98.9; 105.5; 107.0; 127.0; 127.6; 136.6; 140.7; 143.8; 145.1; 152.7; 156.1; 159.5; 166.2; 173.9. ESI-MS: 680 (100, *M*$^-$).

*Ethyl 4-Cyano-6,7-dimethoxy-2-oxoquinoline-1(2*H*)-acetate* (**8**). As described for **6**, with $^i$Pr$_2$NH (0.92 ml, 6.5 mmol, 3.0 equiv.), THF (7 ml), 1.35M BuLi in hexane (4.0 ml, 5.4 mmol, 2.5 equiv.), 1,2-dihydro-6,7-dimethoxy-2-oxoquinoline-4-carbonitrile (**5**; 0.50 g, 2.2 mmol, 1.0 equiv.), THF (10 ml), and BrCH$_2$COOEt (0.50 g, 2.2 mmol, 1.0 equiv.). CC ($SiO_2$, $CH_2Cl_2$ → $CH_2Cl_2$/MeOH 98:2) afforded **8** (0.60 g, 88%). Pale green solid. M.p. 217° ([16]: 220–221°). $^1$H-NMR ((D$_6$)DMSO): 1.22 (*t*, *J* = 7.1,

*Me*$CH_2$); 3.83 (*s*, MeO); 3.94 (*s*, MeO); 4.18 (*q*, *J* = 7.1, Me$CH_2$); 5.19 (*s*, $CH_2N$); 7.00 (*s*, H–C(3)); 7.37 (*s*, H–C(8)); 8.65 (*s*, H–C(5)). APCI-MS: 317 (100, $[M+H]^+$). Spectroscopic data in accordance with [16].

*4-Cyano-6,7-dimethoxy-2-oxoquinoline-1(2*H*)-acetic Acid* (**11**). A soln. of **8** (516 mg, 1.63 mmol, 1.0 equiv.), 1M aq. NaOH (3.4 ml, 3.4 mmol, 2.1 equiv.), and EtOH (35 ml) was heated under reflux for 7 h. The solvent was evaporated, and the residue dissolved in $H_2O$ (10 ml). The mixture was acidified to pH 1–2 with conc. HCl soln. The formed precipitate was filtered, washed with $H_2O$ (100 ml), and dried by co-evaporation with MeCN. The combined aq. layer was extracted with $CH_2Cl_2$ (4 × 100 ml), the combined org. phase dried ($MgSO_4$) and concentrated, and the residue combined with the precipitate to yield **11** (348 mg, 74%; [16]: 80%). Pale green solid. M.p. 280° ([16]: 281–282°). $^1$H-NMR ((D$_6$)DMSO): 3.83 (*s*, MeO); 3.93 (*s*, MeO); 5.08 (*s*, $CH_2N$); 6.99 (*s*, H–C(3)); 7.36 (*s*, H–C(8)); 8.63 (*s*, H–C(5)). ESI-MS: 287 (100, $[M-H]^-$). Spectroscopic data in accordance with [16].

*(2*S*)-6-{2-[4-Cyano-6,7-dimethoxy-2-oxoquinolin-1(2*H*)-yl]acetamido}-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic Acid* (= N$^6$-*{2-[4-Cyano-6,7-dimethoxy-2-oxoquinolin-1(2*H*)-yl]acetyl}*-N$^2$-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]*-L-*lysine*; **17**). As described for **15**, with **11** (188 mg, 0.652 mmol, 1.0 equiv.), THF (15 ml), *N*-hydroxysuccinimide (75.2 mg, 0.652 mmol, 1.0 equiv.), *N*,*N*′-diisopropylcarbodiimide (57.5 µl, 0.652 mmol, 1.0 equiv.), DMF/$H_2O$ 9:1 (20 ml), *Titrisol* buffer® (pH 7.0; 4.5 ml), and Fmoc–Lys–OH (312 mg, 0.848 mmol, 1.3 equiv.). The pH of the suspension in $H_2O$ (70 ml) was adjusted to 1–2 with conc. HCl soln. and the resulting precipitate filtered and washed with ice water (100 ml). The residue was purified by CC ($SiO_2$, $CH_2Cl_2$/MeOH/AcOH 98:2:0.1 → 98:5:0.1): **17** (140 mg, 34% over two steps). Pale green solid. $^1$H-NMR ((D$_6$)DMSO): 1.28–1.48 (*m*, NHCHC$H_2$C$H_2$); 1.54–1.76 (*m*, CONHC$H_2$C$H_2$); 3.06–3.12 (*m*, CONHC$H_2$); 3.81 (*s*, MeO); 3.88 (*s*, MeO); 3.89–3.93 (*m*, H–C(9) of Fmoc); 4.21 (*t*, *J* = 7.01, C*H*$CO_2$H); 4.24–4.31 (*m*, C$H_2$ of Fmoc); 4.97 (*s*, $CH_2N$); 6.81 (*s*, H–C(3)); 7.29–7.34 (*m*, H–C(8), H–C(4,5) of Fmoc); 7.40 (*dd*, *J* = 7.5, 7.4, H–C(2,7) of Fmoc); 7.55 (*d*, *J* = 8.1, N*H*CH$CO_2$H); 7.72 (*dd*, *J* = 7.5, 7.1, H–C (3,6) of Fmoc); 7.88 (*d*, *J* = 7.5, H–C(1,8) of Fmoc); 8.28 (*t*, *J* = 5.4, CON*H*C$H_2$); 12.61 (br. *s*, $CO_2$H). $^{13}$C-NMR ((D$_6$)DMSO): 23.0; 28.6; 30.4; 38.5; 45.4; 46.3; 53.8; 55.8; 56.2; 65.5; 97.9; 101.0; 110.2; 112.1; 116.5; 120.0; 125.2; 127.0; 127.6; 137.7; 140.7; 143.8; 145.4; 147.4; 155.0; 156.1; 158.5; 166.0; 174.0. ESI-MS: 662 (87, $[M+Na]^+$), 639 (100, $[M+H]^+$). Anal. calc. for $C_{35}H_{34}N_3O_8 \cdot 3/2\ H_2O$ (665.69): C 64.61, H 5.47, N 8.61; found: C 64.88, H 5.98, N 8.22.

