Three-dimensional visualization of contact networks in granular material
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Synthesis of the solvatochromic and rigidochromic fluorescent probes

This chapter describes the synthesis and characterization of the solvatochromic and rigidochromic chromophores that are used in this project. For the visualization of force networks in granular systems, molecules are needed that are sensitive to the contact forces between surfaces. As discussed in chapter 2, various molecules possess the desired photophysical properties, and are therefore promising candidates to deal with varying contact forces. Target solvatochromic probe 17 and rigidochromic probe 33 are functionalized with a linking moiety, to be able to connect them to the surfaces. Thus, carboxylic acid-functionalized solvatochromic probe 46 and rigidochromic probe 58 were synthesized in a total yield of 42 % (5 steps) and 19 % (4 steps), respectively. Their basic photophysical properties were shown to be practically identical to those of the parent fluorophores. Linear relationships between the Stokes shift and the medium polarity (for solvatochromic amide 47) and between the log intensity and the log medium viscosity (for rigidochromic amine 33). The carboxylic acid linkers are ideally suited for the functionalization of glass cover slips and PMMA granules via a peptide coupling, as discussed in chapter 5.
4.1. Introduction: medium sensitivity of fluorescence

4.1.1. Solvatochromism

As outlined in chapter 2, a plethora of fluorescent dyes exists that are sensitive to changes in their environment.[1-6] One particular class of those dyes are the solvatochromic probes.[7-9] These molecules show changes in their absorption or emission characteristics, or in both, when the polarity of the medium is changed.[10,11] Solvatochromism occurs when the chromophore undergoes a significant change of dipole moment (ground state $\mu_g$ to excited state $\mu_e$) upon excitation. When $\mu_e > \mu_g$, the excited state is more stabilized in polar solvents, leading to increased solvent relaxation and thus to longer emission wavelengths in more polar solvents. This bathochromic shift is referred to as positive solvatochromism (see section 2.3.1. and figure 2.2). Positive solvatochromism is most pronounced in fluorescence, because solvent relaxation stabilizes the excited states and destabilizes the ground state. An example of positive solvatochromic dyes are the Fluoroprobes.[12]

Opposite to a bathochromic shift with increasing solvent polarity is a hypsochromic shift. This phenomenon is termed negative solvatochromism, in which the absorption and emission maxima shift to the blue. Reichardt’s dye, which has been used to define an empirical solvent polarity scale ($E_I(30)$) is an example of this latter category.[13,14] This dye has a highly dipolar ground state structure, and a less dipolar excited state.

9-Amino substituted perylene monoimides such as 17 have good solvatochromic sensitivity (figure 4.1).[15] This molecule exhibits the behavior of a push-pull conjugated system, with a modest solvatochromic shift in absorption and a significant shift in fluorescence, as is described in section 2.3.3.1.

Figure 4.1. Solvatochromic perylene probe 17.
The absorption and emission spectra of 17 have been recorded in a series of solvents, which showed that its absorption maximum ranges from 521 nm (measured in cyclohexane) to 556 nm (in methanol), and its emission maximum from 637 nm (cyclohexane) to 746 nm (methanol). The quantum yields of 17 decrease with increasing solvent polarity; $\Phi_F = 0.48$ in cyclohexane and $\Phi_F = 0.22$ in methanol.

Granules functionalized with solvatochromic probes are envisioned to display different fluorescent properties at the “contact points”, where the particles touch each other, than at the “non-contact points”. Because of this, solvatochromic probes are promising candidates for the visualization of force networks in granular materials. In this respect, it is expected that the fluorescent properties at the contact points are dependent on the amount of force applied to the granular system. Two assumptions lie behind this idea.

First of all, it is supposed that at the contact points between the particles the polarity is locally lower than at the non-contact points. This is due to the fact that the solvent is squeezed out of this area and therefore less solvent molecules are present to stabilize the excited state of the solvatochromic probe. If this is the case, less solvent relaxation will occur. Therefore, it is expected that at the contact points between particles a hypsochromic shift in emission maxima will be observed. This shift may be larger when more force is applied to the granules, as more solvent will be pushed away, resulting in a less polar environment.

Secondly, the extent of solvatochromism is dependent on the viscosity of the medium. Complete solvent stabilization around the excited state can only be reached when the solvent molecules have enough mobility. Thus, in highly viscous media, relaxation of the solvent takes more time than the lifetime of the excited state and therefore cannot be fully completed. The solvent molecules cannot reorient themselves fully around the dipole. It is envisioned that, when force is applied to a system of granular particles functionalized with a solvatochromic probe, the regions of the particle that experience force, i.e. where they touch each other, will be more rigid. Due to this locally increased viscosity, a solvatochromic probe in that region can be expected to exhibit a hypsochromic shift in emission wavelength.

4.1.2. Rigidochromism

Related to solvatochromism is rigidochromism, in which the fluorescence properties are also dependent on the viscosity of the system. The difference between the two phenomena is that for solvatochromism the wavelength of emission changes, while for rigidochromism the quantum yield varies. Although the term suggests otherwise (chromism refers to color change), rigidochromism does not necessarily lead to change in emission wavelength. A small blue shift is nevertheless often observed for rigidochromic probes in more viscous media.
Probes suitable for rigidochromism experiments typically have a low quantum yield in solution, because non-radiative deactivation pathways are available. Mostly, this is because of the presence of an internal motion, such as a rotation around a single bond. When the viscosity of the medium rises, these motions become more hampered, and thus the non-radiative decay processes become less available. Therefore, more molecules will be deactivated via photon emission, resulting in a rise in quantum yield.

The blue shift is observed for compounds with a large dipole moment in the excited state, analogous to solvatochromic probes. As explained above, the excited state is stabilized by reorientation of the solvent. In rigid media, this reorientation is significantly slowed down or even completely halted. Therefore, the excited state cannot be stabilized completely, which result in a blue shift of the emission wavelength.[17]

Rigidochromic probes should also be effective in visualization of the forces between particles. In chapter 2, various rigidochromic probes were discussed, such as the class of dicyanomethylenedihydrofuran (DCDHF) probes, which was recently developed by the groups of Moerner and Twieg.[18-20] A representative example, which is used in the present project, is compound 33 shown in figure 4.2, which shows excellent rigidochromic behavior.[21]

![33]

Figure 4.2. Rigidochromic reference compound 33; $\lambda_{abs} = 486$ nm; $e = 7.1 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ (toluene); $\Phi_t$ (PMMA) = 0.92; $\Phi_t$ (ethanol) = 0.0066.[21]

Compound 33 was first used in photorefractive organic glasses, where it was shown that it has very high photorefractive gain coefficients in a polyvinylcarbazole matrix and that it is able to form an amorphous glass by itself.[22] Compound 33 has an amine donor group, which is connected to the $\pi$-accepting DCDHF unit, via a $\pi$-conjugated linker, in this case a phenyl ring. This molecule is an example of a push-pull chromophore, like compound 17. Therefore, compounds such as 33 are expected to exhibit solvatochromic behaviour as well, analogous to compound 17. The change in dipole moment, expressed as $\mu_e - \mu_g$, for compound 33 amounts to 4.4 D.[23] The length of the $\pi$-conjugated linker has an influence on the extent of solvatochromism of these probes; when going from a phenyl linker to the larger naphtalene and
anthracene linkers, the solvatochromic sensitivity is increased.\textsuperscript{[18]} Longer $\pi$-conjugated linkers afford greater charge separation in the excited state, leading to enhanced solvatochromism, as discussed in section 4.1.1. Thus, compound 33 exhibits the smallest solvatochromic behavior, when compared to the longer $\pi$-conjugated analogs. Next to the small amount of solvatochromic behavior, compound 33 exhibits strong rigidochromic behavior; the fluorescence quantum yield increases dramatically when the viscosity of the medium increases. In figure 4.3, this rigidochromic behavior of compound 33 is visualized. When dissolved in a solvent (two vials at the top), hardly any fluorescence can be observed. However, when 33 is placed in a more viscous medium, such as a PMMA matrix (two cover slips at the bottom), the quantum yield rises and intense fluorescence can be observed. For example, the quantum yield of 33 in ethanol is only 0.0066, while in a PMMA matrix, it is 0.92, a 140-fold increase.

The mechanism of rigidochromism for compound 33 is depicted in figure 4.4. An important role in the mechanism of this rigidochromic behavior is a twisted intramolecular charge transfer state C that causes non-radiative decay to the $S_0$ state. When the molecule is excited to the $S_1$ state (A), two possible relaxation pathways are available. First of all, the structure of the molecule can relax while the molecule remains in its planar configuration (B). Secondly, a rotation of 90° of the dicyano group around the methylene bond affords twisted structure C. Calculations have shown that the latter pathway gives the most stable structure.\textsuperscript{[23]} In low viscous solvents, like ethanol or toluene, this rotation towards C occurs freely, without an energy barrier. From C, a non-radiative decay to D is available, as the $S_0$ state is very close in energy at this point. The presence of a twisted double bond leads to this dramatic rise in energy of the ground state (D). Thus, in low viscous media, a rigidochromic probe will decay non-radiatively via C and D. However, when the molecule resides in a more viscous medium, such as a PMMA matrix, the rotation around the methylene bond in the excited state is hindered, and the molecule relaxes towards structure B, and starts to fluoresce. No other decay pathways are available.
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Figure 4.4. Rigidochromism of compound 33. Compound 33 can relax from its vertically excited state A to B or to C. When the molecule relaxes to C, a twist around the methylene bond produces a low energy twisted excited state, from which the molecule can relax back non-radiatively to the ground state. In viscous media, this twist is hindered and relaxation to the planar state B becomes more competitive and fluorescence emission can compete with the non-radiative pathway; thus the fluorescence quantum yield increases.[19]

It is expected that a rigidochromic chromophore as force sensitive dye could sense the small variations in viscosities at the contact points. Locally, the viscosity of the medium may be even further increased, because of the application of a force. Such small differences should be observable, as 33 is very sensitive towards viscosity increases. Thus, it is envisioned that at the contact points where the particles touch, the quantum yield is slightly higher than at the non-contact points. The brightness of the spots, as well as the fluorescence lifetimes, may tell something about the magnitude of the force in that specific point.

4.2. The solvatochromic chromophore

4.2.1. Synthesis
To be able to attach perylene monoimide 17 to PMMA beads or glass slides, it is necessary that 17 is modified with a functional unit that is able to perform a coupling reaction without affecting the probe and the different surfaces. Especially PMMA is
sensitive to most organic solvents, and can only withstand DMSO or water. Therefore, a peptide coupling is ideally suited to couple the functionalized probe to the surface. In this respect, it is necessary to functionalize the probe with a carboxylic acid moiety, as PMMA and glass are readily functionalized with a primary amine moiety (see chapter 5).

