Aziridinyl fluorophores demonstrate bright fluorescence and superior photostability through effectively inhibiting twisted intramolecular charge transfer

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1 Computational methods and results

1.1 Computational details

Density functional theory (DFT) and time dependent (TD)-DFT calculations were performed using Gaussian 09.\(^1\) Geometry optimizations for 1 – 7 in the ground state (S\(_0\)) and for 1–3 in the first excited singlet state (S\(_1\)) were performed in vacuo, cyclohexane, ethanol and water. Similar optimizations for 10 – 17 in both the S\(_0\) and S\(_1\) states were performed in ethanol only. These geometry optimizations employ the B3LYP functional,\(^2\)\(^-\)\(^4\) in combination with the 6-31+G(d,p) basis set.\(^5\) Solvent effects were taken into account using the IEFPCM model. Frequency checks were carried out after each geometry optimization to ensure that the minima on the potential energy surfaces (PES) were found.

Atomic contributions to the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) in selected compounds were calculated using Mulliken population analysis, based on the optimized molecular structures at the S\(_0\) state.

We also constructed the PES of 1–3 in ethanol as a function of the dihedral angle between their amino substituents and main scaffolds. Unless otherwise stated, the dihedral angles were fixed at various values, while other geometry parameters were freely optimized employing B3LYP/6-31+G(d,p) in the S\(_1\) state. The LE and TICT minima in the S\(_1\) state were optimised without any constraint. During the geometry optimization of all TICT structures, we pre-twisted the amino groups to a nearly perpendicular conformation to order to reach the TICT minima.

TD-DFT and single point calculations were carried out on the optimized molecular structures using CAM-B3LYP/6-31+G(d,p),\(^6\) in order to determine their relative energy levels in the Franck–Condon (FC), local excited (LE), and twisted intramolecular charge transfer (TICT) states. For all de-excitation processes, such as the LE and TICT emissions and the PES scans in ethanol, the S\(_0\) energy levels were calculated with non-equilibrium solvation.
1.2 Choices of functionals

1.2.1 Geometry optimizations
B3LYP functional offers excellent performance in geometry optimization. This functional was employed to optimize the molecular structures of 1—7 and 10—17 in both the $S_0$ and $S_1$ states.

1.2.2 Excitation energy calculations
While B3LYP predicts the excitation energy of the LE state accurately, it severally underestimates the excitation energy of the TICT state, where a significant extent of charge transfer is involved. In contrast, the CAM-B3LYP functional describes the overall PES profile accurately in systems involving varied amount of charge transfer (i.e., when both LE and TICT states are of concern). We have thus used CAM-B3LYP to calculate the excitation energies of 1—7, based on B3LYP optimized molecular structures.

1.3 Impact of medium polarity on the molecular structures of 1—7 in the $S_0$ state
Figure S1 shows the atom and bond labelling convention of naphthalimide dyes, used in this paper.

![Figure S1. Atom and bond labelling of naphthalimide dyes.](image)

1.3.1 Theoretically optimized molecular structures of 1—7 in ethanol in the $S_0$ state
The molecular structures of 1—7 in the ground state have been theoretically optimized using B3LYP/6-31+G(d,p) in ethanol (Figure 1c). The three-membered aziridine ring in 1 possess the up-up conformation. The four-membered azetidine ring affords the flat conformation. The rest molecules 3—7 all adopt the up-down conformation.

Detailed geometry parameters and the conformations between the amino groups and the fluorophore scaffolds are available in Table S1.
Table S1. Dihedral angles along the N-C\textsubscript{aro} bond in the theoretically optimized molecular structures of 1—7 and their conformations in ethanol in the S\textsubscript{0} state.

<table>
<thead>
<tr>
<th>Compound</th>
<th>θ\textsubscript{Cα-C4-N1-C'} (°)</th>
<th>θ\textsubscript{Cβ-C4-N1-C''} (°)</th>
<th>Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-69.02</td>
<td>40.11</td>
<td>up-up</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>-0.01</td>
<td>flat</td>
</tr>
<tr>
<td>3</td>
<td>-23.29</td>
<td>-5.19</td>
<td>up-down</td>
</tr>
<tr>
<td>4</td>
<td>-65.40</td>
<td>-18.86</td>
<td>up-down</td>
</tr>
<tr>
<td>5</td>
<td>-63.87</td>
<td>-19.75</td>
<td>up-down</td>
</tr>
<tr>
<td>6</td>
<td>-66.49</td>
<td>-24.99</td>
<td>up-down</td>
</tr>
<tr>
<td>7</td>
<td>-55.16</td>
<td>-16.45</td>
<td>up-down</td>
</tr>
</tbody>
</table>

1.3.2 Theoretically optimized molecular structures of 1—3 in the S\textsubscript{0} state in various medium

The molecular structures of 1—7 also depend on the solvent in use. Compounds 1—3 are selected to represent the up-up, flat, and up-down conformations, respectively. These three compounds undergo further theoretical analysis in various medium of different polarities, including vacuo, cyclohexane, ethanol and water.

The molecular structures of 1 adopt the up-up conformation in all studied medium; while 3 always affords the up-down conformation. Slight flattering of the amino groups in both 1 and 3, however, is observed as the solvent polarity rises (Table S2).

Table S2. Dihedral angles along the N-C\textsubscript{aro} bond in the theoretically optimized molecular structures of 1—3 in the ground state in various solvents.

<table>
<thead>
<tr>
<th>Medium</th>
<th>θ\textsubscript{Cα-C4-N1-C'} (°)</th>
<th>θ\textsubscript{Cβ-C4-N1-C''} (°)</th>
<th>Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacuum</td>
<td>-70.87</td>
<td>38.55</td>
<td>up-up</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>-70.16</td>
<td>39.20</td>
<td>up-up</td>
</tr>
<tr>
<td>ethanol</td>
<td>-69.02</td>
<td>40.11</td>
<td>up-up</td>
</tr>
<tr>
<td>water</td>
<td>-69.00</td>
<td>40.13</td>
<td>up-up</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacuum</td>
<td>-33.15</td>
<td>13.63</td>
<td>up-up</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>-26.71</td>
<td>12.02</td>
<td>up-up</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.02</td>
<td>-0.01</td>
<td>flat</td>
</tr>
<tr>
<td>water</td>
<td>0.01</td>
<td>-0.01</td>
<td>flat</td>
</tr>
</tbody>
</table>
The electron-donating group in 2 is a four-membered azetidine ring. This ring exhibits different geometries as a function of solvent polarity (Figure S2).

In non-polar environment (i.e., in vacuo or cyclohexane; Figure S2a, b), the azetidine ring bends up and affords the up-up conformation, owing to its high strain energy.

In polar solvents, (i.e., in ethanol and water; Figure S2c, d), the azetidine ring is planar and affords the flat conformation. This planarization is due to increased intramolecular charge transfer and enhanced resonance effect in 2.

1.4 Impact of medium polarity on the energy levels of 1—3

The energy levels of 1—3 in Franck-Condon (absorption), LE (emission) and TICT states have been calculated in vacuo, cyclohexane, ethanol and water (Figure S3). These compounds are selected to represent the up-up, flat and up-down conformations, respectively.

In non-polar environment (such as in vacuo), our calculations show that dyes 1—3 energetically favor local excited (LE) state, but not the TICT state (Figure S3a). Not surprisingly, our experiments demonstrate that 1—7 all exhibit high quantum yields in non-polar solvents (Table S8).

