Synthesis of Model Chromophores Related to the Gold Fluorescent Protein (GdFP)

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lin-4-ones 8 and 9 were expectedly in the moderate range.4,10 The reaction proceeds stepwise, i.e., via nucleophilic amine-induced azlactone ring opening and subsequent ring closure to the imidazolinone. Using \( N \)-methylamine as amine, ring-opened intermediate 10 (Figure 2) could be isolated if the reaction was stopped after four hours. The assignment of the two distinct amide NH protons was possible and NOE experiments established the spatial proximity of the NHAc group and the proton at C-2 of the indole.

The reduction of the nitro group was eventually conducted with hydrogen in the presence of Lindlar catalyst (5% \( \text{Pd/ CaCO}_3/ \text{Pb} \)). Other attempts to produce 4-aminoindoles 2 and 3 from the nitroindoles 8 and 9 met with little success. Reduction experiments with hydrogen and other catalysts or with \( \text{SnCl}_2 \) in EtOH and EtOAc led to partial or complete degradation.

Product 2 was obtained as intensely red colored solid, and 3 as equally intense oil. They are well soluble in organic solvents and UV spectra of compound 2 were recorded (Figure 3). The compound exhibits a hypsochromic extinction maximum relative to GdFP (Tris-buffer: 20 mM tris(hydroxymethyl)aminomethane HCl, pH 8) with the hypsochromic shift depending on the solvent. Absorption maxima were determined to be at 417 nm in MeCN, at 430 nm in \( \text{H}_2\text{O} \) and at 442 nm in EtOH as the solvent. The peak width at half-height of the absorption band is about 4700 cm\(^{-1}\). It is slightly larger than the peak width at half-height of the GdFP band but independent of the solvent. Similarly, the extinction coefficient \( e \) is identical in EtOH and MeCN (ca. 10000 L mol\(^{-1}\) cm\(^{-1}\)). The fluorescence properties of model compound 2 are currently being studied.

Upon irradiation at 419 nm (irradiation source: RPR 4190 \( \text{Å} \)) product (\( Z \))-2 underwent geometrical isomerization into the corresponding E-isomer (Scheme 2). Under our conditions (DMSO-\( \text{d}_6 \) as solvent, 35 °C) a photostationary state was reached after 30 minutes. The \( Z/E \) ratio was determined by NMR as 60:40. There was no indication for other photochemical processes.

In conclusion, the model chromophores 2 and 3 were obtained by conventional condensation and functional group transformation chemistry. The azlactone formation and nitro group reduction were closely studied. Optimized conditions are reported for these transformations. Further photophysical and biophysical data of the chromophores will be reported in due course.

All reactions involving water-sensitive chemicals were carried out in flame-dried glassware with magnetic stirring under argon. All solvents, EtOAc, \( \text{CH}_2\text{Cl}_2 \) and MeOH for column chromatography.

**Scheme 1** Synthesis of model compounds 2 and 3 starting from commercially available nitroaniline 4

**Scheme 2** \( E/Z \)-Isomerization of compound (\( Z \))-2

**Figure 2** Chemical shift data for the relevant protons in compound 10 and observed NOE contact

**Figure 3** Comparison between the normalized absorption spectra of GdFP and of the model chromophore 2 in various solvents
were distilled prior to use. Acetylglycine was prepared from glycine according to literature precedence.\textsuperscript{14} N-(tert-Butyoxycarbonyl)-1,6-diaminohexane was obtained from 1,6-diaminohexane.\textsuperscript{15} All other chemicals were commercially available and were used without further purification.

TLC: Merck glass sheets (0.25 mm silica gel 60, F\textsubscript{254}, eluent given in brackets. Detection by UV or coloration with cerium ammonium molybdate (CM)). NMR: Bruker AV-250, AV-360, AV-500. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded in DMSO-\textit{d}\textsubscript{6} at ambient temperature, unless stated otherwise. Chemical shifts are reported relative to tetramethylsilane as internal standard. Apparent multiplets which occur as a result of the accidental equality of coupling constants of magnetically nonequivalent protons are marked as virtual (vrt.). The multiplicities of the \textsuperscript{13}C NMR signals were determined by DEPT experiments. IR: PerkinElmer 1600 FT-IR. MS: Finnigan MAT 8200 (EI).

4-(N-Acetyl-4-nitroindol-3-ylmethylene)-2-methyloxazolin-5-one (7)
A suspension of acetylglycine\textsuperscript{14} (358 mg, 3.06 mmol) and NaOAc (233 mg, 2.84 mmol) in Ac\textsubscript{2}O (2.50 mL, 2.72 g, 26.6 mmol) was stirred for 1 h at 80 °C. 3-Formyl-4-nitroindole (6, 500 mg, 2.63 mmol) was added and the mixture was stirred at 140 °C for 30 min. The dark red mixture was cooled overnight in the refrigerator (4 °C). The brownish precipitate was filtered, washed with H\textsubscript{2}O and dried in vacuo; mp 228–235 °C (dec.). The precipitate was filtered, washed with H\textsubscript{2}O and CH\textsubscript{2}Cl\textsubscript{2}, and dried in vacuo; mp >250 °C (dec.).

\textsuperscript{1}H NMR (250 MHz): \( \delta = 2.00 \) (s, 3 H), 2.67 (d, \( J = 4.5 \) Hz, 3 H), 7.32 (t, \( J = 8.2 \) Hz, 1 H), 7.42 (s, 1 H), 7.73 (q, \( J = 4.5 \) Hz 1 H), 7.82 (d, \( J = 8.2 \) Hz, 1 H), 7.84 (d, \( J = 8.2 \) Hz, 1 H), 7.91 (s, 1 H), 9.16 (s, 1 H), 12.28 (br s, 1 H).

\textsuperscript{13}C NMR (62.9 MHz): \( \delta = 23.0 \) (q), 26.1 (q), 117.6 (d), 117.8 (s), 118.3 (d), 120.8 (d), 122.3 (d), 126.2 (s), 130.7 (d), 138.1 (d), 142.1 (s), 165.3 (s), 169.4 (s).

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13C NMR (90.6 MHz): δ = 152.0 (q), 75.9 (q), 71.9 (q), 71.8 (q), 68.9 (q).

UV/Vis (EtOH): λmax (%) = 294 (100), 277 (100), 247 (11), 235 (10), 195 (3), 184 (3).

HRMS (EI): m/z calc for C13H14N3O2: 248.1015; found: 248.1011.

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