The synthetic pathway that is followed to synthesize carboxylic acid functionalized probe 46 is depicted in scheme 4.1. First of all, commercially available perylene derivative 40 is converted into perylene monoimide 41 via a modified literature procedure. Then 41 was converted into 9-bromo-N-(2,6-di-tert-butylphenyl)-perylen-3,4-dicarboximide (42) in quantitative yield via a reported procedure. In this reaction, 41 was treated with Br₂ in the presence of I₂ and acetic acid under reflux. The amine moiety is introduced via a Buchwald-Hartwig reaction between 42 and the butyl ester of piperidine-4-acetic acid 44, resulting in 9-piperidine perylene monoimide 45. In this reaction, Pd(III) precatalyst PEPPSI is in situ reduced to the active Pd(0) with K3PO4, and subsequently couples amine 44 to arylbromide 42 in 95 % yield. It is necessary to protect compound 43, as the carboxylic acid moiety interferes with the Buchwald-Hartwig reaction. The carboxylic acid moiety is regained after hydrolysis of 45 with KOH in water and THF, thus to obtain the suitable building block 46 for the peptide synthesis in 89 % yield.

\[ \text{Scheme 4.1. Synthetic pathway for compound 46.} \]

---

i) tert-butylaniline, imidazole, Zn(OAc)₂, H₂O, 24 h, 190 °C; ii) HOAc, Br₂, I₂, MeOH, 5 h, rt; iii) 1: SOCl₂, 1.5 h, 45 °C; 2: BuOH, 24h, rt; 3: 1M NaOH (aq); iv) PEPPSI, K₃PO₄, butyl 2-(piperidin-4-yl)acetate, toluene/DMF (3/1 (v/v)), 24 h, 120 °C; v) 2M KOH, THF, 80 °C, 48 h.
Compound 46 is subjected to a test reaction with butylamine, to check if the carboxylic acid moiety of compound 46 can be employed in a peptide coupling, which is needed for the surface modification as described in chapter 5. The resulting amide-containing compound 47 (see figure 4.5) was successfully synthesized in high yield, and can be used as a reference for the solvatochromic probe connected to the glassy surfaces. The purity of 47 was confirmed by HPLC-FS to be ~98%. The electronic structure of this compound is very similar to that of the immobilized target probe. Therefore, the solvatochromic properties of 47 were investigated in solvents of different polarities.

Figure 4.5. Reference compound 47.

4.2.2. Photophysical properties
The photophysical properties of carboxylic acid compound 46 were investigated in solvents of varying polarity. Emission maxima ranged from $\lambda_{em} = 699$ nm in toluene ($\Delta f = 0.026$) to $\lambda_{em} = 738$ nm in DMSO ($\Delta f = 0.527$). Fluorescence quantum yields decreased with increasing solvent polarity ($\Phi_F = 0.41$ (toluene); $\Phi_F = 0.18$ (DMSO)). The fluorescence lifetime $\tau$ amounted to 2.9 – 3.3 ns. A lifetime of 3.29 ns was observed at a concentration of $10^{-7}$ M in DMSO, while at $10^{-4}$ M the lifetime was slightly shortened to 3.11 ns. This small difference could imply that some self-quenching occurs at higher concentrations.

Likewise, the absorption and emission spectra were recorded for amide 47 in several solvents, which are depicted in figure 4.6. A bathochromic shift is observed in both absorption and emission spectra upon increasing the polarity of the solvent. In absorption, this is a shift of 23 nm, starting from $\lambda_{abs} = 514$ nm in cyclohexane to $\lambda_{abs} = 538$ nm in methanol. The solvatochromic behavior in the emission is much bigger, shifting from $\lambda_{em} = 632$ nm to $\lambda_{em} = 737$ nm, in the same solvents. These values are comparable with those of compound 17.
Compounds 17 and 47 are examples of push-pull conjugated molecules, having a piperidine donor site and an imide acceptor site. These compounds form a class of fluorophores with a moderate extent of solvatochromic behavior. They exhibit reasonable ground state dipole moments, which enables solvent polarization. As the excited state dipoles are oriented in the same direction as the ground state dipoles, a modest solvatochromic shift is observed in the absorption maxima. The solvent dipoles interact more strongly with the excited state dipoles of the fluorophore, as these dipoles are more pronounced. This results in a larger solvatochromic shift in the emission maxima.

In table 4.1, a comparison is made between the absorption and emission maxima and the quantum yields of compounds 17 and 47. In analogy to compound 17, compound 47 also exhibits solvatochromic behavior. The variations in absorption and emission maxima between the various solvents are comparable to those of compound 17. The most apparent change with respect to the parent compound is a hypsochromic shift of several nanometers over the entire range of solvents, in both absorption and emission maxima. This implies a slightly bigger HOMO-LUMO gap for compound 47, which may be caused by the presence of the amide moiety. This is an electron withdrawing group, which may slightly reduce the donor capacity of the piperidine nitrogen, despite its remoteness. Furthermore, the quantum yields of 47 are slightly higher compared to 17.

To further characterize the solvent sensitivity of fluorophore 47, the Stokes shift is plotted against the orientation polarizability function $\Delta f$ of the solvent in figure 4.7. A comparison is made with the unfunctionalized analogue 17. For 17 and 47, the linear relationship between Stokes shift and $\Delta f$ is given by equations 4.1 and 4.2 respectively.
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17: Stokes shift = 2047 (± 290) × Δf + 3508 (± 129)  
47: Stokes shift = 2222 (± 233) × Δf + 3809 (± 103)  

(Eq. 4.1)  
(Eq. 4.2)

The plot of compound 47 is slightly steeper and located higher in energy than the corresponding plot of compound 17. Thus, compound 47 experiences larger energy differences and can be regarded as slightly more solvatochromic than compound 17. The larger Stokes shift of 47 for all polarities implies that this compound experiences more relaxation, either from the solvent or internally.

Figure 4.7. Stokes shift versus polarity for compounds 17 and 47.

Table 4.1. Photophysical properties of 17 and 47 in several solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Δf[a]</th>
<th>λ_abs (nm)</th>
<th>λ_em (nm)</th>
<th>Φ_F</th>
<th>λ_abs (nm)</th>
<th>λ_em (nm)</th>
<th>Φ_F[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclohexane</td>
<td>0.000</td>
<td>521</td>
<td>637</td>
<td>0.48</td>
<td>514</td>
<td>632</td>
<td>0.54</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.026</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>525</td>
<td>669</td>
<td>---</td>
</tr>
<tr>
<td>Diisopropylether</td>
<td>0.290</td>
<td>526</td>
<td>671</td>
<td>0.40</td>
<td>519</td>
<td>670</td>
<td>0.46</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.291</td>
<td>549</td>
<td>704</td>
<td>0.43</td>
<td>533</td>
<td>693</td>
<td>0.45</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.399</td>
<td>535</td>
<td>704</td>
<td>0.33</td>
<td>523</td>
<td>704</td>
<td>0.39</td>
</tr>
<tr>
<td>THF</td>
<td>0.419</td>
<td>538</td>
<td>709</td>
<td>0.30</td>
<td>524</td>
<td>704</td>
<td>0.44</td>
</tr>
<tr>
<td>DCM</td>
<td>0.434</td>
<td>552</td>
<td>719</td>
<td>0.37</td>
<td>536</td>
<td>710</td>
<td>0.44</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.527</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>540</td>
<td>743</td>
<td>0.33</td>
</tr>
<tr>
<td>DMF</td>
<td>0.549</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>534</td>
<td>735</td>
<td>---</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.569</td>
<td>535</td>
<td>725</td>
<td>0.29</td>
<td>527</td>
<td>722</td>
<td>0.42</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.611</td>
<td>546</td>
<td>735</td>
<td>0.32</td>
<td>532</td>
<td>731</td>
<td>0.38</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.617</td>
<td>556</td>
<td>746</td>
<td>0.22</td>
<td>538</td>
<td>737</td>
<td>0.22</td>
</tr>
</tbody>
</table>

[a] Δf = f(ε) − f(n²); f(ε) according to equation 2.4; f(n²) according to equation 2.5.
[b] using Perylene Red as standard (Φ_F = 0.96).  

[27]
The slope of the linear relationship between $\Delta f$ and the Stokes shift for 47 is determined to be 2222 cm$^{-1}$, which results in a $\mu_e - \mu_g$ of 5.57 D using equation 2.7. This value is slightly higher than the corresponding value of 5.35 D for compound 17.\textsuperscript{[28]} Thus, the difference in dipole moment upon excitation is larger for compound 47, resulting in a larger extent of solvatochromism.

Hydrostatic pressure ranging from 1 to 3900 bar was applied to a solution of 47 in acetonitrile, and fluorescence emission and excitation spectra were recorded. The spectra are depicted in figure 4.8 and the corresponding maxima and associated Stokes shifts are listed in table 4.2.

The pressure is envisioned to influence the polarity of the medium; the dielectric constant of the solvent rises slightly with increasing pressure, as the density increases and the intermolecular distances shorten. Likewise, the refractive index rises with increasing pressure in view of the increasing density. Srinivasan and Kay developed Tait-like equations for determining the dielectric constant $\varepsilon$ and density $\rho$ of acetonitrile at a given pressure $P$ (equations 4.3 and 4.4).\textsuperscript{[29]} The refractive index ($n$) in turn is dependent on the density via the Clausius-Mossotti equation 4.5.\textsuperscript{[30]}

\[
1 - \frac{\varepsilon}{\varepsilon_p} = 0.10879 \ln \left( \frac{952.6 + P}{953.6} \right) \quad \text{(Eq. 4.3)}
\]

\[
1 - \frac{\rho}{\rho_p} = 0.10185 \ln \left( \frac{920 + P}{921} \right) \quad \text{(Eq. 4.4)}
\]

\[
\frac{n^2 - 1}{n^2 + 2} = \frac{\rho N_A \alpha}{3 M \varepsilon_0} \quad \text{(Eq. 4.5)}
\]

In the Clausius-Mossotti equation, $N_A$ is Avogadro’s constant, $\alpha$ is the polarizability of the medium, $M$ is the molar mass of the acetonitrile molecules and $\varepsilon_0$ is the vacuum

![Figure 4.8](image)

**Figure 4.8.** Excitation and emission spectra of compound 47 in acetonitrile at various pressures in bar; $10^{-6}$ M; $\lambda_{exc} = 530$ nm; $\lambda_{em} = 730$ nm.
permittivity. The values for the dielectric constant and the refractive index of acetonitrile are determined at each pressure and given in Table 4.2. Using \( f(\varepsilon) \) according to equation 2.4 and \( f(n^2) \) according to equation 2.5, the \( \Delta f \) is determined (\( \Delta f = f(\varepsilon) - f(n^2) \)) and also given in Table 4.2. An increase in pressure thus leads to a small decrease in \( \Delta f \).