In polar environment (such as in ethanol and water), our calculations show that 1 remains resistant to TICT rotation. The TICT state of 2, however, becomes slightly more stable than the LE state. A strong tendency to the TICT state is most evident in 3. Consequently, 1 (the up-up conformation) exhibits the highest quantum yields (0.708 in ethanol), followed by 2 (the flat conformation, 0.631 in ethanol). Compounds 3 (as

<table>
<thead>
<tr>
<th></th>
<th>vacuum</th>
<th>cyclohexane</th>
<th>ethanol</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td>TICT</td>
<td>-5.30</td>
<td>-5.06</td>
<td>-5.19</td>
<td>-5.23</td>
</tr>
</tbody>
</table>

Figure S2. Side view of theoretically optimized molecular structures of 2 in the ground state in (a) vacuo; (b) cyclohexane; (c) ethanol and (d) water.
well as 4—7; the up-down conformation) affords very low quantum yields (Figure S3c, d; Table S8).

Figure S3. Calculated relative $S_0$ (blue) and $S_1$ (red) energy levels corresponding to the Franck-Condon (absorption), LE (emission) and TICT states of 1, 2, and 3 in (a) vacuo, (b) cyclohexane, (c) ethanol and (d) water.

We have also quantitatively compared the excitation energy in the FC, LE and TICT states, derived from both experimental measurements and theoretical calculations (using both B3LYP and CAM-B3LYP; Table S3). Indeed, B3LYP predicts the excitation energy of the FC and LE states quite accurately. However, it severally underestimates the excitation energy of the TICT state. While CAM-B3LYP generally overestimates the excitation energy, it has been shown that CAM-B3LYP correctly
describes the overall PES profile. We thus use CAM-B3LYP results to construct the PES of 1—3.

Table S3. Comparison of the experimental and theoretical (calculated using both B3LYP and CAM-B3LYP functional) excitation energy.

<table>
<thead>
<tr>
<th></th>
<th>experimental FC (absorption)</th>
<th>B3LYP</th>
<th>CAM-B3LYP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.2457 eV (382 nm)</td>
<td>3.0704 eV (404 nm)</td>
<td>3.4764 eV (357 nm)</td>
</tr>
<tr>
<td>2</td>
<td>2.7862 eV (445 nm)</td>
<td>2.8324 eV (438 nm)</td>
<td>3.1977 eV (388 nm)</td>
</tr>
<tr>
<td>3</td>
<td>2.7675 eV (448 nm)</td>
<td>2.8494 eV (435 nm)</td>
<td>3.2136 eV (386 nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.4358 eV (509 nm)</td>
<td>2.5165 eV (493 nm)</td>
<td>2.9012 eV (427 nm)</td>
</tr>
<tr>
<td>2</td>
<td>2.3262 eV (533 nm)</td>
<td>2.3688 eV (523 nm)</td>
<td>2.7470 eV (451 nm)</td>
</tr>
<tr>
<td>3</td>
<td>2.3437 eV (529 nm)</td>
<td>2.3742 eV (522 nm)</td>
<td>2.7584 eV (449 nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>1.1258 eV (1101 nm)</td>
<td>1.2120 eV (585 nm)</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>0.7144 eV (1736 nm)</td>
<td>1.5482 eV (801 nm)</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>0.6392 eV (1940 nm)</td>
<td>1.4911 eV (832 nm)</td>
</tr>
</tbody>
</table>

1.5 Molecular structures and energy levels of 1 in the TICT state

The theoretically optimized molecular structures of 1 are different from those of other dyes (Figure S4). In the LE state, 1 exhibits the unique up-up conformation (Figure S4a). In the TICT state, the perpendicular alignment between the amino group and the main fluorophore scaffold is stable for 2—7, but not for 1 (Figure S4b). When reaching a stable TICT state in 1, its amino group exhibits a pyramidal structure (Figure S4c).

Figure S4. Theoretically optimized molecular structures of 1 in the S1 state. (a) in the LE state; (b) in the nonstable TICT state; and (c) in the stable TICT state.

The stable TICT (pyramidal) states have lower energy levels in comparison to the nonstable TICT (perpendicular) states in 1 (Figure S5). Both TICT states have higher energy levels than the corresponding LE (emission) states. As a result, compound 1 is resistant to TICT rotation in all studied solvents.
Figure S5. Calculated relative $S_0$ (blue) and $S_1$ (red) energy levels corresponding to the Franck-Condon (absorption), LE (emission) and TICT (perpendicular, nonstable) and TICT (pyramidal, stable) states of 1 in (a) vacuo, (b) cyclohexane, (c) ethanol and (d) water.

1.6 Potential energy surfaces of 1—3 in ethanol

Forming the TICT state requires two conditions: (1) the TICT state has a lower energy level than the LE state in the $S_1$ PES, and (2) the energy barrier between the LE and TICT states is small (comparable to thermal energy at room temperature; Figure S6). Note that we use the LE state to denote the more planar conformation with less charge transfer (compared to the TICT state). In some studies, this “LE” state is referred as the intramolecular charge transfer (ICT) state.

Figure S6. Illustration of the $S_0$ and $S_1$ potential energy surfaces, as well as the corresponding Franck-Condon, local excited (LE), and twisted intramolecular charge transfer (TICT) states.
In order to study the TICT tendency of 1—3, we have computed their $S_0$ and $S_1$ PES in ethanol as well as the corresponding energy barriers between their LE and TICT states (Figure S7).

![Figure S7](image)

Figure S7. Calculated potential energy surfaces (PES) of the ground ($S_0$, in blue) and the first excited singlet ($S_1$, in red) states of 1 (a), 2 (b) and 3 (c) in ethanol, as a function of the rotational angle ($\theta$) between the amino substituent and the main fluorophore scaffold. $\theta$ is defined as the average value of $\angle C_\alpha-C_4-N_1-C'$ and $\angle C_\beta-C_4-N_1-C''$ (see Figure S1).

In the case of 1, the energy barrier is particularly large, indicating significant TICT resistance. Moreover, the TICT state of 1 is energetically unfavourable with respect to the LE state (even in ethanol and water). Compound 1 is thus not likely to enter the TICT state upon photoexcitation (Figure S7a).

For compound 2, our calculations suggest that the TICT state corresponds to a lower energy level than the LE state in the $S_1$ PES in ethanol (Figure S7b, c). Nevertheless, a large energy barrier (0.246 eV) exists between the LE and TICT states (Figure S7b). Compound 2 is thus non prone to TICT rotation, affording a high quantum yield in ethanol (0.631).

However, substantial TICT rotations in 2 could be activated in more polar solvents. In fact, a complete charge transfer from the donor to the acceptor moiety of a fluorophore occurs in the TICT state. This TICT state is highly polarized and become stabilized through intensive dye-solvent electrostatic interactions. This stabilization effect is particularly strong in highly polar solvents, such as water. Consequently, significant TICT formation (along with hydrogen bond interactions) substantially lowers the quantum yield of 2 to 0.199 in water.

In the case of 3, its TICT state is more stable and the energy barrier to the TICT state is very small (0.056 eV). This small value is comparable to the thermal energy at
room temperature (0.039 eV; Figure S7c). Substantial TICT formation is thus expected, leading to negligible quantum yields of 3 in polar solvents.

Note that the assignment of the LE and TICT states is further corroborated by their associated charge transfer upon photoexcitation (Figure S8). The TICT state exhibits a greater extent of charge transfer than the LE state.

Figure S8. LUMOs and HOMOs of 1—3 in ethanol (red: positive; blue: negative; isovalue: 0.02).

It is also worth noting that calculating PES, excitation energy, and oscillator strength of higher energy states (such as S2) will provide more insights about the photophysics of azacyclic ring substituted fluorophores and the relative stability of the LE and (twisted) ICT states.

1.7 Geometry change in aziridinyl naphthalimide 1 upon photoexcitation
The aziridine ring in 1 exhibits the up-up conformation. After photoexcitation, the C4—N1 bond gains more double-bond character in conjunction with intramolecular charge transfer from the aziridine ring to naphthalimide scaffold. Consequently, a slight flatterting of the aziridine ring occurs in 1. This structural change is reflected in the associated dihedral angles, $\angle C_\alpha$-C4-N1-C’ and $\angle C_\beta$-C4-N1-C’’ (Table S4). In contrast, the molecular structure of 2 remains flat both before and after photoexcitation. The greater geometry relaxation in 1 leads to a larger Stokes shift (127 nm in ethanol), in comparison to that of 2 (88 nm in ethanol).

