Co-crystallization of organic chromophore roseolumiflavin and effect on its optical characteristics

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Electronic Supplementary Information

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1. Synthesis of **R**

All reagents were used in reagent grade without further purification. The solvents were purchased in reagent grade or purified by conventional methods. For reactions requiring an inert atmosphere the glassware was dried in a compartment dryer at 120 °C and standard Schlenk techniques were used to work under a dry nitrogen atmosphere. Reactions were monitored by thin-layer chromatography using aluminium foil-backed silica gel from Macherey-Nagel (ALUGRAM® Xtra SIL G/ UV254) with fluorescence indicator. To concentrate solutions under reduced pressure rotary evaporators (Heidolph Instruments) in combination with membrane pumps by VACUUBRAND were used. For purifications by column chromatography silica gel 60 from Macherey-Nagel (Kieselgel 60 M, 0.04-0.063 mm) was used.

¹H- and ¹³C-NMR-spectra were measured at room temperature using a Bruker Avance III – 300 (300 MHz). The chemical shifts were standardized with traces of chloroform $(\delta(\text{CDCl}_3) = 7.26 \text{ ppm})$ or dimethylsulfoxide $(\delta(\text{DMSO-d}_6) = 2.50 \text{ ppm})$ in ¹H-spectra and with the signal of deuterated chloroform $(\delta(\text{CDCl}_3) = 77.0 \text{ ppm})$, dimethylsulfoxide $(\delta(\text{DMSO-d}_6) = 39.52 \text{ ppm})$ or formic acid $(\delta(\text{HCO}_2\text{H}) = 166.3 \text{ ppm})^1$ in ¹³C-spectra. The coupling constants *J* are given in Hertz (Hz) and the chemical shifts δ in ppm.

IR spectra were recorded using a Bruker Tensor FT/IR 37 spectrometer with ATR-unit or a Jasco FT/IR-6200 spectrometer as KBr pellet. High resolution mass spectra (ESI-HRMS) were measured with a *Bruker Daltonics* UHR-QTOF maXis 4G. For melting point determination, a Büchi Melting Point B-540 apparatus was used. DSC measurements were performed with a Linkam DSC600 at 5°C/min heating rate.

N^1 , N^3 , N^3 , 4-Tetramethylbenzene-1, 3-diamine



A modified procedure by Brown and Rizzo was followed². In a 250 mL flask N^1 , N^1 ,6-trimethylbenzene-1,3-diamine (5.00 g, 30.1 mM) was dissolved in dichloromethane (150 mL) and triethylamine (4.57 g, 6.29 mL, 45.1 mM) as well as trifluoroacetic anhydride (7.58 g, 5.02 mL, 36.1 mM) added at 0 °C. After stirring for 15 min, water (50 mL) was added and the organic layer separated. After drying over sodium sulfate, the solvent was evaporated.

The residue was dissolved in toluene (60 mL) and benzyltriethylammonium chloride (6.86 g, 30.1 mM) and dimethyl sulfate (4.74 g, 3.57 mL, 37.6 mM) added at 0 °C. Sodium hydroxide solution (50%, 30 mL) was added and stirring continued for 4 h. The reaction was quenched by addition of conc. ammonia (5 mL) and water (100 mL). The aqueous layer was extracted with toluene (3 x 100 mL), the combined organic layers dried over sodium sulfate and the solvent evaporated. Flash column chromatography (silica gel, hexane/ethyl acetate 6:1) gave the product as an orange oil (2.16 g, 12.0 mM, 40%).