\( \Delta f \) may be further lowered when the density of the medium increases in view of the increased formation of dipole-dipole dimers, in which two molecules of acetonitrile align their dipoles antiparallel. As a consequence, dipole moments cancel to some extent, lowering the polarity of the medium with increasing density.

When the pressure is increased from 1 bar to 3900 bar, a 14 nm bathochromic shift in the emission maximum occurs, and a 12 nm shift in excitation maximum, which are consistent with a rise in polarity of the medium (Table 4.2). A reverse correlation is observed with \( \Delta f \) because its value decreases while a red-shift is observed in the emission and excitation maxima. The \( \Delta f \) of 0.567 of the medium at 3000 bar is comparable with that of acetone at 1 bar (\( \Delta f = 0.569 \)). However, the emission wavelength is significantly red-shifted at 3000 bar in acetonitrile (\( \lambda_{em} = 740 \text{ nm} \)), compared to the 1 bar values in acetone (\( \lambda_{em} = 722 \text{ nm} \); see Table 4.1). However, as the excitation maxima shift to a lesser extent than the emission maxima, the observed Stokes shift decreases with increasing pressure (see Table 4.2). Thus, the change in Stokes shift agrees with a lowering in \( \Delta f \) with increasing pressure. Figure 4.9
shows a plot of the observed Stokes shifts versus the calculated values of $\Delta f$ (using equations 4.3 – 4.5). A linear relationship could be established as given in equation 4.6.

Stokes shift = 4584 (± 352) × $\Delta f$ + 2209 (± 208)  
(Eq. 4.6)

Strikingly, the slope of this relationship is significantly increased compared to the relationship of equation 4.2, which describes the extent of solvatochromity of compound 47 in varying solvents. Thus, it seems that the fluorescence of compound 47 is more sensitive towards changes in pressure than towards changes in solvent.

![Figure 4.9. Stokes shifts of compound 47 versus medium polarity at various pressures.](image)

4.3. The rigidochromic chromophore

4.3.1. Synthesis
The synthesis of 33 is depicted in scheme 4.2. First of all, 48 is protected via the method by Kobler and Effenberger. In this procedure, DMF was used as solvent, but as this is difficult to remove, DCM was used instead. After addition of the reactants at 0 °C, the mixture was stirred overnight at room temperature. Then, the reaction mixture was extracted with DCM and dried over MgSO$_4$. Compound 49 was obtained as light brown oil in 73 % yield.

Compound 51 was synthesized via commercially available compound 50. In this reaction, a large excess of base and bromohexane is needed, especially to accomplish the second alkyl coupling. In the first attempt, 4.5 equivalents of base and 5 equivalents of bromohexane were used, but such excess turned out to be insufficient, as $^1$H NMR showed that the major product was the mono-coupled product. Therefore, the reaction was done again with additional base and bromohexane. $^1$H NMR analysis showed that bromohexane had completely reacted away. Probably, this compound co-
evaporates with hexane during work-up, as the boiling point of bromohexane is only 155 °C. After 16 hours stirring at 115 °C, the $^1$H NMR proved the exclusive presence of the di-coupled product. After column chromatography, (eluent PE/EA 50/1 (v/v)), the fractions with $R_f = 0.76$ were collected and 51 was obtained as yellow oil in 45 % yield.

In the next step, 51 was lithiated and reacted with 2-methyl-2-trimethylsilyloxypropionitrile (49) to form compound 52.[22] In this reaction, compound 51 was dissolved in dry THF and cooled to −78 °C. Then, n-BuLi was added dropwise and after 1 hour of stirring, compound 49 was added. The reaction mixture was stirred for 12 hours at room temperature after which the reaction was quenched with water and HCl and stirred for another 12 hours. After work-up and column chromatography (eluent gradient from PE/EA 25/1 (v/v) to EA 100 %), the last fractions were collected ($R_f = 0$ in PE/EA 50/1 (v/v)) and dried. The product 52 was obtained as brown oil in 74 % yield.

In the last step to obtain probe 33, a Knoevenagel reaction was performed, followed by a ring closure and another Knoevenagel reaction. In a first attempt, the conditions described by Lu et al.[20] were used, but this attempt was unsuccessful. During crystallization, only a very minor amount of red compound 33 precipitated from the solution. Another reaction procedure by Gubler et al. was successful.[22] Molecular sieves were introduced in the reaction mixture to capture the liberated water, and crystallization from hexane afforded 33 a red powder in 12 % yield.

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {50};
\node (B) at (1.5,0) {51};
\node (C) at (3,0) {52};
\node (D) at (4.5,0) {33};
\node (E) at (0,1.5) {48};
\node (F) at (1.5,1.5) {49};

\draw[->] (A) -- (B);
\draw[->] (B) -- (C);
\draw[->] (C) -- (D);
\draw[->] (E) -- (F);
\end{tikzpicture}
\end{center}

\textit{i) 2-hydroxy-2-methylpropanenitrile, imidazole, Cl-TMS, DCM, rt, overnight ii) 1-bromohexane, $K_2CO_3$, DMF, 110 °C, 10 h; iii) 1: n-BuLi, −78 °C, 1 h; 2: 49, −78 °C to rt, overnight; 3: HCl (2M), rt, 6 h; iv) CH$_2$(CN)$_2$, pyridine, rt, overnight.}

\textbf{Scheme 4.2.} Reaction pathway to obtain compound 33.

In order to be suitable for the present project, compound 33 has to be functionalized with a linking moiety to enable coupling to the surfaces of glass or PMMA. Again, the carboxylic acid moiety is employed, which is perfectly suited for a peptide coupling with an amine-functionalized surface. This carboxylic acid moiety is best introduced as substituent of one of the amine linkers of 33, as these provide the most flexibility. The dicyano part of the target probe is rigidochromically sensitive.
Synthesis of the solvatochromic and rigidochromic fluorescent probes

Carboxylic acid-functionalized analogues of 33 are synthesized as depicted in scheme 4.3. Here, a fluorine atom is used to allow the introduction of the amine in the last step. Amines functionalized with a carboxylic acid moiety enable attachment of the probe to the solid support.

A Grignard reaction is performed between compounds 53 and 49, which is sensitive to water and air. After work-up, $^1$H NMR showed that the desired product (54) was formed, but also some starting material 53 was still present. Based on the ratios between the peaks of product and starting material, the yield of the reaction was determined to be 66%.

No further purification was performed, instead 54 was reacted with malononitrile in pyridine, in the presence of a few drops of acetic acid and ammonium acetate. The mixture was stirred for 24 h at room temperature, after which $^1$H NMR showed that compound 54 was completely converted to 55, while starting material 53 remained unchanged in the product mixture.
Next, three carboxylic acid functionalized rigidochromic probes $56 \rightarrow 58$ were synthesized. For the synthesis of $56$, sarcosine was introduced via a nucleophillic aromatic substitution. Wang et al. reported the synthesis of a closely related analogue of $56$, having a second acetic acid moiety instead of a methyl, which they obtained in 45 % yield.$^{[33]}$ Using their approach, i.e. reacting $55$ and the amine for 24 h at 40 °C in pyridine, compound $56$ was obtained together with a lot of by-products. Better results were expected for $57$ and $58$, as the carboxylic acid moiety is placed further away from the amine group, as such enhancing its nucleophilicity. In addition, the temperature of the reaction was raised to 60 °C. The reaction towards compound $58$ was completed within 30 minutes, which was indicated by a color change of the reaction mixture to red. For compound $57$, the reaction mixture had completely turned red in 24 h. For the reaction towards compound $56$, the mixture still had not turned completely red after 48 h. Thin Layer Chromatography analysis showed that $57$ and $58$ were formed almost exclusively, while compound $56$ was formed together with several by-products. Because of the increased efficiency in terms of rate and purity, it was chosen to use compound $58$ for the functionalization of the surfaces of glass and PMMA.

4.3.2. Photophysical properties

In figure 4.10, absorption and emission spectra of rigidochromic probe $33$ are depicted in several solvents with different polarities. A bathochromic shift is observed in both absorption and emission spectra upon increasing the polarity of the solvent. For the absorption maxima, a shift of 18 nm is observed between 486 nm for toluene and 504 nm for DMSO. A more pronounced shift of 32 nm is observed in the emission maxima, ranging from 507 nm in toluene to 539 nm in DMSO. Similarly, the absorption and emission spectra of the carboxylic acid-functionalized compound $58$ are depicted in figure 4.11. Compound $58$ behaves more or less similarly as unmodified $33$. These compounds are far less solvatochromic than compound $46$ and $47$, the carboxylic acid and amide derivatives of the solvatochromic probe $17$.

Table 4.3 lists photophysical properties of compounds $33$ and $58$ in several solvents of varying polarity and viscosity. As already observed in figures 4.11 and 4.12, both compounds exhibit moderate solvatochromism. In addition, a slight decrease in quantum yield with increasing solvent polarity could be observed, for both compounds. No correlation between the viscosity of the solvent and the emission wavelength or the quantum yield could be observed. A plot of the Stokes shift versus the orientation polarizability $\Delta f$ of the solvent is given in figure 4.12. Despite the large spread, linear correlations could be established, which are given in equation 4.7 and 4.8 for compound $33$ and $58$ respectively.
Synthesis of the solvatochromic and rigidochromic fluorescent probes

Figure 4.10. Normalized absorbance (solid line) and emission (dashed line) spectra of compound 33 in several solvents; $\lambda_{exc} = 475$ nm.

Figure 4.11. Normalized absorbance (solid line) and emission (dashed line) spectra of compound 58 in several solvents; $\lambda_{exc} = 475$ nm.