Table S4. Dihedral angles along the N-C$_{aro}$ bond in the theoretically optimized molecular structures of 1 in both the S$_0$ and S$_1$ state in ethanol.

<table>
<thead>
<tr>
<th>Dihedral Angle</th>
<th>the S$_0$ state</th>
<th>the S$_1$ state</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\angle C_\alpha$-C4-N1-C’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\angle C_\beta$-C4-N1-C’’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.8 Relative energy levels of the Franck–Condon (FC, absorption), local excited (LE), and twisted intramolecular charge transfer (TICT) states in coumarin, phthalimide and NBD dyes in ethanol

Comparing the $S_1$ energy level differences between the LE and TICT states, we notice that aziridinyl fluorophores demonstrate the highest TICT resistance, followed by azetidinyl dyes (Figure S9). In contrast, conventional dimethylamino groups lead to the largest energy reduction via LE-to-TICT transitions, indicating a strong TICT tendency.

The assignment of the LE and TICT states is corroborated by their associated charge transfer upon photoexcitation (Figures S10—S12). The TICT state exhibits a greater extent of charge transfer than the LE state.

Figure S9. Calculated relative $S_0$ (blue) and $S_1$ (red) energy levels corresponding to the Franck-Condon (absorption), LE (emission) and TICT (non-radiative) states of selected coumarin, phthalimide and NBD dyes in ethanol.
1.9 Quantum yield vulnerability to hydrogen bond interactions
We have found one plausible mechanism to explain quantum yield vulnerabilities to hydrogen bond interactions among different fluorophores. This vulnerability is closely related to the partial charge increase upon photoexcitation, at hydrogen bond
formation sites in the fluorophore scaffolds. We denote the total change at these sites during HOMO→LUMO transition as Δ.

In fluorophores with small Δ changes, the quantum yields are relatively high in general (Figure S13). In stark contrast, in fluorophores with large Δ changes, their quantum yields are significantly lower, conceivably due to significant hydrogen bond interactions upon the photoexcitation of these fluorophores (Figure S14). This contrast becomes even more obvious in aqueous solution (Figure 2c). Note that quantum yields of compounds A (2), E (15), and F (17) are from our in-house measurements, while other known values are reported by Lavis et al.9

Based on this mechanism we predict that compounds H, I, and J (and other compounds from these fluorophore families) possess relatively low quantum yields in protonic solvents (Figure S14).

Figure S13. The atomic contributions to the HOMO and LUMO electron densities of representative compounds whose quantum yields are less vulnerable to hydrogen bond interactions. The blue/pink circle size represents the atomic contribution; only contributions greater than 0.02 are shown. Hydrogen bond formation sites are highlighted by arrows.
Figure S14. The atomic contributions to the HOMO and LUMO electron densities of representative compounds whose quantum yields are considerably vulnerable to hydrogen bond interactions. The blue/pink circle size represents the atomic contribution; only contributions greater than 0.02 are shown. Hydrogen bond formation sites are indicated by arrows. Molecular sites that experience significant partial charge increase upon photoexcitation are highlighted by red arrows.
2 Chemical synthesis and characterizations

2.1 Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification.

$^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker 400 spectrometer, using TMS as an internal standard. Chemical shifts were given in ppm and coupling constants ($J$) in Hz. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer.

HPLC was monitored on Waters e2695 (separations module), 2998PDA detector and SunFire @ C18 (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; MeOH/H$_2$O, linear gradient, 30 min run; 1 mL/min flow; UV detection at 254 nm). High-resolution mass spectrometry (HRMS) data were obtained using ESI (6540 UHD Q-TOF, positive ion).

UV—vis absorption spectra were collected on an Agilent Cary 60 UV-Vis Spectrophotometer. Fluorescence measurements were performed on an Agilent CARY Eclipse fluorescence spectrophotometer.

The fluorescence lifetime of 1—7 in various solvents were measured using Fluoromax-4 spectro-fluorometer equipped with a NanoLED-370 pulsed diode (excitation wavelength, 368 nm) and a DeltaHub TCSPC controller. The fluorescence lifetime of all samples was monitored at their respective peak emission wavelengths.

The quantum yields of 1—17 were determined via the relative determination method, with Coumarin 153, quinine sulphate and Rhodamine 101 as a reference compound.\textsuperscript{10}

For photostability tests under white light, a 500W tungsten lamp was used to irradiate dyes 1, 2, and Rhodamine B dissolved in DMSO/water mixture (volume ratio, 30:70) continuously. Their emission intensities were measured after 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12 hours of irradiation.
2.2 Chemical synthesis

150 mg (3.5 mmol, 5 eq.) aziridine was added dropwise to a solution of 200 mg (0.69 mmol) N-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to 80 °C for 10 h. The solvent was removed under reduced pressure and the crude product was then purified by column chromatography (SiO2, CHCl3) to give 1 as a yellow solid in 18% yield (32 mg). 1H-NMR (CDCl3, 400 MHz) δ 2.47 (s, 4H), 3.54 (s, 3H), 7.15 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 8.48 (d, J = 8.0 Hz, 1H), 8.65 (d, J = 8.0 Hz, 1H). 13C-NMR (CDCl3, 100 MHz) δ 26.91, 28.79, 115.95, 116.77, 122.99, 126.06, 126.17, 129.03, 129.06, 131.30, 132.51, 156.89, 164.22, 164.71. Analytical HPLC: 99.51% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 55–45% CH3OH/H2O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C15H13N2O2 [MH+] 253.0977, found 253.0984.

2.2.1 N-Methyl-4-(aziridin-1-yl)-1,8-naphthalimide (1)

150 mg (3.5 mmol, 5 eq.) aziridine was added dropwise to a solution of 200 mg (0.69 mmol) N-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to 80 °C for 10 h. The solvent was removed under reduced pressure and the crude product was then purified by column chromatography (SiO2, CHCl3) to give 1 as a yellow solid in 18% yield (32 mg). 1H-NMR (CDCl3, 400 MHz) δ 2.47 (s, 4H), 3.54 (s, 3H), 7.15 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 8.48 (d, J = 8.0 Hz, 1H), 8.65 (d, J = 8.0 Hz, 1H). 13C-NMR (CDCl3, 100 MHz) δ 26.91, 28.79, 115.95, 116.77, 122.99, 126.06, 126.17, 129.03, 129.06, 131.30, 132.51, 156.89, 164.22, 164.71. Analytical HPLC: 99.51% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 55–45% CH3OH/H2O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C15H13N2O2 [MH+] 253.0977, found 253.0984.

2.2.2 N-Methyl-4-(azetidin-1-yl)-1,8-naphthalimide (2)
200 mg (3.5 mmol, 5 eq.) azetidine was added dropwise to a solution of 200 mg (0.69 mmol) \(N\)-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give compound 2 in 66% yield (120 mg). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta\) 2.56 (m, \(J = 7.6\) Hz, 2H), 3.53 (s, 3H), 4.50 (t, \(J = 7.6\) Hz, 4H), 6.38 (d, \(J = 8.8\) Hz, 1H), 7.51 (t, \(J = 8.0\) Hz, 1H), 8.25 (d, \(J = 8.4\) Hz, 1H), 8.39 (d, \(J = 8.4\) Hz, 1H), 8.55 (d, \(J = 7.2\) Hz, 1H). \(^{13}\)C-NMR (CDCl\(_3\), 100 MHz) \(\delta\) 17.08, 26.80, 55.38, 106.25, 110.15, 120.93, 122.59, 123.69, 130.09, 130.46, 131.12, 133.31, 152.55, 164.36, 165.06. Analytical HPLC: 98.96% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 70–30% CH\(_3\)OH/H\(_2\)O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C\(_{16}\)H\(_{15}\)N\(_2\)O\(_2\) [M+H\(^+\)] 267.1134, found 267.1145.