¹**H NMR** (300 MHz, CDCl₃): δ [ppm] = 6.99 (dd, J = 8.0, 0.8 Hz, 1H, C(5)*H*), 6.34 (d, J = 2.4 Hz, 1H, C(2)*H*), 6.27 (dd, J = 8.0, 2.4 Hz, 1H, C(6)*H*), 3.56 (s, 1H, N*H*), 2.83 (s, 3H, NH*Me*), 2.69 (s, 6H, N*Me*₂), 2.23 (s, 3H, Ar-*Me*). ¹³**C NMR** (75 MHz, CDCl₃): δ [ppm] = 153.57, 148.40, 131.79, 120.79, 106.61, 103.62, 44.24, 31.23, 17.66. IR (thin film): v [cm⁻¹] = 3406, 2397, 2822, 2781, 1614, 1515, 1327, 1252, 1146, 1106, 796. **HRMS** (ESI): calc'd. for C₁₀H₁₇N₂ [M+H]⁺, 165.1386; found: 165.1388.

8-(Dimethylamino)-7,10-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione; Roseolumiflavin



A modified procedure by Kasai, Miura and Matsui was followed³. In a 100 mL flask, N^1 , N^3 , N^3 ,4-tetramethylbenzene-1,3-diamine (2.16 g, 12.0 mM) was dissolved in methanol (50 mL) and violuric acid added (2.77 g, 15.8 mM). The mixture was heated to reflux for 2.5 h. After cooling to rt, the brick-red precipitate was filtered off and washed with ice-cold methanol. Repeated recrystallization from acetic acid/methanol gave roseolumiflavin (1.62 g, 5.68 mM, 47%).

Mp/DSC: no melting until 330 °C, decomposition at ca. 350 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ [ppm] = 11.10 (s), 7.80 (s), 6.88 (s), 3.95 (s), 3.06 (s), 2.45 (s). ¹³C NMR (75 MHz, HCO₂H/10% D₂O): δ [ppm] = 161.21, 160.83, 151.21, 143.93, 138.07, 136.19, 135.62, 135.43, 122.34, 99.25, 44.76, 35.37, 22.06. **IR** (ATR): v [cm⁻¹] = 3380, 3250, 3129, 3063, 2984, 2799, 1690, 1633. **IR** (KBr): v [cm⁻¹] = 3570, 3517, 2835, 1719, 1700, 1620, 1548, 1522, 1382, 1272, 1096, 941. **HRMS** (ESI): calc'd. for C₁₄H₁₆N₅O₂ [M+H]⁺, 286.1299; found: 286.1304.

2. Characterization of R

The ¹H-NMR (600 MHz) shows weak signals yet significant ones are visible to confirm the presence of respective functional groups. For the magnetically equivalent methyl groups 6 and 7 at the dimethylamine substituent, we locate a singlet at 3.06 ppm (normalized integral: 6.00). Both other methyl substituents on the rings can be detected at 2.45 ppm (3.03) and 3.95 ppm (2.94). We further detect the labile amine group hydrogen 1 at 11.10 ppm (0.94) and both aryl hydrogen signals 2 and 3 at 6.88 ppm (1.02) and 7.80 (1.01), see Figure S1.



Figure S1: ¹H-NMR (600 MHz) of **R** in DMSO-d₆



Figure S2: ¹³C-NMR (75 MHz) of **R** in HCO₂H/10% D₂O



Figure S3: Infrared spectrum (ATR) of R recorded from 400 cm-1 to 4000 cm-1



Figure S4: DSC of **R** recorded up to 330°C at 5°C/min heating rate. (red – heating, blue – cooling). No melting or crystallization is detected.



Figure S5: (ESI)-HRMS spectrum of R.

3. Powder diffraction patterns of **R** and co-crystals

Purity and crystallinity of **R** are confirmed via PXRD analysis displayed in Figure S6 where one can identify characteristic peaks at 8.64°, 10.11°, 12.48°, 14.09°, 17.32° and 25.85° 2 θ . The simulated peak at 26.3° is assigned to the signal at 25.85° since the pattern is slightly shifted in the range between 25° to 35° 2 θ . The remaining peaks match well with simulated data.