*Peptide Syntheses.* Standard peptide synthesis was employed on a 0.025 mmol scale, by using the Fmoc/$^t$Bu protocol [22] and *TentaGel S Ram* resin (*Rapp Polymere*; loading 0.24 mmol/g) with *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) [17] as a coupling reagent. Standard enantiomeric pure Boc and $^t$Bu side-chain-protected amino acids were employed. The modified amino acid building blocks **1**, **3**, **16**, and **17** were introduced with an 1.2-fold excess, with TBTU as coupling reagent and the standard protocol with an elongated coupling time of 15 h. The peptide was deprotected and cleaved from the solid support by exposure to $CF_3COOH/H_2O/^i Pr_3SiH$ 95:2.5:2.5 for 2 h. The cleavage cocktail was treated with $Et_2O$ to precipitate the peptides. The peptides **18**–**20** were finally isolated by prep. HPLC.

*Sodium {*L*-Histidyl-*L*-alanyl-*N$^6$*-{1-oxo-5-[4-(7-phenyl-1,10-phenanthrolin-4-yl-*κN$^1$,*κN$^{10}$*)phenyl]pentyl}-*L*-lysyl-*L*-tyrosyl-*L*-histidyl-*N$^6$*-{2-[6,7-dimethoxy-2-oxo-4-(trifluoromethyl)quinolin-1(2*H*)-yl]acetyl}-*L*-lysylglycinamide}bis{(1,10-phenanthroline-4,7-diyl-*κN$^1$,*κN$^{10}$*)bis[benzenesulfonato](2–)}ruthenate(2–) Chloride (4:1:2)* (**18**·2 Cl$^-$): ESI-MS: 1326 (100, $[M-4\,Na+4\,H-2\,Cl]^{2+}$), 884 (10, $[M-4\,Na+5\,H-2\,Cl]^{3+}$).

*Sodium {*L*-Histidyl-*L*-alanyl-*N$^6$*-{1-oxo-5-[4-(7-phenyl-1,10-phenanthrolin-4-yl-*κN$^1$,*κN$^{10}$*)phenyl]pentyl}-*L*-lysyl-*L*-tyrosyl-*L*-histidyl-*N$^6$*-{2-[4-cyano-6,7-dimethoxy-2-oxoquinolin-1(2*H*)-yl]acetyl}-*L*-lysylglycinamide}bis{(1,10-phenanthroline-4,7-diyl-*κN$^1$,*κN$^{10}$*)bis[benzenesulfonato](2–)}ruthenate(2–) Chloride (4:1:2)* (**19**·2 Cl$^-$): ESI-MS: 1305 (100, $[M-4\,Na+4\,H-2\,Cl]^{2+}$), 870 (20, $[M-4\,Na+5\,H-2\,Cl]^{3+}$).

*Sodium {*L*-Histidyl-*L*-alanyl-*N$^6$*-{1-oxo-5-[4-(7-phenyl-1,10-phenanthrolin-4-yl-*κN$^1$,*κN$^{10}$*)phenyl]pentyl}-*L*-lysyl-*L*-tyrosyl-*L*-histidyl-4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}-*L*-phenylalanylglycinamide}bis{(1,10-phenanthroline-4,7-diyl-*κN$^1$,*κN$^{10}$*)bis[benzenesulfonato](2–)}ruthenate(2–) Chloride (4:1:2)* (**20**·2 Cl$^-$): ESI-MS: 1355 (100, $[M-4\,Na+4\,H-2\,Cl]^{2+}$).

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