2.2.3 \(N\)-Methyl-4-(pyrrolidin-1-yl)-1,8-naphthalimide (3)

245 mg (3.5 mmol, 5 eq.) pyrrolidine was added dropwise to a solution of 200 mg (0.69 mmol) \(N\)-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give compound 3 in 87% yield (168 mg). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta\) 2.09 (t, \(J = 6.0\) Hz, 4H), 3.51 (s, 3H), 3.75 (t, \(J = 6.0\) Hz, 4H), 6.72 (d, \(J = 8.4\) Hz, 1H), 7.47 (t, \(J = 8.0\) Hz, 1H), 8.34 (d, \(J = 8.4\) Hz, 1H), 8.51 (m, 2H). \(^{13}\)C-NMR (CDCl\(_3\), 100 MHz) \(\delta\) 26.08, 26.73, 53.15, 108.38, 110.40, 122.29, 122.46, 122.90, 130.91, 131.93, 133.31, 152.52, 164.24, 165.10. Analytical HPLC: 99.86% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 70–30% CH\(_3\)OH/H\(_2\)O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C\(_{17}\)H\(_{17}\)N\(_2\)O\(_2\) [M+H\(^+\)] 281.1290, found 281.1295.

2.2.4 \(N\)-Methyl-4-morpholino-1,8-naphthalimide (4)

\(\text{O} \quad \text{N} \quad \text{O} \)

\(\text{O} \quad \text{N} \quad \text{O} \)
300 mg (3.5 mmol, 5 eq.) morpholine was added dropwise to a solution of 200 mg (0.69 mmol) N-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give compound 4 in 91% yield (186 mg). $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ 3.27 (t, $J = 4.4$ Hz, 4H), 3.53 (s, 3H), 4.02 (t, $J = 4.4$ Hz, 4H), 7.22 (d, $J = 8.0$ Hz, 1H), 7.69 (t, $J = 7.8$ Hz, 1H), 8.41 (d, $J = 8.4$ Hz, 1H), 8.50 (d, $J = 8.0$ Hz, 1H), 8.56 (d, $J = 7.2$ Hz, 1H). $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$ 26.88, 53.44, 66.97, 114.93, 117.02, 123.16, 125.81, 126.10, 129.70, 130.05, 131.12, 132.47, 155.61, 164.18, 164.64. Analytical HPLC: 99.47% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 55–45% CH$_3$OH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calemd for C$_{17}$H$_{15}$N$_2$O$_3$ [M+H]$^+$ 297.1239, found 297.1246.

2.2.5 N-Methyl-4-(piperidin-1-yl)-1,8-naphthalimide (5)

293 mg (3.5 mmol, 5 eq.) piperidine was added dropwise to a solution of 200 mg (0.69 mmol) N-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give compound 5 in 90% yield (182 mg). $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ 1.73 (m, 2H), 1.89 (m, 4H), 3.23 (t, $J = 5.0$ Hz, 4H), 3.53 (s, 3H), 7.15 (d, $J = 8.4$ Hz, 1H), 7.66 (t, $J = 8.0$ Hz, 1H), 8.37 (d, $J = 8.4$ Hz, 1H), 8.47 (d, $J = 8.4$ Hz, 1H), 8.55 (d, $J = 7.2$ Hz, 1H). $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$ 24.35, 26.23, 26.85, 54.55, 114.69, 115.77, 122.95, 125.33, 126.23, 129.78, 130.63, 130.98, 132.66, 157.32, 164.39, 164.88. Analytical HPLC: 99.73% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 80-20% CH$_3$OH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calemd for C$_{18}$H$_{19}$N$_2$O$_2$ [M+H]$^+$ 295.1447, found 295.1454.

2.2.6 N-Methyl-4-(hexamethyleneimine-1-yl)-1,8-naphthalimide (6)
341 mg (3.5 mmol, 5 eq.) hexamethylenimine was added dropwise to a solution of 200 mg (0.69 mmol) N-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give compound 6 in 92% yield (197 mg).

1H-NMR (CDCl₃, 400 MHz) δ 1.79 (m, 4H), 1.94 (m, 4H), 3.53 (s, 3H), 3.59 (t, J = 5.4 Hz, 4H), 7.13 (d, J = 8.4 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 8.42 (m, 2H), 8.54 (d, J = 7.2 Hz, 1H). 13C-NMR (CDCl₃, 100 MHz) δ 26.81, 27.68, 28.57, 55.67, 113.66, 113.77, 122.78, 124.31, 125.34, 130.39, 130.96, 131.53, 132.63, 157.69, 164.34, 165.01.

Analytical HPLC: 99.70% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 80–20% CH₃OH/H₂O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C₁₉H₂₁N₂O₂ [M+H]+ 309.1603, found 309.1606.

2.2.7 N-Methyl-4- dimethylamino-1,8-naphthalimide (7)

155 mg (3.5 mmol, 5 eq.) dimethylamine was added dropwise to a solution of 200 mg (0.69 mmol) N-methyl-4-Bromo-1,8-naphthalimide in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give compound 7 in 90% yield (157 mg).

1H-NMR (CDCl₃, 400 MHz) δ 3.10 (s, 6H), 3.53 (s, 3H), 7.09 (d, J = 8.4 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 8.42 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 8.0 Hz, 1H), 8.54 (d, J = 7.2 Hz, 1H). 13C-NMR (CDCl₃, 100 MHz) δ 26.82, 44.78, 113.27, 114.85, 122.92, 124.84, 125.24, 130.07, 130.94, 131.17, 132.59, 156.94, 164.31, 164.86. Analytical HPLC: 99.81% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 70–30% CH₃OH/H₂O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C₁₅H₁₅N₂O₂ [MH⁺] 255.1134, found 255.1133.

2.2.8 2-(6-(aziridin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetic acid (8)
4-Bromo-1,8-naphthalic anhydride (2.77 g, 10 mmol), glycine hydrochloride (1.12 g, 10 mmol) and TEA (1.52 g, 15 mmol) were added into 50 mL ethanol. The solution was heated to reflux for 6 h. The mixture was cooled to room temperature, and the precipitate was collected as grey solid (3.2 g) without further purification in 88% yield. $^1$H NMR (400 MHz, DMSO) δ 8.69 – 8.54 (m, 2H), 8.39 (d, $J$ = 7.9 Hz, 1H), 8.27 (d, $J$ = 7.9 Hz, 1H), 8.10 – 7.95 (m, 1H), 4.82 (s, 2H), 4.17 (q, $J$ = 7.0 Hz, 2H), 1.22 (t, $J$ = 7.1 Hz, 3H).

Aziridine (119 mg, 2.76 mmol) was added dropwise to the solution of N-ethoxycarbonylmethyl-4-Bromo-1,8-naphthalimide (200 mg, 0.55 mmol) in 5 mL 2-methoxyethanol. The mixture was heated to 80°C for 12 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO$_2$, DCM/MeOH, 200:1, V/V) to give light yellow powder (60 mg) in 34% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.69 (d, $J$ = 8.4 Hz, 1H), 8.63 (d, $J$ = 7.2 Hz, 1H), 8.50 (d, $J$ = 8.0 Hz, 1H), 7.77 (t, $J$ = 7.9 Hz, 1H), 7.17 (d, $J$ = 8.0 Hz, 1H), 4.93 (s, 2H), 4.24 (q, $J$ = 7.1 Hz, 2H), 2.48 (s, 4H), 1.29 (t, $J$ = 7.1 Hz, 3H).