Figure S6: Experimental and simulated from single crystal data PXRD patterns of **R** (Cu K α radiation)

The powder patterns of **R:A** (Figure S7) are in good agreement with the simulated single crystal patterns and fair crystallinity and purity can be confirmed. We identify significant peaks at 6.29° , 11.59° , 13.55° , 25.22° , 25.97° and 27.78° 2 θ . Figure S7 illustrates the powder patterns of the experimental and simulated data. The highest signals are slightly shifted by 0.1° to 0.4° 2 θ , however, intensities and positions match well overall. Since merely the peak at $7.75^{\circ}2\theta$ stands out and does not fit either compound (Figure S10) we assume it emerged from impurities.



Figure S7: Experimental patterns of R:A and R and simulated from single crystal powder pattern of R:A (Cu Ka radiation)

The diffraction patterns of both single crystal and experimental powder sample of **R:B** match well, suggest good crystallinity and do not indicate any phase impurity. Most significant peaks are detected at 12.93° , 16.63° , 16.94° , 24.28° , 24.96° , 26.65° and 26.94° 2 θ , see Figure S8.



Figure S8: Experimental patterns of **R:B** and **R** and simulated from single crystal powder pattern of **R:B** (Cu K α radiation) The significant reflexes of **R:C** are found at 6.14°; 11.47°; 12.64°; 24.84° and 27.15° 2 θ (Figure S9). The shift of the reflections at higher angles could be caused either by the sample preparation or by differences in measurement temperatures (SCXRD at 100 K and PXRD at approx. 298 K).



Figure S9: Experimental powder patterns of R:C and R and simulated from single crystal pattern of R:C (Cu Ka radiation)



Figure S10: Experimental powder pattern of R:A compared to experimental patterns of R and A (Cu Ka radiation)



Figure S11: Experimental powder pattern of R:B compared to experimental patterns of R and B (Cu Ka radiation)



Figure S12: Experimental powder pattern of R:C compared to experimental patterns of R and C (Cu Ka radiation)

4. Thermograms of **R** and co-crystals



Figure S13: Thermograms of R:A and R measured between 30-600 °C, nitrogen purge gas, heating rate 10 °C/min.



Figure S14: Thermograms of **R:B** and **R** measured between 30-600 °C, nitrogen purge gas, heating rate 10 °C/min.



Figure S15: Thermograms of R:C and R measured between 30-600 °C, nitrogen purge gas, heating rate 10 °C/min.

5. IR-spectra of co-crystals

The infrared spectrum of **R**:**A** (Figure S16) contains two broad bands in the range between 3600 cm⁻¹ to 3300 cm⁻¹ as well as 3300 cm⁻¹ to 2600 cm⁻¹ which overlap most of the characteristic bands of **R**. This observation is a good indication for the presence of O - H bonds, which occur ubiquitously in the crystal structure. We further notice blue-shifted C = O bands at 1694 cm⁻¹ by +4 cm⁻¹, indicating a shortening of the C = O bond, which may be the effect of the newly built hydrogen bonds with adjacent co-former molecules that leads to the reorganization of the interaction strength and causes a significant shift of the vibrational band. A comparison with the **A** component indicates significant broadening of the O - H band in the co-crystal compared to pure **R**, yet the occurrence of two bands at 3505 cm⁻¹ and 3439 cm⁻¹ suggests that the extent of overall hydrogen bonding decreased, so these bands are originally likely overlapped in pure **A** which due to the presence of two carboxylic functional groups assumably contains extensive amounts of hydrogen bonding interactions.



Figure S16: Comparison of IR spectra of **R**: **A** with **R** and **A**. The fingerprint area (wavenumbers $<1500 \text{ cm}^{-1}$) is cut out for clarity. Characteristic bands are highlighted.