33: Stokes shift = $502 \pm 216 \times \Delta f + 1107 \pm 98$ (Eq. 4.7)
58: Stokes shift = $-1284 \pm 492 \times \Delta f + 2727 \pm 224$ (Eq. 4.8)

The slope of this linear relationship for 33 of 502 cm$^{-1}$ could be used to determine a dipole moment difference $\mu_e - \mu_g$ of 1.5 D (using equation 2.7). Notably, the slope for 58 is negative ($-1284$ cm$^{-1}$), which is not in accordance with equation 2.7. The error in the linear relationship between the observed Stokes shifts and $\Delta f$ is relatively big, and we may conclude that the value for $\mu_e - \mu_g$ is negligible for compound 58. Alternatively, the negative slope could be related to a solvent-polarity-induced change in electronic structure.\cite{15}
### Table 4.3. Photophysical properties of compounds 33 and 58.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\Delta f$ [^a]</th>
<th>$\eta$ [^b] (mPa\ s)</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\Phi_t$ [^c]$</th>
<th>$\tau$ [^d]$ (ns)</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\Phi_t$</th>
<th>$\tau$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-dioxane</td>
<td>0.023</td>
<td>1.18</td>
<td>482</td>
<td>518</td>
<td>0.081</td>
<td>---</td>
<td>457</td>
<td>514</td>
<td>0.090</td>
<td>0.44</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.026</td>
<td>0.56</td>
<td>486</td>
<td>508</td>
<td>0.035</td>
<td>0.40</td>
<td>463</td>
<td>508</td>
<td>0.028</td>
<td>0.23</td>
</tr>
<tr>
<td>Diisopropylether</td>
<td>0.290</td>
<td>0.30</td>
<td>482</td>
<td>507</td>
<td>0.021</td>
<td>---</td>
<td>477</td>
<td>510</td>
<td>0.027</td>
<td>0.29</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>0.345</td>
<td>0.69</td>
<td>486</td>
<td>520</td>
<td>0.075</td>
<td>---</td>
<td>468</td>
<td>522</td>
<td>0.053</td>
<td>0.38</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.399</td>
<td>0.42</td>
<td>488</td>
<td>523</td>
<td>0.029</td>
<td>---</td>
<td>482</td>
<td>525</td>
<td>0.018</td>
<td>0.22</td>
</tr>
<tr>
<td>Cyclohexanol</td>
<td>0.467</td>
<td>57.5</td>
<td>---</td>
<td>537</td>
<td>0.106</td>
<td>---</td>
<td>495</td>
<td>529</td>
<td>0.118</td>
<td>0.83</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.527</td>
<td>1.99</td>
<td>504</td>
<td>540</td>
<td>0.008</td>
<td>0.026</td>
<td>489</td>
<td>533</td>
<td>0.006</td>
<td>0.016</td>
</tr>
<tr>
<td>1-butanol</td>
<td>0.527</td>
<td>2.54</td>
<td>497</td>
<td>528</td>
<td>0.023</td>
<td>---</td>
<td>498</td>
<td>528</td>
<td>0.018</td>
<td>0.15</td>
</tr>
<tr>
<td>DMF</td>
<td>0.549</td>
<td>0.79</td>
<td>500</td>
<td>533</td>
<td>0.004</td>
<td>---</td>
<td>496</td>
<td>537</td>
<td>0.004</td>
<td>---</td>
</tr>
<tr>
<td>2-propanol</td>
<td>0.551</td>
<td>2.04</td>
<td>495</td>
<td>524</td>
<td>0.018</td>
<td>---</td>
<td>501</td>
<td>528</td>
<td>0.015</td>
<td>0.17</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.611</td>
<td>0.37</td>
<td>495</td>
<td>530</td>
<td>0.002</td>
<td>---</td>
<td>474</td>
<td>527</td>
<td>0.002</td>
<td>---</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.617</td>
<td>0.54</td>
<td>496</td>
<td>531</td>
<td>0.003</td>
<td>---</td>
<td>494</td>
<td>531</td>
<td>0.003</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\[^a\] $\Delta f = f(\varepsilon) - f(n^2)$; $f(\varepsilon)$ according to equation 2.4; $f(n^2)$ according to equation 2.5.

\[^b\] Literature values are: $\Phi_t = 0.003$ (acetonitrile); 0.005 (methanol); 0.011 (DMSO); 0.100 (toluene); 0.034 (2-propanol); 0.049 (1-butanol). \[^23\]

\[^c\] Value in toluene is from the literature, \[^23\] and in DMSO is measured using SPC.

\[^d\] Measurements with streak camera, except for DMSO for which SPC was used.

---

![Figure 4.12. Stokes shift versus polarity for compounds 33 and 58.](image)

The rigidochromic behavior of compound 33 is investigated in several solvents with a melting point between 10 °C and 26 °C. Compound 33 was first excited at room temperature and emission spectra were recorded in the liquid state (low viscosity). Subsequently, the samples were cooled to below the melting point of the solvent (high
viscosity), and the emission spectra were recorded again. Table 4.4 displays the emission maxima and intensities of 33 at the different temperatures, from which a significant increase in fluorescence intensity upon freezing is evident. Quantitative determination of the rigidochromic behavior was not possible, as the solid solvents were melting during recording of the emission spectra.

Table 4.4. Photophysical properties of 33 in liquid and frozen solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Mp</th>
<th>(\lambda_{em}) (nm)</th>
<th>(I) ((10^4) counts)</th>
<th>(\lambda_{em}) (nm)</th>
<th>(I) ((10^4) counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-dioxane</td>
<td>11.8</td>
<td>512</td>
<td>21</td>
<td>515</td>
<td>67</td>
</tr>
<tr>
<td>Cyclooctane</td>
<td>14.6</td>
<td>495</td>
<td>0.8</td>
<td>504</td>
<td>3.1</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>16.6</td>
<td>528</td>
<td>0.6</td>
<td>526</td>
<td>3.1</td>
</tr>
<tr>
<td>DMSO</td>
<td>18.4</td>
<td>542</td>
<td>2.6</td>
<td>540</td>
<td>3.3</td>
</tr>
<tr>
<td>Cyclohexanol</td>
<td>25.2</td>
<td>526</td>
<td>27</td>
<td>526</td>
<td>147</td>
</tr>
<tr>
<td>tert-Butanol</td>
<td>25.5</td>
<td>528</td>
<td>8.4</td>
<td>530</td>
<td>18</td>
</tr>
</tbody>
</table>

The pressure sensitivity of compound 33 is investigated by recording emission spectra at various pressures. These measurements were done using the high pressure set-up, as discussed in chapter 3, which enables the sample to experience pressures up to 4000 bar. Compound 33 is dissolved in acetonitrile, and the emission spectra at the different pressures are depicted in figure 4.13. Clearly, the application of greater pressure causes an increase in emission intensity. Strangely, the intensity at 1 bar is significantly higher than at 200, 400 and 600 bar. This deviation is caused by a defect in the high pressure set-up below 1000 bar, which leads to a strongly reduced light transmission. Figure 4.14 shows the excitation spectra at various pressures.

A full description of the relationship between the pressure on a liquid and the viscosity of that liquid is not yet available. Numerous intermolecular interactions, such as Van der Waals forces, hydrogen bonding, coulombic interactions and entanglement interplay to determine the viscosity. To date, several (semi-)empirical expressions of the pressure-viscosity relationship have been established, such as the Arrhenius plot, the rolling sphere technique, the power law and the Free Volume equation.\(^{35-37}\) A relatively simple linear relationship between pressure \(P\) and viscosity \(\eta\) is given by equation 4.9.\(^{38}\)

\[
\eta = \eta_0 \left(1 + \alpha (P - P_0)\right) \tag{Eq. 4.9}
\]
Figure 4.13. Emission spectra of compound 33 in acetonitrile at various pressures in bar; $10^{-6}$ M; $\lambda_{\text{exc}} = 495$ nm.

Figure 4.14. Excitation spectra of compound 33 in acetonitrile at various pressures in bar; $10^{-6}$ M; $\lambda_{\text{em}} = 530$ nm.

In equation 4.9, $\eta_0$ is the viscosity of the liquid at reference pressure $P_0$ (for acetonitrile: $\eta_0 = 0.324$ mPa s at $P_0 = 1$ bar) and $\alpha$ is the empirically determined viscosity pressure coefficient, which amounts to 0.624 mPa s kbar$^{-1}$ for acetonitrile at a temperature of 25 °C. Increasing the pressure from 1 bar to 4000 bar gives rise to an increase in viscosity from 0.324 to 1.133 mPa s.

In table 4.5, the pressure and associated emission wavelength and relative intensity are given. The theoretical relationship between the quantum yield $\Phi_F$ of a dye and the viscosity $\eta$ of the medium is given by the Förster-Hoffmann equation (equation 4.10), in which $C$ is a temperature dependent constant and $X$ is a dye dependent constant. The larger $X$, the larger the viscosity dependency of the quantum yield and the larger the rigidochromic nature of the dye. Assuming that fluorescence intensity $I$ is
directly proportional to $\Phi_F$, the values of table 4.5 can be used to determine $X$ for compound 33. Using the values at 1000 bar and above, a linear relationship between \( \log(\eta) \) and \( \log(I) \) is established (figure 4.15 and equation 4.11). Using equation 4.11, $X$ for compound 33 is determined to be 1.04. In comparison with two extensively studied julolidine derivatives, bearing two cyano moieties or one cyano and one carboxylic acid moiety (both have $X = 0.60$), compound 33 is significantly more rigidochromic.\[^{40-42}\]

\[ \log(\Phi_F) = X \times \log(\eta) + C \]  
\[ \log(I) = 1.04 (\pm 0.01) \times \log(\eta) + 1.123 (\pm 0.002) \]

(Eq. 4.10)  
(Eq. 4.11)

**Table 4.5.** Photophysical properties of 33 in acetonitrile at various pressures.

<table>
<thead>
<tr>
<th>$P$ (bar)</th>
<th>$\eta$[^a]</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$I/I_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.324</td>
<td>527</td>
<td>1.0</td>
</tr>
<tr>
<td>200</td>
<td>0.364</td>
<td>530</td>
<td>0.4</td>
</tr>
<tr>
<td>400</td>
<td>0.405</td>
<td>531</td>
<td>0.4</td>
</tr>
<tr>
<td>600</td>
<td>0.445</td>
<td>532</td>
<td>0.5</td>
</tr>
<tr>
<td>800</td>
<td>0.486</td>
<td>535</td>
<td>0.8</td>
</tr>
<tr>
<td>1000</td>
<td>0.526</td>
<td>534</td>
<td>1.4</td>
</tr>
<tr>
<td>1500</td>
<td>0.627</td>
<td>532</td>
<td>1.7</td>
</tr>
<tr>
<td>2000</td>
<td>0.728</td>
<td>535</td>
<td>1.9</td>
</tr>
<tr>
<td>2500</td>
<td>0.829</td>
<td>536</td>
<td>2.2</td>
</tr>
<tr>
<td>3000</td>
<td>0.930</td>
<td>536</td>
<td>2.5</td>
</tr>
<tr>
<td>3500</td>
<td>1.031</td>
<td>536</td>
<td>2.8</td>
</tr>
<tr>
<td>4000</td>
<td>1.133</td>
<td>537</td>
<td>3.2</td>
</tr>
</tbody>
</table>

\[^a\] calculated using $\eta = \eta_0 \left[ 1 + \alpha(P - P_0) \right]$ with $\alpha = 0.624$ mPa s kbar$^{-1}$.\[^{38}\]  

![Figure 4.15.](image) Log-log relationship between viscosity and fluorescence intensity of compound 33 in acetonitrile.
4.4. Conclusion
In this chapter, the synthesis of two potentially pressure sensitive probes is described. The carboxylic acid derivatives of solvatochromic probe 17 and rigidochromic probe 33 have been successfully prepared. Compound 46, bearing a carboxylic acid moiety on the amine donor part of solvatochromic 17, was synthesized in five steps from 40 and 43, in a total yield of 42%. Similarly, compound 58, bearing a carboxylic acid moiety on the amine donor part of rigidochromic 33, was synthesized in four steps from 48 and 53, in a total yield of 19%. Compounds 47 and 58 are modified in such a way that easy coupling to glass and PMMA surfaces is feasible, as will be discussed in the next chapter.