2 mL 0.1 M NaOH was slowly added to the solution of N-ethoxycarbonylmethyl-4-azetidinly-1,8-naphthalimide (50 mg, 0.15 mmol) in 10 mL methanol at room temperature. After 4 h, the solvent was removed under reduced pressure, and the product was purified by column chromatography (SiO$_2$, DCM/MeOH, 10:1, V/V) to give yellow powder (25 mg) in 55% yield. $^{13}$C NMR (101 MHz, DMSO) δ 172.40, 163.77, 163.29, 157.31, 132.40, 131.06, 129.51, 128.85, 126.79, 126.15, 123.18, 116.52, 116.50, 43.99, 28.93. HRMS (ESI) calcd for C$_{16}$H$_{13}$N$_2$O$_4$ [MH$^+$] 297.0875, found 297.0863.
2.2.9 2-(6-(azetidin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetic acid (9)

Azetidine (157 mg, 2.76 mmol) was added dropwise to the solution of N-ethoxycarbonylmethyl-4-Bromo-1,8-naphthalimide (200 mg, 0.55 mmol) in 5 mL 2-methoxyethanol. The mixture was heated to reflux for 2 h, while monitored by TLC. After the reaction was completed, the solution was cooled to room temperature to give yellow needle crystals. The product was filtered off and then washed with hexane to give N-ethoxycarbonylmethyl-4-azetidinly-1,8-naphthalimide in 59% yield (110 mg).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.49 (d, $J = 6.8$ Hz, 1H), 8.33 (d, $J = 8.5$ Hz, 1H), 8.17 (d, $J = 8.0$ Hz, 1H), 7.45 (t, $J = 7.9$ Hz, 1H), 6.29 (d, $J = 8.5$ Hz, 1H), 4.92 (s, 2H), 4.45 (t, $J = 7.5$ Hz, 4H), 4.24 (q, $J = 7.1$ Hz, 2H), 2.61 – 2.45 (m, 2H), 1.29 (t, $J = 7.1$ Hz, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.56, 164.42, 163.53, 152.54, 133.59, 131.42, 130.80, 130.51, 123.59, 122.04, 120.81, 109.21, 106.07, 61.35, 55.31, 41.26, 17.00, 14.19.

5 mL 0.1 M NaOH was slowly added into the solution of N-ethoxycarbonylmethyl-4-azetidinly-1,8-naphthalimide (100 mg, 0.30 mmol) dissolved in 20 mL MeOH at room temperature. After 2 h, 0.1 M HCl was added into the solution to make its pH value to 2. MeOH was removed under reduced pressure, and the product was filtered off and washed with DCM to give 80 mg yellow powder in 87% yield. $^1$H NMR (400 MHz, DMSO) $\delta$ 12.92 (s, 1H), 8.45 (d, $J = 7.7$ Hz, 2H), 8.24 (d, $J = 8.5$ Hz, 1H), 7.64 (t, $J = 7.9$ Hz, 1H), 6.51 (d, $J = 8.6$ Hz, 1H), 4.69 (s, 2H), 4.53 (t, $J = 7.4$ Hz, 4H), 2.44-2.52 (2H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 170.12, 163.94, 162.92, 153.02, 133.59, 131.79, 131.48, 130.63, 124.45, 121.71, 120.68, 108.03, 106.63, 55.79, 41.34, 16.91. HRMS (ESI) calcd for C$_{17}$H$_{15}$N$_2$O$_4$ [MH$^+$] 311.1032, found 311.1026.
2.2.10 7-(aziridin-1-yl)-4-methyl-coumarin (10)

3-Bromophenol (1.73 g, 10 mmol) was slowly added to 40 mL 80% H₂SO₄ at 0 °C. The mixture was stirred for over 20 min, and ethyl acetoacetate (1.30 g, 10 mmol) was added dropwise in 30 min. After stirring for 24 h at room temperature, the reaction mixture was poured into 50 mL ice water and stirred for another 30 min. The precipitate was collected and dried under reduced pressure to give 500 mg white solid, yield 21%.

¹H NMR (400 MHz, CDCl₃) δ 7.52 (d, J = 1.3 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.43 (dd, J = 8.5, 1.6 Hz, 1H), 6.31 (d, J = 0.9 Hz, 1H), 2.43 (d, J = 1.0 Hz, 3H).

HRMS (ESI) calcd for C₁₀H₈BrO₂ [MH⁺] 238.9708, found 238.9700.

7-Bromo-4-methylcoumarin (100 mg, 0.42 mmol), Cs₂CO₃ (411 mg, 1.26 mmol), Pd(PPh₃)₂Cl₂ (19 mg, 5% mmol), aziridine (90 mg, 2.10 mmol) were dissolved in 8 mL dry toluene under N₂. The reaction mixture was slowly heated to 100 °C and stirred for 10 h. The solvent was removed under reduced pressure, and the residue was further purified by column chromatography (SiO₂, DCM/MeOH, 400:1, V/V) to give 15 mg white solid, yield 18%. ¹H NMR (400 MHz, CDCl₃) δ 7.46 (d, J = 8.5 Hz, 1H), 6.97 (dd, J = 8.5, 1.8 Hz, 1H), 6.91 (d, J = 1.8 Hz, 1H), 6.14 (s, 1H), 2.39 (s, 3H), 2.21 (s, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 161.23, 158.87, 154.54, 152.39, 125.20, 117.75, 115.03, 112.52, 108.62, 28.09, 18.67. Analytical HPLC: 98.13% purity (4.6 mm × 150 mm 5 µm C18 column; 20 µL injection; 45-55% MeOH/H₂O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C₁₂H₁₂NO₂ [MH⁺] 202.0868, found 202.0865.

2.2.11 7-(azetidin-1-yl)-4-methyl-coumarin (11)

7-Bromo-4-methylcoumarin (100 mg, 0.42 mmol), Cs₂CO₃ (411 mg, 1.26 mmol), Pd(PPh₃)₂Cl₂ (19 mg, 5% mmol), azetidine (120 mg, 2.10 mmol) were dissolved in 8 mL dry toluene under N₂. The reaction mixture was slowly heated to 100 °C and stirred for 10 h. The solvent was removed under reduced pressure, and the residue was further purified by column chromatography (SiO₂, DCM/MeOH, 200:1, V/V) to give 62 mg white solid, yield 66%. ¹H NMR (400 MHz, CDCl₃) δ 7.37 (d, J = 8.6 Hz, 1H), 6.30 (dd, J = 8.6, 2.0 Hz, 1H), 6.20 (d, J = 1.9 Hz, 1H), 5.96 (s, 1H), 3.98 (t, J = 7.3 Hz, 4H), 2.97 (q, J = 7.3 Hz, 4H), 2.20 (s, 3H).
Hz, 4H), 2.48 – 2.38 (m, 2H), 2.34 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 161.97, 155.59, 153.94, 153.04, 125.37, 110.27, 109.37, 107.68, 97.04, 51.75, 18.60, 16.50. Analytical HPLC: 99.05% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 60-40% MeOH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C$_{13}$H$_{14}$NO$_2$ [MH$^+$] 216.1025, found 216.1015.