Infrared data of **R:B** (Figure S17) shows significant red-shifts for the amide N – H stretching bands at 3359 cm⁻¹ and 3237 cm⁻¹ with -13 cm⁻¹ to -21 cm⁻¹ compared to the **R** spectrum. Further, the C = 0 vibrational bands are also red-shifted to 1683 cm⁻¹ and 1623 cm⁻¹. The significant prolongation of the C = 0 and N – H bonds likely arise from both halogen bonds and hydrogen bonds connecting to the same donor atoms, so that the halogen bonds weaken the hydrogen bond interaction strength. A similar effect was reported by Vasylyeva et al. where N-methylacetamid was co-crystallized with several XB donors including **B** and both XB and HB connected to the same C = 0 functional group, causing red-shifted C = 0 stretching bands⁴.

A comparison with the co-former spectrum in fingerprint area is shown Figure S19. The C – I asymmetrical stretch at 762 cm⁻¹ signifies a blue-shift by +4 cm⁻¹ and the C – F stretch at 941 cm⁻¹ is slightly blue-shifted by +2 cm⁻¹. The C – C stretching vibrational band at 1455 cm⁻¹ is slightly red-shifted with -3 cm⁻¹ compared to the free co-former indicating increased electron density in the fluorophenyl rest.



Figure S17: Comparison of IR spectra of **R:B** with **R**. The fingerprint area (wavenumbers $<1500 \text{ } \text{cm}^{-1}$) is cut out for clarity. Characteristic bands are highlighted.



Figure S18: Comparison of IR spectra of **R:B** with **B**. Wavenumbers >2000 cm^{-1} are cut out for clarity. Characteristic bands are highlighted.

The IR spectrum of **R**:**C** displays a broad, red-shifted band at the 3400 cm⁻¹ to 3300 cm⁻¹ region which represents the symmetric and asymmetric N - H valence vibrations of the respective compounds. The red-shift is a result of longer N - H bonds in the co-crystal due to the formation of HB between the amino groups of **C** and the imide group of **R** or between the

co-formers. The band representing the C = 0 vibration at 1688 cm⁻¹ is red-shifted by -2 cm⁻¹ compared to **R** indicating a slight increase in the bond length.



Figure S19: Comparison of IR spectra of **R**:**C** with **R** and **C**. The fingerprint area (wavenumbers $<1500 \text{ cm}^{-1}$) is cut out for clarity. Characteristic bands are highlighted.



6. Lifetimes, excitation and emission spectra of **R**, co-crystals and coformers

Figure S20: Normalized absorption and emission spectra of R, R:A, R:B and R:C in acetonitrile at room temperature



Figure S21: Normalized absorption and emission spectra of R, R:A, R:B and R:C in chloroform at room temperature



Figure S22: Normalized absorption and emission spectra of R, R:A, R:B and R:C in methanol at room temperature



Figure S23: Normalized absorption and emission spectra of A in acetonitrile, chloroform and methanol at room temperature



Figure S24: Normalized absorption and emission spectra of B in acetonitrile, chloroform and methanol at room temperature



Figure S25: Normalized absorption and emission spectra of C in acetonitrile, chloroform and methanol at room temperature



Figure S26: Normalized solid-state excitation and emission spectra of A at room temperature



Figure S27: Normalized solid-state excitation and emission spectra of **B** at room temperature



Figure S28: Normalized solid-state excitation and emission spectra of C at room temperature



Figure S29: Normalized solid-state excitation and emission spectra of **R** at room temperature



Figure S30: Normalized solid-state excitation and emission spectra of R:A at room temperature



Figure S31: Normalized solid-state excitation and emission spectra of R:B at room temperature



Figure S32: Normalized solid-state excitation and emission spectra of R:C at room temperature



Figure S33: Normalized solid-state luminescence decay curves of **A** with instrument response function (IRF), acquired at room temperature



Figure S34: Normalized solid-state luminescence decay curves of C with instrument response function (IRF), acquired at room temperature

No luminescence decay curves for co-former **B** could be measured as the required excitation wavelength is below 375 nm and no pulsed EPL laser diode sources with UV wavelengths were available for respective time-resolved measurements at our institute.

7. References

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