Fluorescence spectroscopy provided evidence of the solvatochromic behavior of functionalized compounds 46 and 47, as well as of the rigidochromic behavior of compounds 58 and 33. Reference amide 47 exhibited pressure dependent emission and excitation maxima. Notably, the variation in Stokes shift could be attributed to a decrease in Δf of the medium with increasing pressure. Rigidochromic reference compound 33 exhibits a linear relationship between the logarithm of viscosity of the medium and the logarithm of intensity of the fluorescence, in accordance with the Förster-Hoffmann equation. The rigidochromic constant X could be determined to be 1.04 for 33. As such, the intensity could be used to determine the pressure applied to the system. These findings are promising for the ultimate goal of the present project, the visualization of force networks in granular systems, caused by the application of force.

4.5. Experimental section

4.5.1. General remarks
Unless stated otherwise, reactions were carried out under an atmosphere of nitrogen or argon. Dry solvents were distilled using standard procedures. All commercially available chemicals were used without further purification.

Flash chromatography refers to chromatographic purification on a column of Biosolve silica gel 60 Å (30 – 65 μm) with the eluent indicated. Rf values were obtained by using thin layer chromatography (TLC) on silica coated plastic sheets (Merck Silica Gel 60 F254). The compounds were visualized by UV light (254 or 366 nm).

Nuclear magnetic resonance spectra (1H NMR and 13C NMR) were determined in the indicated solvent using a Bruker ARX 400 (1H: 400 MHz, 13C: 100 MHz) at a temperature of 25 °C. Peak shapes in the 1H NMR spectra are indicated with the symbols: ‘s’ (singlet), ‘bs’ (broad singlet), ‘d’ (doublet), ‘dd’ (double doublet), ‘t’ (triplet), ‘dq’ (double quartet), ‘quint’ (quintet), ‘sext’ (sextet) and ‘m’ (multiplet). Chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane (TMS) and coupling
constants $J$ are given in Hertz (Hz). $^{13}$C NMR spectra are measured in the APT mode. The numbering of the atoms of the perylene core of the solvatochromic molecules and the rigidochromic chromophores are depicted in figure 4.16 (A) and figure 4.16 (B) respectively.

![Figure 4.16. General numbering of the atoms of (A) the solvatochromic probes and (B) the rigidochromic probes for $^1$H and $^{13}$C NMR characterization.](image)

**High resolution mass spectra** (HRMS) were recorded using Fast Atom Bombardment (FAB) ionization on a JEOL JMS SX/SX102A four-sector mass spectrometer, coupled to a JEOL MS-MP9021D/UPD system, equipped with a Xenon primary atom beam utilizing a 3-nitrobenzyl matrix. Electron Impact (EI) spectra were recorded on a JEOL DX-303 double focusing mass spectrometer (operating at an ionization potential of 70 eV) coupled to a JEOL MP-7000 data system.

**High performance liquid chromatography** (HPLC) was performed using a LC-10AT liquid chromatograph (Shimadzu) over a silica column (ReproSil 100Si, $\varnothing = 5$ $\mu$m, Dr. Maisch) controlled by system controller SCL-10A VP (eluent = mixture of heptane/DCM/acetone 8/1/1 (v/v/v), pressure = 20 bar, time of measurement = 30 min). Emission was set at 700 nm and excitation spectra were recorded, which showed a signal at 500 nm (RF-10A XL fluorescence detector, Shimadzu).

**Steady state absorption** is described in detail in section 3.1.1. All solvents used for recording the absorption spectra were of spectrograde quality. Absorption spectra were recorded on a Varian Cary 3E spectrophotometer (190 – 900 nm). The spectra were recorded in 1 cm quartz cuvettes, using a scan speed of 100 nm/min.

**Steady state fluorescence** is described in detail in section 3.1.2. All solvents used for recording the fluorescence spectra were of spectrograde quality. Emission and excitation spectra were recorded on a Spex Fluorolog 3 spectrometer. Fluorescence was detected in the right angle mode, with a concentration where the absorbance at
\( \lambda_{\text{exc}} \) was low (< 0.1), using 1 cm quartz cuvettes. An excitation wavelength of 525 nm was used for the solvatochromic compounds and 495 nm for the rigidochromic compounds. Perylene Red in chloroform (\( \Phi_f = 0.96 \))\(^{[27]} \) or Perylene Orange in acetonitrile (\( \Phi_f = 0.98 \))\(^{[44]} \) were used as reference for the determination of the quantum yields. Quantum yields were calculated with equation 3.3.

**Streak Camera** measurements are described in detail in section 3.3. All solvents used for determining the fluorescence life times were of spectrograde quality. Quartz cuvettes of 1 cm were used.

**Fluorescence lifetimes** for compound 46 were recorded using the confocal microscopy set-up described in section 3.2.3.1. The measurements in DMSO for compounds 33, 47 and 58 are recorded using an SPC set-up as described in literature.\(^{[45]} \)

A detailed description of the **high pressure set-up** measurements is given in section 3.4. Emission and excitation spectra were recorded in the right angle mode, using a concentration of 10\(^{-6}\) M in acetonitrile. An excitation wavelength of 525 nm was used for the solvatochromic compounds and 495 nm for the rigidochromic compound. The integration time was 1 sec/nm. Waiting times in between the measurements were at least 15 minutes, to allow pressure to equilibrate.

### 4.5.2. Synthesis of the solvatochromic compounds

**N-(2,5-di-tert-butylphenyl)perylene-3,4-dicarboximide (41)**\(^{[24]} \)

![N-(2,5-di-tert-butylphenyl)perylene-3,4-dicarboximide (41)]

Compound 41 was prepared using a modification of a literature procedure.\(^{[24]} \) A 100 ml autoclave glass vessel was filled with 15.0 g (38.2 mmol, 1.7 eq) perylene bisanhydride, 4.50 g (21.9 mmol, 1 eq) tert-butylaniline, 77.0 g (1.13 mol, 52 eq) imidazole, 18.5 g (84.3 mmol, 3.8 eq) Zn(OAc)\(_2\) \( \cdot \) \( \text{H}_2\text{O} \) and 33 ml distilled \( \text{H}_2\text{O} \). The autoclave, closed and put into an oven at 190 °C for 24 h. After cooling to room temperature, the autoclave was carefully opened and the reaction mixture was collected by rinsing with EtOH. The solvent was evaporated and the solid placed in the fridge overnight. The black solid was grinded and purified using soxhlet extraction with 200 ml DCM overnight. 50 ml of 2 M HCl were added and the emulsion filtered over celite. The organic phase was washed three times with 50 ml of 2 M HCl and with 50 ml brine. The organic layer was dried over MgSO\(_4\) and the solvent evaporated. A dark brown powder was obtained. The yield was not determined.

\( ^{1}H\text{ NMR} \) (400 MHz, CDCl\(_3\)):

<table>
<thead>
<tr>
<th>Peak</th>
<th>ppm</th>
<th>J (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.67</td>
<td>(d, 3J(_{HH}) = 8.0 Hz, 2H, ( \text{H}^2\text{S} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>8.49</td>
<td>(d, 3J(_{HH}) = 7.2 Hz, 2H, ( \text{H}^{1,6} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>8.47</td>
<td>(d, 3J(_{HH}) = 7.2 Hz, 2H, ( \text{H}^{7,12} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>7.94</td>
<td>(d, 3J(_{HH}) = 8.1 Hz, 2H, ( \text{H}^{8,10} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>7.67</td>
<td>(t, 3J(_{HH}) = 7.9 Hz, 2H, ( \text{H}^{8,11} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>7.61</td>
<td>(d, 3J(_{HH}) = 8.5 Hz , 1H, ( \text{H}^{3} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>7.48</td>
<td>(dd, 3J(<em>{HH}) = 10.8 Hz, 4J(</em>{HH}) = 2.2 Hz, 1H, ( \text{H}^{4} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>7.06</td>
<td>(d, 3J(_{HH}) = 2.2 Hz, 1H, ( \text{H}^{6} ))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>1.36</td>
<td>(s, 9H, ortho-C(CH(_3))(_3))</td>
<td>( ^{1}H )</td>
</tr>
<tr>
<td>1.32</td>
<td>(s, 9H, meta-C(CH(_3))(_3))</td>
<td>( ^{1}H )</td>
</tr>
</tbody>
</table>
9-bromo-N-(2,5-di-tert-butylphenyl)perylen-3,4-dicarboximide (42)[25]

1.00 g (1.96 mmol, 1 eq) of 41 was suspended in 13 ml of acetic acid for 30 min. After adding 24.0 mg (94.5 μmol, 0.05 eq) of I₂ and 1.25 g (7.82 mmol, 4 eq) of Br₂, the mixture was stirred for 4.5 h at room temperature under light exclusion. The excess of bromine was removed by bubbling argon into the flask for 15 minutes and the mixture was precipitated by addition of 15 ml of MeOH and stirred overnight. The product was filtrated and washed with 20 ml MeOH. The filtrate was collected by dissolving it in DCM. Removal of the solvent afforded 1.21 g of a red powder (2.06 mmol, 100%).

**1H NMR** (400 MHz, CDCl₃): δ (ppm) = 8.68 (d, 3JHH = 6.4 Hz, 1H, H7'), 8.66 (d, 3JHH = 6.4 Hz, 1H, H7'), 8.50 (d, 3JHH = 7.5 Hz, 1H, H6'), 8.47 (d, 3JHH = 8.1 Hz, 1H, H7'), 8.43 (d, 3JHH = 8.1 Hz, 1H, H7'), 8.33 (d, 3JHH = 8.4 Hz, 1H, H7'), 8.25 (d, 3JHH = 8.2 Hz, 1H, H6'), 7.92 (d, 3JHH = 8.1 Hz, 1H, H10'), 7.74 (t, 3JHH = 8.0 Hz, 1H, H11'), 7.61 (d, 3JHH = 8.6 Hz, 1H, H3'), 7.48 (dd, 3JHH = 10.7 Hz, 4JHH = 2.2 Hz, 1H, H5'), 7.06 (d, 3JHH = 2.1 Hz, 1H, H6'), 1.36 (s, 9H, ortho-C(CH₃)₃), 1.32 (s, 9H, meta-C(CH₃)₃).