2.2.12 3'-((aziridin-1-yl)-6'-(diethylamino)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (12)

Solution of 2-(4-diethylamino-2-hydroxybenzoyl) benzoic acid (313 mg, 1.00 mmol) and 3-iodophenol (260 mg, 1.18 mmol) in 10 mL CH$_3$SO$_3$H was slowly heated to 150 °C. After 8 hours, saturated Na$_2$CO$_3$ was added till the pH value of the solution rises to 9. The mixture was then extracted with DCM (3×50 mL). The organic product was dried with anhydrous Na$_2$SO$_4$ overnight and concentrated by rotary evaporation. The residue was further purified by column chromatography (SiO$_2$, DCM) to give light pink solid 380 mg, yield 77%. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.01 (d, $J$ = 7.3 Hz, 1H), 7.69 – 7.63 (m, 3H), 7.61 (td, $J$ = 7.4, 1.0 Hz, 1H), 7.30 (dd, $J$ = 8.3, 1.7 Hz, 1H), 7.16 (t, $J$ = 7.2 Hz, 1H), 6.56 (d, $J$ = 8.9 Hz, 1H), 6.48 (d, $J$ = 8.3 Hz, 1H), 6.44 (d, $J$ = 2.6 Hz, 1H), 6.40 – 6.34 (m, 1H), 3.36 (q, $J$ = 7.1 Hz, 4H), 1.17 (t, $J$ = 7.1 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 169.45, 152.92, 152.43, 152.04, 149.75, 134.95, 132.31, 129.68, 129.44, 128.84, 126.95, 126.15, 125.02, 123.96, 119.41, 108.68, 104.61, 97.57, 95.02, 83.42, 44.51, 12.50.

Compounds RI (100 mg, 0.20 mmol), Cs$_2$CO$_3$ (196 mg, 0.60 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (9 mg, 5%mmol), aziridine (43 mg, 1.0 mmol) were dissolved in 8 mL dry toluene under N$_2$. The mixture was heated to 100 °C and stirred for 18 h. The solvent was then removed under reduced pressure, and the residue was further purified by column chromatography (SiO$_2$, DCM/MeOH, 300:1, V/V) to give light pink solid 15 mg, yield 18%. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.00 (d, $J$ = 7.0 Hz, 1H), 7.69 – 7.62 (m, 1H), 7.62 – 7.55 (m, 1H), 7.20 (d, $J$ = 7.5 Hz, 1H), 6.86 (d, $J$ = 1.9 Hz, 1H), 6.72 – 6.65 (m, 1H), 6.61 (d, $J$ = 8.4 Hz, 1H), 6.55 (d, $J$ = 8.9 Hz, 1H), 6.44 (d, $J$ = 2.4 Hz, 1H), 6.35 (d, $J$ = 8.8 Hz, 1H), 3.36 (dd, $J$ = 14.1, 7.1 Hz, 4H), 2.14 (s, 4H), 1.21 – 1.07 (m, 6H). HRMS (ESI) calcd for C$_{26}$H$_{25}$N$_2$O$_3$[MH$^+$] 413.1865, found 413.1847.
2.2.13 3’-(azetidin-1-yl)-6’-(diethylamino)-3H-spiro[isobenzofuran-1,9’-xanthen]-3-one (13)

Compounds **RI** (100 mg, 0.20 mmol), Cs₂CO₃ (196 mg, 0.60 mmol), Pd(PPh₃)₂Cl₂ (9 mg, 5% mmol), azetidine (57 mg, 1.0 mmol) were dissolved in 8 mL dry toluene under N₂. The mixture was heated to 100 °C and stirred for 12 h. The solvent was removed under reduced pressure, and the residue was further purified by column chromatography (SiO₂, DCM/MeOH, 30:1, V/V) to give purple powder 40 mg, yield 47%.¹ H NMR (400 MHz, MeOD) δ 8.13 – 8.05 (m, 1H), 7.68 – 7.57 (m, 2H), 7.27 – 7.16 (m, 3H), 6.97 (dd, J = 9.5, 2.4 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 6.57 (dd, J = 9.1, 2.1 Hz, 1H), 6.50 (d, J = 2.1 Hz, 1H), 4.27 (t, J = 7.6 Hz, 4H), 3.64 (q, J = 7.1 Hz, 4H), 2.60 – 2.40 (m, 2H), 1.28 (t, J = 6.9 Hz, 6H). HRMS (ESI) calcd for C₂₇H₂₇N₂O₃[MH⁺] 427.2022, found 427.2028.

2.2.14 4-(aziridin-1-yl)-N-methylphthalimide (14)

4-Br-phthalimide (1.0 g, 4.41 mmol) in 30 mL glacial acetic acid was slowly added into 1 mL methylamine alcohol solution at room temperature. The mixture was heated and stirred for 2 h to reflux. The precipitate was collected and washed with water. The product was obtained as 900 mg white powder, yield 85%.¹ H NMR (400 MHz, CDCl₃) δ 7.98 (d, J = 0.5 Hz, 1H), 7.85 (d, J = 7.5 Hz, 1H), 7.71 (d, J = 7.9 Hz, 1H), 3.18 (s, 3H).

4-Br-N-methylphthalimide (100 mg, 0.42 mmol), Cs₂CO₃ (411 mg, 1.26 mmol), Pd(PPh₃)₂Cl₂ (19 mg, 5% mmol), aziridine (90 mg, 2.10 mmol) were dissolved in 5 mL dry toluene under N₂. The mixture was heated to 100 °C and stirred for 10 h. The solvent was removed under reduced pressure, and the residue was further purified by column chromatography (SiO₂, DCM/MeOH, 200:1, V/V) to give 30 mg white solid, yield 36%.¹ H NMR (400 MHz, CDCl₃) δ 7.62 (d, J = 8.0 Hz, 1H), 7.35 (s, 1H), 7.18
(dd, J = 8.1, 1.8 Hz, 1H), 3.07 (s, 3H), 2.18 (s, 4H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 168.42, 168.30, 161.00, 133.99, 125.67, 125.53, 124.24, 115.64, 28.25, 23.91. Analytical HPLC: 98.65% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 45—55% MeOH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C$_{11}$H$_{11}$N$_2$O$_2$ [MH$^+$] 203.0821, found 203.0816.

2.2.15 4-(azetidin-1-yl)-N-methylphthalimide (15)

4-Br-N-methylphthalimide (100 mg, 0.42 mmol), Cs$_2$CO$_3$ (411 mg, 1.26 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (19 mg, 5% mmol), azetidine (120 mg, 2.10 mmol) were dissolved in 5 mL dry toluene under N$_2$. The mixture was heated to 100 °C and stirred for 10 h. The solvent was removed under reduced pressure, and the residue was further purified by column chromatography (SiO$_2$, DCM/MeOH, 200:1, V/V) to give 65 mg light yellow powder, yield 72%. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.60 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.46 (dd, J = 8.2, 2.0 Hz, 1H), 4.04 (t, J = 7.4 Hz, 4H), 3.12 (s, 3H), 2.54 – 2.36 (m, 2H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 169.16, 168.97, 155.10, 134.73, 124.59, 118.41, 113.25, 104.48, 51.70, 23.74, 16.50. Analytical HPLC: 99.40% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 70-30% MeOH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C$_{12}$H$_{13}$N$_2$O$_2$ [MH$^+$] 217.0977, found 217.0977.

2.2.16 4-(aziridin-1-yl)-7-nitrobenzofurazan (16)

Aziridine (108 mg, 2.50 mmol) was slowly added into the solution of 4-chloro-7-nitrobenzofurazan (100 mg, 0.50 mmol) in 10 mL EA at room temperature. The mixture was stirred for 4 h, and the precipitate was collected and washed with EA. The product was obtained as 60 mg yellow powder, yield 58%. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.46 (d, J = 8.1 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 2.64 (s, 4H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 151.41, 146.84, 143.62, 133.30, 130.03, 113.44, 29.23. Analytical HPLC: 97.31% purity (4.6 mm × 150 mm 5 μm C18 column; 10 μL injection; 45–55% MeOH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C$_8$H$_7$N$_4$O$_3$ [MH$^+$] 207.0518, found 207.0516.
2.2.17 4-(azetidin-1-yl)-7-nitrobenzofurazan (17)

Azetidine (143 mg, 2.50 mmol) was slowly added into the solution of 4-chloro-7-nitrobenzofurazan (100 mg, 0.50 mmol) in 10 mL EA at room temperature. The mixture was stirred for 2 h, and the precipitate was collected and washed with EA. The product was obtained as 90 mg red powder, yield 82%. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.40 (d, $J = 8.7$ Hz, 1H), 5.77 (d, $J = 8.8$ Hz, 1H), 4.84 (s, 2H), 4.39 (s, 2H), 2.87 – 2.49 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 145.63, 145.04, 144.12, 136.88, 119.58, 99.92, 56.89, 53.47, 17.00. Analytical HPLC: 98.90% purity (4.6 mm $\times$ 150 mm 5 $\mu$m C18 column; 10 $\mu$L injection; 45-55% MeOH/H$_2$O, linear gradient; 30 min run; 1 mL/min flow; detection at 254 nm). HRMS (ESI) calcd for C$_9$H$_9$N$_4$O$_3$ [MH$^+$] 221.0675, found 221.0670.
2.3 $^{1}$H-NMR, $^{13}$C-NMR, HRMS and analytical HPLC spectra of 1—17 and other intermediate products

Figure S16. $^{1}$H-NMR spectrum of 1 in CDCl$_3$.

Figure S17. $^{13}$C-NMR spectrum of 1 in CDCl$_3$. 
Figure S18. HRMS spectrum of 1.

Figure S19. Analytical HPLC spectrum of 1.
Figure S20. $^1$H-NMR spectrum of 2 in CDCl$_3$.

Figure S21. $^{13}$C-NMR spectrum of 2 in CDCl$_3$. 
Figure S22. HRMS spectrum of 2.

Figure S23. Analytical HPLC spectrum of 2.
Figure S24. $^1$H-NMR spectrum of 3 in CDCl$_3$.

Figure S25. $^{13}$C-NMR spectrum of 3 in CDCl$_3$. 
Figure S26. HRMS spectrum of 3.

Figure S27. Analytical HPLC spectrum of 3.
Figure S28. $^1$H-NMR spectrum of 4 in CDCl$_3$.

Figure S29. $^{13}$C-NMR spectrum of 4 in CDCl$_3$. 

S35
Figure S30. HRMS spectrum of 4.

Figure S31. Analytical HPLC spectrum of 4.
Figure S32. $^1$H-NMR spectrum of 5 in CDCl$_3$.

Figure S33. $^{13}$C-NMR spectrum of 5 in CDCl$_3$. 
Figure S34. HRMS spectrum of 5.

Figure S35. Analytical HPLC spectrum of 5.
Figure S36. $^1$H-NMR spectrum of 6 in CDCl$_3$.

Figure S37. $^{13}$C-NMR spectrum of 6 in CDCl$_3$. 
Figure S38. HRMS spectrum of 6.

Figure S39. Analytical HPLC spectrum of 6.
Figure S40. $^1$H-NMR spectrum of 7 in CDCl$_3$.

Figure S41. $^{13}$C-NMR spectrum of 7 in CDCl$_3$.
Figure S42. HRMS spectrum of 7.

Figure S43. Analytical HPLC spectrum of 7.
Figure S44. $^1$H NMR of N-ethoxycarbonylmethyl-4-Bromo-1,8-naphthalimide in DMSO-$d_6$.

Figure S45. $^1$H NMR of N-ethoxycarbonylmethyl-4-aziridinly-1,8-naphthalimide 8 in CDCl$_3$. 
Figure S46. $^1$H NMR of 8 in DMSO-$d_6$.

Figure S47. $^{13}$C NMR of 8 in DMSO-$d_6$. 
Figure S48. HRMS spectrum of 8.

Figure S49. $^1$H NMR of N-ethoxycarbonylmethyl-4-azetidinly-1,8-naphthalimide 9 in CDCl$_3$. 
Figure S50. $^{13}$C NMR of N-ethoxycarbonylmethyl-4-azetidinly-1,8-naphthalimide 9 in CDCl$_3$.

Figure S51. $^1$H NMR of 9 in DMSO-$d_6$. 
Figure S52. $^{13}$C NMR of 9 in DMSO-$d_6$.

Figure S53. HRMS of 9.
Figure S54. $^1$H NMR of 7-Br-4-methylcoumarin in CDCl$_3$.

Figure S55. HRMS of 7-bromo-4-methylcoumarin.
Figure S56. $^1$H NMR of 7-aziridinyl-4-methylcoumarin 10 in CDCl$_3$.

Figure S57. $^{13}$C NMR of 7-aziridinyl-4-methylcoumarin 10 in CDCl$_3$. 
Figure S58. HRMS of 7-aziridinyl-4-methylcoumarin 10.

Figure S59. Analytical HPLC spectrum of 7-aziridinyl-4-methylcoumarin 10.
Figure S60. $^1$H NMR of 7-azetidinyl-4-methylcoumarin 11 in CDCl$_3$.

Figure S61. $^{13}$C NMR of 7-azetidinyl-4-methylcoumarin 11 in CDCl$_3$. 
Figure S62. HRMS of 7-azetidinyl-4-methylcoumarin 11.

Figure S63. Analytical HPLC spectrum of 7-azetidinyl-4-methylcoumarin 11.
Figure S64. $^1$H NMR of RI in CDCl$_3$.

Figure S65. $^{13}$C NMR of RI in CDCl$_3$. 
Figure S66. $^1$H NMR of 12 in CDCl$_3$.

Figure S67. HRMS of 12.
Figure S68. $^1$H NMR of 13 in CD$_3$OD.

Figure S69. HRMS of 13.
Figure S70. $^1$H NMR of 4-Br-N-methylphthalimide in CDCl$_3$.

Figure S71. $^1$H NMR of 4-aziridinly-N-methylphthalimide 14 in CDCl$_3$. 
Figure S72. $^{13}$C NMR of 4-aziridinly-N-methylphthalimide 14 in CDCl$_3$.

Figure S73. HRMS of 4-aziridinly-N-methylphthalimide 14.
Figure S74. Analytical HPLC spectrum of 4-aziridinly-N-methylphthalimide 14.

Figure S75. $^1$H NMR of 4-azetidinly-N-methylphthalimide 15 in CDCl$_3$. 

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.69 (d, $J$ = 8.2 Hz, 1H), 6.75 (d, $J$ = 2.0 Hz, 1H), 6.46 (dd, $J$ = 8.2, 2.0 Hz, 1H), 4.04 (t, $J$ = 7.6 Hz, 4H), 3.12 (s, 3H), 2.54 – 2.34 (m, 2H).
Figure S76. $^{13}$C NMR of 4-azetidinly-N-methylphthalimide 15 in CDCl$_3$.

Figure S77. HRMS of 4-azetidinly-N-methylphthalimide 15.
Figure S78. Analytical HPLC spectrum of 4-azetidinly-N-methylphthalimide 15.

Figure S79. $^1$H NMR of 4-aziridinly-7-nitrobenzofurazan 16 in CDCl$_3$. 
Figure S80. $^{13}$C NMR of 4-aziridinly-7-nitrobenzofurazan 16 in CDCl$_3$.

Figure S81. HRMS of 4-aziridinly-7-nitrobenzofurazan 16.
Figure S82. Analytical HPLC spectrum of 4-aziridinly-7-nitrobenzofurazan 16.

Figure S83. $^1$H NMR of 4-azetidinly-7-nitrobenzofurazan 17 in CDCl$_3$. 
Figure S84. $^{13}$C NMR of 4-azetidinly-7-nitrobenzofurazan 17 in DMSO-$d_6$.

Figure S85. HRMS of 4-azetidinly-7-nitrobenzofurazan 17.
Figure S86. Analytical HPLC spectrum of 4-azetidinly-7-nitrobenzofurazan 17.
2.4 UV—vis and fluorescence spectral data of 1—9
Table S5. Peak UV—vis absorption wavelengths ($\lambda_{\text{abs}}$, nm), peak emission wavelengths ($\lambda_{\text{em}}$, nm) and the Stokes shifts ($\Delta\lambda$, nm) of 1—7 in various solvents.