**13C NMR** (100 MHz, CDCl₃): δ (ppm) = 164.8 (C=O), 150.0 (C9'), 143.8 (Cq), 136.8 (Cq), 136.7 (Cq), 133.0 (Cq), 132.0 (CH), 131.9 (CH), 131.3 (CH), 130.2 (Cq), 130.0 (CH), 129.7 (Cq), 129.0 (Cq), 129.1 (Cq), 128.7 (CH), 128.2 (CH), 127.8 (CH), 126.6 (Cq), 126.2 (CH), 124.4 (CH), 123.7 (CH), 123.3 (CH), 121.8 (Cq), 121.8 (Cq), 120.7 (CH), 120.5 (CH), 35.5 (ortho-C(CH₃)₃), 34.2 (meta-C(CH₃)₃), 31.7 (ortho-C(CH₃)₃), 31.3 (meta-C(CH₃)₃).

**butyl 2-(piperidin-4-yl)acetate (44)**

2-(piperidin-4-yl)acetic acid hydrochloride (43) (181 mg, 1.01 mmol, 1 eq) was stirred in 10 ml SOCl₂ for 2 h at 45 °C. The SOCl₂ was evaporated and 20 ml of butanol was added. This mixture was stirred for 24 h at room temperature. The butanol was evaporated and ethyl acetate was added. The organic layer was washed with 1 M NaOH solution. 185 mg of a yellow oil was obtained (0.93 mmol, 92%).

**1H NMR** (400 MHz, CDCl₃): δ (ppm) = 4.09 (t, 3JHH = 6.7 Hz, 2H, H9') 3.08 (d, 3JHH = 12.1 Hz 2H, H7'), 2.64 (t, 3JHH = 11.4 Hz, 2H, H7'), 2.24 (d, 3JHH = 7.1 Hz, 2H, H6'), 1.96-1.90 (m, 1H, H7'), 1.87 (bs, 1H, H5'), 1.71 (d, 3JHH = 13.3 Hz, 2H, H3'), 1.62 (quint, 3JHH = 7.1 Hz, 2H, H10'), 1.40 (sex, 3JHH = 7.6 Hz, 2H, H11'), 1.19 (dq, 3JHH = 12.2 Hz, 4JHH = 3.6 Hz, 2H, H5'), 0.95 (t, 3JHH = 7.3 Hz, 3H, H12').

**13C NMR** (100 MHz, CDCl₃): δ (ppm) = 172.6 (C=O), 64.0 (C9'), 46.2 (C7'), 41.7 (C11'), 33.2 (C6'), 32.9 (C10'), 30.5 (C3'), 18.8 (C11'), 13.5 (C12').

**HRMS** (FAB) m/z = 200.1655 (calculated for C₁₁H₁₂NO₂: 200.1651); 200.17 [M+H]⁺ (100).
9-(4-(butylcarboxymethyl)piperidin-1-yl)-N-(2,5-di-tert-butylphenyl)perylene-3,4-dicarboximide (45)

Compound 42 (908 mg, 1.54 mmol, 1 eq), compound 44 (307 mg, 1.54 mmol, 1 eq), PEPSI (64.0 mg, 94.2 µmol, 0.06 eq), K$_3$PO$_4$ (925 mg, 4.36 mmol, 2.8 eq) were dissolved in toluene/DMF mixture 3/1 (v/v). The mixture was heated to 120 °C with stirring for 24 h, subsequently cooled to room temperature and the solvent removed under reduced pressure. The organic layer was dissolved in DCM and washed with water. The organic layer was dried with MgSO$_4$ and the solvent evaporated. The crude product was flash chromatographed (eluent: PE/DCM 1/4 (v/v)). The purple spot ($R_t = 0.26$) was collected and the solvent evaporated. 550 mg of a dark purple powder was obtained (0.78 mmol, 51 %), together with 158 mg recovered 42 ($R_t = 0.80, 0.27$ mmol, 17 %).

$^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) = 8.65 (t, $^3$J$_{HH}$ = 7.9 Hz, 2H, H$^{2,5}$), 8.52 (d, $^3$J$_{HH}$ = 7.2 Hz, 1H, H$^3$), 8.45 (t, $^3$J$_{HH}$ = 7.4 Hz, 2H, H$^{1,12}$), 8.38 (d, $^3$J$_{HH}$ = 8.4 Hz, 1H, H$^1$), 8.25 (d, $^3$J$_{HH}$ = 8.2 Hz, 1H, H$^8$), 7.67 (t, $^3$J$_{HH}$ = 7.9 Hz, 1H, H$^{11}$), 7.60 (d, $^3$J$_{HH}$ = 8.5 Hz, 1H, H$^3$), 7.46 (dd, $^3$J$_{HH}$ = 8.8 Hz, $^4$J$_{HH}$ = 2.4 Hz, 1H, H$^4$), 7.24 (d, $^3$J$_{HH}$ = 8.5 Hz, 1H, H$^{10}$), 7.03 (d, $^3$J$_{HH}$ = 2.0 Hz, 1H, H$^5$), 4.16 (t, $^3$J$_{HH}$ = 6.6 Hz, 2H, H$^9$') 3.56 (d, $^3$J$_{HH}$ = 11.4 Hz, 2H, H$^{2,6'}$), 2.93 (quint, $^3$J$_{HH}$ = 11.1 Hz, 2H, H$^{3,5'}$), 2.43 (d, $^3$J$_{HH}$ = 7.1 Hz, 2H, H$^{7''}$), 2.12-2.10 (m, 1H, H$^{11''}$), 2.00 (d, $^3$J$_{HH}$ = 6.9 Hz, 2H, H$^{3''}$), 1.98-1.64 (m, 4H, H$^{3'',5'',10''}$), 1.47-1.39 (m, 2H, H$^{11''}$), 1.36 (s, 9H, ortho-C(CH$_3$)$_3$), 1.32 (s, 9H, meta-C(CH$_3$)$_3$), 1.01 (t, $^3$J$_{HH}$ = 7.7 Hz, 3H, H$^{12''}$).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ (ppm) = 172.6 (C=O), 165.0 (C=O), 153.6 (C$^0$), 149.9 (C$^3$), 143.7 (C$^6$), 138.0 (C$_q$), 137.9 (C$_q$), 133.2 (C$_q$), 132.0 (CH), 131.8 (CH), 130.5 (C$_q$), 129.6 (C$_q$), 129.2 (C$_q$), 129.1 (C$_q$), 128.6 (CH), 127.8 (CH), 127.0 (CH), 126.6 (C$_q$), 126.1 (CH), 126.0 (CH), 124.9 (CH), 124.1 (CH), 123.7 (C$_q$), 120.9 (C$_q$), 119.9 (C$_q$), 119.7 (CH), 118.9 (CH), 115.8 (CH), 64.3 (C$^9$), 53.6 (C$^{2''}$,6$''$), 41.3 (C$''$), 35.5 (ortho-C(CH$_3$)$_3$), 34.2 (meta-C(CH$_3$)$_3$), 33.1 (C$^{4''}$), 32.5 (C$^{10''}$), 31.7 (ortho-C(CH$_3$)$_3$), 31.2 (meta-C(CH$_3$)$_3$), 30.7 (C$^{3''}$), 19.2 (C$^{11''}$), 13.7 (C$^{12''}$).

HRMS (FAB): m/z = 707.3846 (calculated for C$_{44}$H$_{52}$N$_2$O$_4$: 707.3849); 706.38 [M$^+$] (100); 707.38 [M+H$^+$] (95).

9-(4-(carboxymethyl)piperidin-1-yl)-N-(2,5-di-tert-butylphenyl)perylene-3,4-dicarboximide (46)

Compound 45 (118 mg, 167 µmol, 1 eq) was dissolved in 10 ml THF. 1.0 ml of a solution of 2 M KOH (115 mg, 2.05 mmol, 12 eq) in water was added. This was refluxed for 24 h. After 16 h and 19 h, water (1 ml) was added to keep the KOH in solution. After cooling to room temperature, the crude product was precipitated in HCl (2 M, 75 ml), and the red precipitate was filtered. Then the compound was extracted with DCM and
washed with water, dried over MgSO₄ and the solvent evaporated. The product was obtained as a purple powder (94 mg, 144 µmol, 89%).

1H NMR (400 MHz, CDCl₃): δ (ppm) = 8.64 (t, JHH = 7.8 Hz, 2H, H₃), 8.50 (d, JHH = 7.6 Hz, 1H, H₄), 8.43 (t, JHH = 7.8 Hz, 2H, H₅), 8.35 (d, JHH = 8.3 Hz, 1H, H₆), 8.26 (d, JHH = 8.3 Hz, 1H, H₇), 7.67 (t, JHH = 7.9 Hz, 1H, H₈), 7.61 (d, JHH = 8.6 Hz, 1H, H₃), 7.47 (dd, JHH = 8.6 Hz, JHH = 2.1 Hz, 1H, H₄), 7.25 (d, JHH = 8.3 Hz, 1H, H₅), 7.05 (d, JHH = 2.1 Hz, 1H, H₆), 3.59 (d, JHH = 11.2 Hz, 2H, H''''), 2.97 (q, JHH = 10.3 Hz 2H, H''''), 2.50 (d, JHH = 6.8 Hz, 2H, H''), 2.11 (m, 1H, H'''), 2.05 (d, JHH = 13.4 Hz, 2H, H''''), 1.79 (q, JHH = 12.7 Hz, 2H, H''''''), 1.35 (s, 9H, ortho-C(CH₃)₃), 1.32 (s, 9H, meta-C(CH₃)₃).

13C NMR (100 MHz, CDCl₃, HSQC): δ (ppm) = 132.1 (C'), 131.8 (C'), 128.7 (C'), 127.9 (C'), 127.0 (C'), 126.3 (C'), 126.2 (C'), 124.7 (C'''), 124.1 (C'), 119.8 (C'), 119.2 (C'), 115.9 (C'), 53.6 (C'''''), 53.5 (C''''''), 40.1 (C''), 33.9 (C'''), 31.7 (C'''''), 31.6 (C'''''), 31.3 (ortho-C(CH₃)₃), 30.9 (meta-C(CH₃)₃).

HRMS (FAB): m/z = 651.3231 (calculated for C₄₃H₄₃N₂O₄: 651.3223); 650.32 [M]+ (100); 651.32 [M+H]+ (97).