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<th>Chloroform</th>
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<td>479</td>
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<tr>
<td>7</td>
<td>388</td>
<td>477</td>
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<table>
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<th>Compound</th>
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<th>Ethanol</th>
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<td>381</td>
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<td>431</td>
<td>526</td>
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<td>7</td>
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<table>
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<th>Compound</th>
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<td>6</td>
<td>442</td>
<td>535</td>
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<tr>
<td>7</td>
<td>425</td>
<td>525</td>
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</table>
Figure S87. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 1—7 in n-hexane.

Figure S88. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 1—7 in chloroform.
Figure S89. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 1—7 in acetonitrile. Weak emission spectra are not shown, due to high noise levels.

Figure S90. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 1—7 in ethanol. Weak emission spectra are not shown, due to high noise levels.
Figure S91. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 1—7 in DMSO. Weak emission spectra are not shown, due to high noise levels.

Figure S92. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 1—7 in water. Weak emission spectra are not shown, due to high noise levels.

Figure S93. Normalized UV—vis absorption (dotted lines) and fluorescence (solid lines) spectra of 8 and 9 in water.
2.5 Fluorescence lifetime of 1—7

2.5.1 Fluorescence lifetime of 1—7 in ethanol
The fluorescence lifetime of 1—7 was measured in ethanol (Table S6).

Both 1 and 2 demonstrate large fluorescence lifetime (τ), in conjunction with bright fluorescence intensities. The bright fluorescence of 1 and 2 is from the local excited (LE) emission (Table S6).

The fluorescence decay dynamics of 3—7 fit better to double-exponential decays. These fittings suggest the presence of two emissive states: the LE and weakly emissive twisted-intramolecular-charge-transfer (TICT) states. The TICT emission has a long lifetime, but its intensity/contribution is rather low. The contribution of the LE emission is significantly higher in 3—7. However, the LE emission lifetime of 3—7 is much lower than that of 1 and 2, owing to substantial TICT formation rates in 3—7. Consequently, the overall emission intensities of 3—7 are very weak.

Table S6. Fluorescence lifetime (τ) of 1—7 in ethanol, and the associated statistical goodness-of-fitting, χ². For double-exponential fluorescence decays, the relative contributions of both time constants are given in brackets.

<table>
<thead>
<tr>
<th>Compound</th>
<th>τ (ns)</th>
<th>χ²</th>
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<tr>
<td>1</td>
<td>9.58</td>
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<tr>
<td>2</td>
<td>9.36</td>
<td>1.183</td>
</tr>
<tr>
<td>3</td>
<td>0.24 (93.4%)</td>
<td>8.39 (6.6%)</td>
</tr>
<tr>
<td>4</td>
<td>0.62 (98.5%)</td>
<td>4.31 (1.5%)</td>
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<tr>
<td>5</td>
<td>0.08 (98.2%)</td>
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<tr>
<td>6</td>
<td>0.04 (84.0%)</td>
<td>8.06 (16.0%)</td>
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<tr>
<td>7</td>
<td>0.08 (94.7%)</td>
<td>7.79 (5.3%)</td>
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2.5.2 Fluorescence lifetime of 1 and 2 in various solvents
The fluorescence decay dynamics of both 1 and 2 fit well to single-exponential decays in various tested solvents (Table S7). The lifetime of 1 and 2 is high in general.

However, the lifetime of 2 drops substantially in water, indicating a large TICT formation rate. In contrast, the lifetime of 1 remains high in water. These results show that 1 is resistant to TICT, even in water. These observations are in excellent agreement with our theoretical calculations.
Table S7. Fluorescence lifetime (ns) of 1 and 2 in various solvents.

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<th>Solvent</th>
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<td>Chloroform</td>
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<td>Acetonitrile</td>
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<td>9.64</td>
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<tr>
<td>Ethanol</td>
<td>9.58</td>
<td>9.36</td>
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<tr>
<td>DMSO</td>
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<tr>
<td>Water</td>
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<td>4.37</td>
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2.6 Quantum yields of 1—7 in various solvents
Table S8. Quantum yields of 1—7 in various solvents at room temperature.

<table>
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<tr>
<th>Compound</th>
<th>n-Hexane</th>
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<th>Ethanol</th>
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<th>Water</th>
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<td>0.1985</td>
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<td>0.7698</td>
<td>0.8413</td>
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2.7 Photo-physical properties of 8 and 9 in water
Table S9. Peak UV—vis absorption wavelengths (λ_{abs}, nm), peak emission wavelengths (λ_{em}, nm), the Stokes shifts (Δλ, nm), molar extinction coefficients (ε) and quantum yields of 8 and 9 in water.

<table>
<thead>
<tr>
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<th>λ_{abs} (nm)</th>
<th>ε (M^{-1} cm^{-1})</th>
<th>λ_{em} (nm)</th>
<th>Δλ (nm)</th>
<th>φ</th>
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<td>464</td>
<td>16840</td>
<td>561</td>
<td>97</td>
<td>0.2730</td>
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</table>

2.8 Photostability of 8 and 9 in water
The fluorescence photostability tests of 8 and 9 were conducted on an IX81 microscope (Olympus, Japan) coupled with a EMCCD camera (DU-897U-CS0-#BV, Andor, UK). Aqueous solution of 8 and 9 (5 µM) was injected into polydimethylsiloxane (PDMS) microfluidic channels via a syringe, separately. The microfluidic channels were designed with AutoCAD (software) and fabricated using conventional soft lithographic techniques.11
These samples were excited with a supercontinuum white-light laser (SC400-PP, Fianium, UK). The excitation wavelength was set to 416 nm, since the excitation spectra of 8 and 9 intersected at this wavelength. The excitation laser beam was defocused to form a homogeneous excitation spot of ~350 µm in diameter through a 10× air objective lens (NA=0.4, Olympus UPLSAPO). The laser intensity was adjusted via a neutral density filter and monitored with a power meter (PM100D S130VC, ThorLabs, USA). The excitation intensity was 3.3 W cm$^{-2}$ during the experiments. The fluorescence signal was collected using the EMCCD equipped with a band pass filter (545±37.5 nm; Figure S94).

The up-up conformed 8 demonstrates a stronger photostability than the planar 9 (Figure 1g; Figure S95).

![Diagram](image)

Figure S94. Illustration of the experimental set-up to test the photostability of 8 and 9 in water (laser wavelength = 416 nm).

![Images](image)

Figure S95. Representative fluorescence images of 8 and 9 during the laser photostability tests in water. Compound 9 experienced a more substantial intensity drop.

### 2.9 Chemical stability of 1 in water

We also investigated the chemical stability of 1 in aqueous solutions of various pH values. The pH values of these samples were adjusted using HCl and NaOH. After 10 min of settling time, the fluorescence spectra and peak emission intensities of these
samples were measured. The fluorescence intensities of 1 remained stable from pH 4.24 to 10.80 (Figure S96).

Figure S96. (a) Fluorescence spectra and (b) peak emission intensities of 1 in aqueous solutions of various pH values.

2.10 Quantum yields of conventional dialkylamino fluorophores
The quantum yields of conventional dialkylamino fluorophores in aqueous solution have been compiled from previous reports (Table S10).

Table S10. Quantum yields of selected dialkylamino fluorophores in aqueous solution.

<table>
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<th>Molecular structure</th>
<th>Quantum yields</th>
<th>References</th>
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<td>12</td>
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<td><img src="image3" alt="Molecular structure 3" /></td>
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<td><img src="image4" alt="Molecular structure 4" /></td>
<td>0.008—0.010</td>
<td>14,15</td>
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3 References

(1) Frisch, M.; Trucks, G.; Schlegel, H. B.; Scuseria, G.; Robb, M.; Cheeseman, J.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, Gaussian 09 (software), 2009.