9-(4-(N-butylacetamide)piperidin-1-yl)-N-(2,5-di-tert-butylphenyl)perylene-3,4-dicarboximide (47)

Compound 46 (49.7 mg, 76.1 µmol, 1 eq), 14.8 mg (110 µmol, 1.37 eq) HOBt, 38.4 mg (86.8 µmol, 1.09 eq) BOP, 50 µl (0.29 mmol, 3.6 eq) DIPEA and 3 ml DMF were mixed and stirred at room temperature. After 10 minutes, 10 µl (0.11 mmol, 1.26 eq) butylamine was added and the mixture was stirred for 3 h at room temperature. Then, DMF was evaporated and the residue was dissolved in DCM, washed three times with water, dried with MgSO₄ and the solvent evaporated. The product was purified with column chromatography (eluent: DCM). Compound 47 was obtained as a purple powder (Rf = 0.32; 49 mg, 0.07 mmol, 87 %).

1H NMR (400 MHz, CD₂Cl₂): δ (ppm) = 8.55 (d, JHH = 8.2 Hz, 1H, H₃), 8.52 (d, JHH = 8.2 Hz, 1H, H₄), 8.41 (d, JHH = 7.5 Hz, 1H, H₅), 8.34 (t, JHH = 7.3 Hz, 2H, H₆), 8.25 (d, JHH = 7.9 Hz, 1H, H₇), 8.20 (d, JHH = 8.3 Hz, 1H, H₈), 7.64 (d, JHH = 8.6 Hz, 1H, H₉), 7.61 (t, JHH = 8.7 Hz, 1H, H₁₀), 7.52 (d, JHH = 8.6 Hz, JHH = 2.2 Hz, 1H, H₁₁), 7.20 (d, JHH = 8.2 Hz, 1H, H₁₂), 7.10 (d, JHH = 2.1 Hz, 1H, H₁₃), 5.68 (bs, 1H, NH), 3.56 (d, JHH = 10.6 Hz, 2H, H'''''), 3.29 (q, JHH = 6.7 Hz 2H, H''''), 2.95 (t, JHH = 11.5 Hz, 2H, H'''), 2.25 (d, JHH = 7.0 Hz, 2H, H'''''), 2.08-2.13 (m, 1H, H'''''), 1.99 (d, JHH = 12.4 Hz 2H, H'''''''), 1.67-1.73 (m, 2H, H''''''''), 1.54 (quint, JHH = 6.8 Hz, 2H, H'''''''), 1.39-1.47 (m, 2H, H''''''''), 1.39 (s, 9H, ortho-C(CH₃)₃), 1.31 (s, 9H, meta-C(CH₃)₃), 0.99 (t, JHH = 7.3 Hz, 3H, H₁₂).
13C NMR (100 MHz, CDCl3): δ (ppm) = 171.2 (C=O), 165.0 (C=O), 153.9 (C=O), 150.2 (C=O), 144.2 (C=O), 138.2 (C=O), 138.1 (C=O), 133.9 (C=O), 131.7 (C=O), 131.5 (C=O), 130.4 (C=O), 129.3 (C=O), 129.0 (C=O), 129.0 (C=O), 128.7 (C=O), 128.0 (C=O), 127.1 (C=O), 126.5 (C=O), 126.0 (C=O), 125.8 (C=O), 125.1 (C=O), 124.1 (C=O), 123.2 (C=O), 120.8 (C=O), 119.7 (C=O), 118.9 (C=O), 115.8 (C=O), 51.1 (C=O), 46.6 (C=O), 43.7 (C=O), 39.1 (C=O), 35.4 (C=O), 34.1 (meta-C(CH3)3), 33.3 (C=O), 32.5 (C=O), 31.8 (C=O), 31.4 (C=O), 30.0 (meta-C(CH3)3), 29.7 (C=O), 20.1 (C=O), 13.6 (C=O).

HPLC: t = 6.7 min (2 %); 17.3 min (98 %, 47).

HRMS (FAB): m/z = 705.3932 (calculated for C47H52N3O3 = 705.3930); 705.39 [M+H]+ (100); 706.39 [M+H]+ (98).

N-(2,5-di-tert-butylphenyl)-9-piperidinoperylene-3,4-dicarboximide (17)

An oven dried Schlenk was charged with 10.5 mg [Pd2(dba)3] (11.5 µmol, 0.06 eq), 22.2 mg BINAP (35.7 µmol, 0.19 eq), 25 mg NaOt-Bu (0.26 mmol, 1.4 eq) and 110 mg of 42 (187 µmol, 1 eq) and then purged with nitrogen. Then, 21 µl of piperidine (0.20 mmol, 1.1 eq) and dry toluene (5 ml) were added. The mixture was heated to 100 °C with stirring for 48 h, subsequently cooled to room temperature and the solvent removed under reduced pressure. The organic layer was dissolved in DCM and washed with water. The organic layer was dried with MgSO4 and the solvent evaporated. The crude product was chromatographed (eluent: PE/DCM 4/1 to 0/1 (v/v)). The purple spot (Rf (DCM) = 0.69) was collected and the solvent evaporated. 20 mg of a dark purple powder was obtained (33.7 µmol, 20 %).

1H NMR (400 MHz, CD2Cl2): δ (ppm) = 8.60 (d, 3JHH = 8.1 Hz, 1H, H5), 8.57 (d, 3JHH = 8.1 Hz, 1H, H2), 8.51 (d, 3JHH = 7.4 Hz, 1H, H6), 8.45 (d, 3JHH = 4.9 Hz, 1H, H1), 8.43 (d, 3JHH = 5.0 Hz, 1H, H12), 8.35 (d, 3JHH = 8.2 Hz, 1H, H7), 8.29 (d, 3JHH = 8.3 Hz, 1H, H10), 7.68 (t, 3JHH = 7.7 Hz, 1H, H11), 7.64 (d, 3JHH = 8.6 Hz, 1H, H3), 7.52 (dd, 3JHH = 8.6 Hz, 4JHH = 2.2 Hz, 1H, H6), 7.24 (d, 3JHH = 8.2 Hz, 1H, H5), 7.07 (d, 3JHH = 2.2 Hz, 1H, H6), 3.23 (bs, 4H, H2″,H3″), 1.93 (quint, 3JHH = 5.4 Hz, 4H, H3″,H5″), 1.76 (bs, 2H, H4″), 1.38 (s, 9H, ortho-C(CH3)3), 1.31 (s, 9H, meta-C(CH3)3).

4.5.3. Synthesis of the rigidochromic compounds

2-methyl-2-(trimethylsilyloxy)propanenitrile (49)[31]

To an ice cooled solution of 2-hydroxy-2-methylpropanenitrile (2.85 g, 33.5 mmol, 1 eq) and imidazole (4.56 g, 67.1 mmol, 2 eq) in dry DCM (84 ml) was added dropwise chlorotrimethylsilane (5 ml, 39.1 mmol, 1.2 eq). The mixture was stirred at room temperature overnight. Then, water was added (30 ml) and the resulting mixture was extracted with DCM and washed with saturated NaHCO3 solution
(3 x 30 ml). Then, the mixture was dried over MgSO₄, filtered and the solvent was evaporated. The product (3.87 g, 24.6 mmol, 73 %) was a light brown oil.

**<sup>1</sup>H NMR** (400 MHz, CDCl₃): δ (ppm) = 1.61 (s, 6H, C(CH₃)₂), 0.25 (s, 9H, Si(CH₃)₃).

**<sup>13</sup>C NMR** (100 MHz, CDCl₃): δ (ppm) = 122.5 (CN), 66.1 (C₆), 30.5 (CH₃).

**HRMS** (El): m/z = 157.0939 (calculated for C₇H₁₅ONSi = 157.0923); 157 [M]⁺ (2.7), 142 [M - CH₃]⁺ (66), 131 [M - CN]⁺ (6.6), 116 [M - CH₃ - CN]⁺ (19), 84 [(CH₃)₂C(CN)O]⁺ (9.1), 73 [Si(CH₃)₃]⁺ (17), 70 [(CH₃)₂C(O)C]⁺ (100), 68 [(CH₃)₂C(CN)]⁺ (5.3).

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4-bromo-<i>N</i>,<i>N</i>-dihexylaniline (51)<sup>20</sup>

4-bromoaniline (2.28 g, 13.3 mmol, 1 eq), 1-bromohexane (15.8 ml, 81.8 mmol, 8.5 eq), potassium carbonate (13.7 g, 99.1 mmol, 7.5 eq) and DMF (40 ml) were added together and the mixture was stirred under nitrogen overnight at 115 °C. The reaction was cooled to room temperature and water (50 ml) was added to the reaction mixture. The product was extracted with hexane. The organic layers were dried over MgSO₄ and purified by column chromatography (eluent: PE/EtOAc 50/1 (v/v)). The fractions with <i>R<sub>f</sub></i> = 0.76 were collected and the solvent evaporated. The product was a brown oil/liquid (2.01 g, 5.92 mmol, 45 %).

**<sup>1</sup>H NMR** (400 MHz, CDCl₃): δ (ppm) = 7.26 (d, <i>j</i>ₗ₁ₘ = 12.1 Hz, 2H, <i>H<sup>16</sup></i>), 6.51 (d, <i>j</i>ₗ₁ₘ = 7.9 Hz, 2H, <i>H<sup>5</sup></i>), 3.23 (t, <i>j</i>ₗ₁ₘ = 6.9 Hz, 4H, <i>H<sup>2</sup></i>), 1.56-1.59 (m, 4H, <i>H<sup>3</sup></i>), 1.28-1.32 (m, 12H, <i>H<sup>4</sup></i>), 0.91 (s, 6H, <i>H<sup>7</sup></i>).

**HRMS** (FAB): m/z = 340.1635 (calculated for C₁₉H₃₁NBr = 340.1640).

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1-(4-(<i>N</i>,<i>N</i>-dihexylanilino)phenyl)-2-hydroxy-2-methylpropan-1-one (52)<sup>22</sup>

4-bromo-<i>N</i>,<i>N</i>-dihexylaniline (51) (1.0 g, 3.0 mmol, 1 eq) and 12 ml THF were placed in a dry 200 ml round bottom flask. The mixture was cooled to −78 °C and 1.4 ml (3.6 mmol) of <i>n</i>-BuLi (2.5 M in hexane) was added to this stirred mixture over a period of 10 min. The resulting mixture was stirred for an additional hour at −78 °C and then 2-methyl-2-trimethylsilyloxy-propionitrile (0.95 g, 6.0 mmol, 2 eq) was added to the mixture over a period of 10 min. After the addition was complete, the reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with 25 ml of water and 6 ml of 6 M HCl (aq), stirred overnight, and then extracted with ethyl acetate. The organic layer was washed with brine and dried over MgSO₄. The solvent was evaporated and the residue was purified by column chromatography (eluent: PE/EtOAc 50/1 to 0/1 (v/v)) to obtain a yellow oil (0.77 g, 2.2 mmol, 74 % yield).

**<sup>1</sup>H NMR** (400 MHz, CDCl₃): δ (ppm) = 7.99 (d, <i>j</i>ₗ₁ₘ = 9.2 Hz, 2H, <i>H<sup>16</sup></i>), 6.64 (d, <i>j</i>ₗ₁ₘ = 7.7 Hz, 2H, <i>H<sup>5</sup></i>), 4.79 (bs, 1H, OH), 3.35 (t, <i>j</i>ₗ₁ₘ = 7.8 Hz, 4H, <i>H<sup>2</sup></i>), 1.67-1.61 (m, 4H, <i>H<sup>3</sup></i>), 1.66 (s, 6H, C(CH₃)-OH), 1.40-1.34 (m, 12H, <i>H<sup>4</sup></i>), 0.93 (t, <i>j</i>ₗ₁ₘ = 6.6 Hz, 6H, <i>H<sup>7</sup></i>).
HRMS (FAB): m/z = 348.2900 (calculated for C$_{22}$H$_{38}$O$_2$N = 348.2903).

2-(3-cyano-4-(4-(dihexylamino)phenyl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (33)$^{[22]}$

A mixture of compound 52 (386 mg, 1.11 mmol, 1 eq), malononitrile (220 mg, 3.33 mmol, 3 eq), acetic acid (few drops), ammonium acetate (1.4 mg), 3 Å molecular sieves (360 mg) and pyridine (2.1 ml) was stirred at room temperature for 24 h. The reaction mixture was then poured into 21 ml of ice water with vigorous stirring and the resulting mixture was left standing in a refrigerator overnight. The molecular sieves containing red precipitate were collected by vacuum filtration, the precipitate dissolved in ethyl acetate and dried over MgSO$_4$. After removing the solids by filtration through a pad of celite and evaporation of the solvent, the oily crude product was crystallized by the addition of hexane. The red crystals were collected (60 mg, 0.13 mmol, 12 %).

$^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) = 8.01 (d, $^3$J$_{HH}$ = 9.4 Hz, 2H, H$_2$,6), 6.72 (d, $^3$J$_{HH}$ = 9.3 Hz, 2H, H$_3$,5), 3.42 (t, $^3$J$_{HH}$ = 7.8 Hz, 4H, H$_{2''}$), 1.85 (s, 6H, CH$_3$), 1.66-1.61 (m, 4H, H$_{3''}$), 1.37-1.31 (m, 12H, H$_{4''}$,5'',6''), 0.92 (m, 6H, H$_{7''}$).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ (ppm) = 177.02 (C$_{5'}$), 173.47 (C$_{3'}$), 152.74 (C$_1$), 132.45 (C$_{3,5}$), 113.25 (C$_8$), 113.12 (C$_7$), 112.98 (C$_9$), 111.98 (C$_4$), 111.95 (C$_5$), 97.09 (C$_2'$), 90.67 (C$_2''$), 54.03 (C$_6''$), 51.4 (C$_{3''}$), 31.55 (C$_3''$), 27.66 (CH$_3$), 27.30 (C$_{4''}$), 26.65 (C$_5''$), 22.60 (C$_6''$), 13.98 (C$_{7''}$).

HRMS (FAB): m/z = 445.2961 (calculated for C$_{28}$H$_{37}$N$_4$O = 444.2967); 444.29 [M$^+$] (61); 445.30 [M+H]$^+$ (100).

1-(4-fluorophenyl)-2-hydroxy-2-methylpropan-1-one (54)$^{[22]}$

To 372 mg (15.3 mmol, 1.2 eq) magnesium in 1 ml dry THF was added dropwise 1.40 ml (12.7 mmol, 1 eq) 4-bromofluorobenzene in 2.5 ml dry THF over a period of 10 min. Because the reaction did not start immediately, a few drops of 1,2-dibromoethane were added. The heat of the reaction was controlled by cooling in an ice bath. The reaction mixture was stirred for 1 h at room temperature. A solution of compound 49 (2.00 g, 12.7 mmol, 1 eq) in 2.5 ml dry THF was added dropwise and the mixture was stirred overnight at room temperature. The reaction mixture turned green. Then, 16 ml of 6 M HCl (aq) was added at 0 °C. The solution became yellow. After 4 h of stirring at room temperature, saturated NaHCO$_3$ (aq) was added until the pH was 7. The mixture was filtered over celite and the filtrate was extracted with EtOAc. The organic layer was dried over MgSO$_4$ and the solvent was evaporated. $^1$H NMR analysis showed that the product was formed in 66 % yield. The crude product was used without purification.
Synthesis of the solvatochromic and rigidochromic fluorescent probes

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) = 8.15-8.10 (m, 2H, $H^3,5$), 7.17-7.13 (m, 2H, $H^{2,6}$), 3.91 (bs, 1H, OH), 1.63 (s, 6H, CH$_3$).

2-(3-cyano-4-(4-fluorophenyl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (55)\(^{[22]}\)

An intense dark blue mixture of 1.00 g of the crude product of compound 54 (667 mg, 3.65 mmol, 1 eq), 1.01 ml malononitrile (17.1 mmol, 4.7 eq), a few drops of acetic acid and 12.0 mg ammonium acetate (0.16 mmol, 0.04 eq) in 4.5 ml pyridine was stirred for 24 h at room temperature. 50 ml of ice-water was added to the mixture during vigorous stirring. The mixture turned green and was left standing in the refrigerator overnight. The green precipitate was filtered and the residue was dissolved in DCM. The solution was dried over MgSO$_4$ and the solvent was evaporated. Compound 55 was obtained as a green powder (1.05 g, 3.77 mmol, 62\%)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) = 7.85 (dd, 2H, $3^J_{HH} = 8.6$ Hz, $3^J_{HF} = 5.0$ Hz, $H^{3,5}$), 7.34 (t, 2H, $3^J_{HH} = 8.6$ Hz, $H^{2,6}$), 1.84 (s, 6H, CH$_3$).

2-((4-(4-cyano-5-(dicyanomethylene)-2,2-dimethyl-2,5-dihydrofur-3-yl)(methyl)amino)acetic acid (56)

A mixture of 55.4 mg of compound 55 (199 \(\mu\)mol, 1 eq) and 90.4 mg of sarcosine (1.01 mmol, 5 eq) was dried on the oil pump for 6 h. 2.5 ml of dry pyridine was added and the reaction was stirred for 20 h at 40 °C. Pyridine was evaporated and $^1$H NMR showed that compound 56 was formed. An attempt was made to purify the product with column chromatography. Several fractions were collected, but none of these contained compound 56. Probably, the product decomposed on the column.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) = 8.03 (dd, 2H, $3^J_{HH} = 9.4$ Hz, 2H, $H^{3,5}$), 6.84 (d, $3^J_{HH} = 9.4$ Hz, 2H, $H^{2,6}$), 4.25 (s, 2H, $H^{2''}$), 3.29 (s, 3H, NCH$_3$), 1.85 (s, 6H, CH$_3$).

3-((4-(4-cyano-5-(dicyanomethylene)-2,2-dimethyl-2,5-dihydrofur-3-yl)(methyl)amino)acetic acid (57)

Compound 55 (62.6 mg, 225 \(\mu\)mol, 1 eq) and 3-(methylamino)-propanoic acid (98.2 mg, 1.05 mmol, 4.7 eq) were dissolved in 4.0 ml pyridine. The reaction mixture was heated to 60 °C and stirred for 48 h. Pyridine was evaporated and $^1$H NMR showed that compound 57 was formed. The yield was not determined.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) = 9.60 (bs, 1H, OH), 8.02 (d, $3^J_{HH} = 9.4$ Hz, 2H, $H^{3,5}$), 6.84 (d, $3^J_{HH} = 9.4$ Hz, 2H, $H^{2,6}$), 3.88 (t, $3^J_{HH} = 7.1$ Hz, 2H, $H^{2''}$), 3.20 (s, 3H, NCH$_3$), 2.70 (d, $3^J_{HH} = 7.0$ Hz, 2H, $H^{3''}$), 1.84 (s, 6H, CH$_3$).
2-(1-(4-(4-cyano-5-(dicyanomethylene))-2,2-dimethyl-2,5-dihydrofuran-3-yl)phenyl)piperidin-4-yl)acetic acid (58)

Compound 55 (64.3 mg, 219 µmol, 1 eq) and 4-piperidine acetic acid (137 mg, 957 µmol, 4 eq) were dissolved in 4.0 ml pyridine. The reaction mixture was heated to 60 °C and stirred for 24 h. After 1 h, the reaction mixture had turned red. Pyridine was evaporated and an extraction with DCM was performed. The organic layer was washed with 0.5 M HCl solution (aq) with some extra added NaCl and dried over MgSO₄. Solvent was evaporated and the residue was purified by column chromatography (eluent: DCM/acetone 1/0 to 9/1 (v/v), Rf = 0.1). Compound 58 was formed in 65 % yield (530 mg, 1.32 mmol).

¹H NMR (400 MHz, CDCl₃): δ (ppm) = 7.99 (d, JHH = 9.2 Hz, 2H, H₂, H₂), 6.92 (d, JHH = 9.3 Hz, 2H, H₂), 4.09 (d, JHH = 13.4 Hz, 2H, H₃, H₃), 3.12 (t, JHH = 12.4 Hz, 2H, H₅, H₅), 2.35 (d, JHH = 7.0 Hz, 2H, H₇), 2.19 (m, 2H, H₄), 1.99 (d, JHH = 12.2 Hz, 2H, H₃, H₃), 1.84 (s, 6H, CH₃), 1.35 (t, JHH = 12.1 Hz, 2H, H₅, H₅).

¹³C NMR (100 MHz, CDCl₃): δ (ppm) = 177.9 (C⁵), 174.8 (C⁶), 173.5 (C³), 155.2 (C¹), 133.3 (C⁴, 5), 115.5 (C⁸), 114.2 (C², 6), 113.7 (C⁶), 113.4 (C⁷), 112.4 (C⁴) 98.7 (C⁴), 93.5 (C²), 47.9 (C², 6), 41.0 (C⁷), 34.0 (C⁵), 32.6 (C³, 5), 27.3 (CH₃).

HRMS (FAB): m/z = 403.1770 (calculated for C₂₃H₂₃N₄O₃ = 403.1770); 402.17 [M]+ (88); 403.18 [M+H]+ (100).

4.6. Acknowledgement

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4.7. References


28. The analogous value for $\mu_e - \mu_g$ of compound 17, as it is obtained in reference [26], is higher (7.5 D). This discrepancy can be attributed to the fact that more solvents are examined in this reference.


34. http://www.wolframalpha.com


