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Fluorescent visualization of the conformational change of aromatic amide or urea induced by N-methylation

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ABSTRACT

The conformational change of amide structure of benzanilide or urea structure of *N*,*N*′-diphenylurea induced by methylation of the secondary nitrogen was visualized by introduction of pyrene moieties on the benzene ring. In contrast to the extended trans-form of secondary amide and urea, which showed monomer fluorescence emission of pyrene, the corresponding N-methylated compounds exist in cis-form, which exhibited excimer emission.

The amide linkage is an important building block of functional molecules, since conformations with restricted rotation can often be obtained by an appropriate choice of substituents. Previously, we reported that N-methylation of benzanilide, which has trans-conformation, causes conformational change, resulting in cis-conformational preference of *N*-methylbenzanilide.[1] Similarly, *N*,*N*′-diphenylurea exists in extended (trans, trans) conformation, while *N*,*N*′-dimethyl-*N*,*N*′-diphenylurea exists in folded (cis, cis) conformation (Fig. 1).[2] The cis-conformational preference is a general steric feature of various aromatic N-methylated amides and ureas, and some amide structures can be altered by external stimuli, such as temperature,[3] pH,[4] and redox potential.[5]

Fluorescence measurement has distinct advantages in terms of sensitivity, selectivity, and response time for sensing certain analytes. Consequently, many fluorescent sensors have been developed and applied to detect ions, enzymes, and environmental changes in various fields of scientific research, including analytical chemistry, biology, and materials science.[6] By means of the introduction of suitable fluorophores on appropriate sites, the structural changes of certain molecules can be fluorescently visualized. In this Letter, we show that the conformational change of benzanilide and *N*,*N*′-diphenylurea skeletons induced by N-methylation can be visualized via a change of fluorescence properties. Therefore, these structures are potentially available as scaffolds for obtaining fluorescent sensor candidates by suitable derivatization.

Pyrene is a polycyclic aromatic fluorophore, and exhibits monomer fluorescence at shorter wavelength (370–390 nm) and excimer fluorescence at longer wavelength (near 480 nm).[7] The efficiency of each fluorescence depends on concentration, that is, the ratio of excimer fluorescence increases at higher concentration, or the distance and orientation of two pyrene units in the molecule. When molecular frameworks, whose structures are changed by external environments, such as temperature and viscosity, or binding with analytes such as ions and DNA, are labeled with

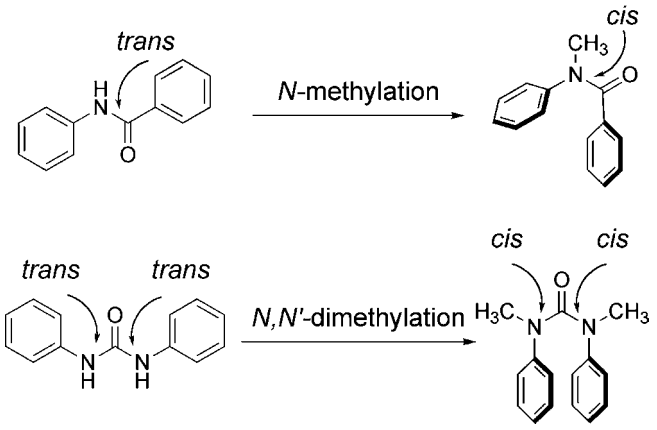

Figure 1. Conformational change by N-methylation of aromatic amide and urea.

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two pyrenes at appropriate positions via appropriate spacer, they can be used as fluorescent sensors for these analytes.[8] Because aromatic urea and amide structures are candidates for such molecular frameworks, we designed benzanilide and diphenylurea derivatives substituted with pyrenes via an ethynylene group or ethylene group as a spacer structure (Fig. 2).

1-Bromopyrene (**6**) was reacted with 4-ethynylaniline (**7**) by means of the Sonogashira coupling reaction to yield **8** (Scheme 1).[9] Reduction of the ethynylene group of **8** with palladium hydroxide on carbon catalyst afforded 4-[2-(1-pyrenyl)ethyl]aniline (**9**). Similarly, methyl 4-[2-(1-pyrenyl)ethynyl]benzoate (**5a**) was prepared from **6** and methyl 4-ethynylbenzoate (**10**), and hydrolyzed to **11** or reduced to **5b**, which was also hydrolyzed to yield **12**. The carboxylic acid **11** was converted to the acid chloride, and then reacted with **8** to afford the secondary amide **1a**, which was methylated using methyl iodide and sodium hydride as a base to afford the tertiary amide **2a**. Ethylene-spacered secondary amide (**1b**) and its N-methylated derivative (**2b**) were similarly prepared from carboxylic acid **12** and amine **9**. The secondary ureas **3a** and **3b** were synthesized from amines **8** and **9**, respectively, with triphosgene, and were similarly converted to dimethylated ureas **4a** and **4b**, respectively.

The conformational differences between secondary amide **1** and its N-methylated amide **2** or between secondary urea **3** and its *N*,*N*′-dimethylated urea **4** were examined by ^{1}H NMR spectroscopy. In each pair of secondary and N-methylated compounds, upfield changes of the protons on the phenyl rings on going from secondary to N-methylated amide or urea were observed (Fig. S1). Such chemical shift differences are similar to those of unsubstituted benzanilide versus *N*-methylbenzanilide or of *N*,*N*′-diphenylurea versus *N*,*N*′-dimethyl-*N*,*N*′-diphenylurea, respectively,[10] which suggested the extended trans-structures of compounds **1** and **3**, and the folded cis-structures of N-methylated compounds **2** and **4** in solution. The signals of the pyrene ring protons of N-methylated compounds were also shifted to a little higher field, compared to the corresponding secondary amides or ureas. ^{1}H NMR spectra of **2b** at low temperature revealed that **2b** is in equilibrium between cis- and trans-conformers in the ratio of 98:2 at 213 K in CD_2Cl_2, while **4b** did not show such an equilibrium between conformers, presumably due to the lower rotational barrier of the urea bond (data not shown).

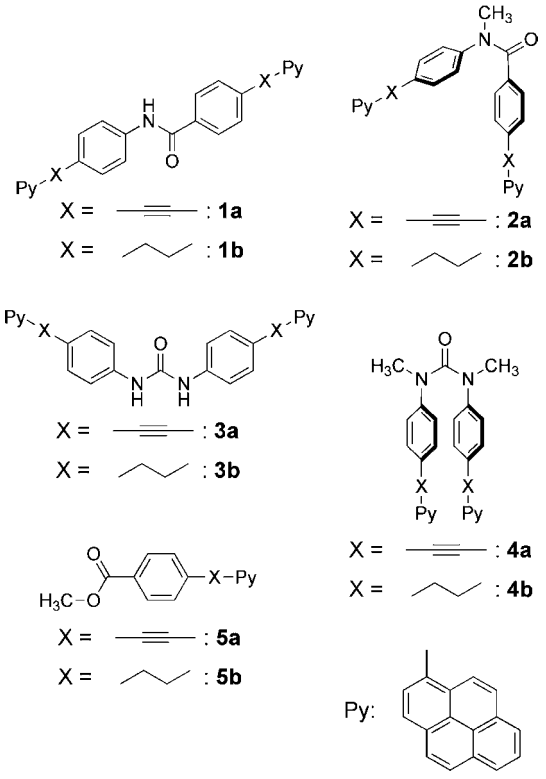
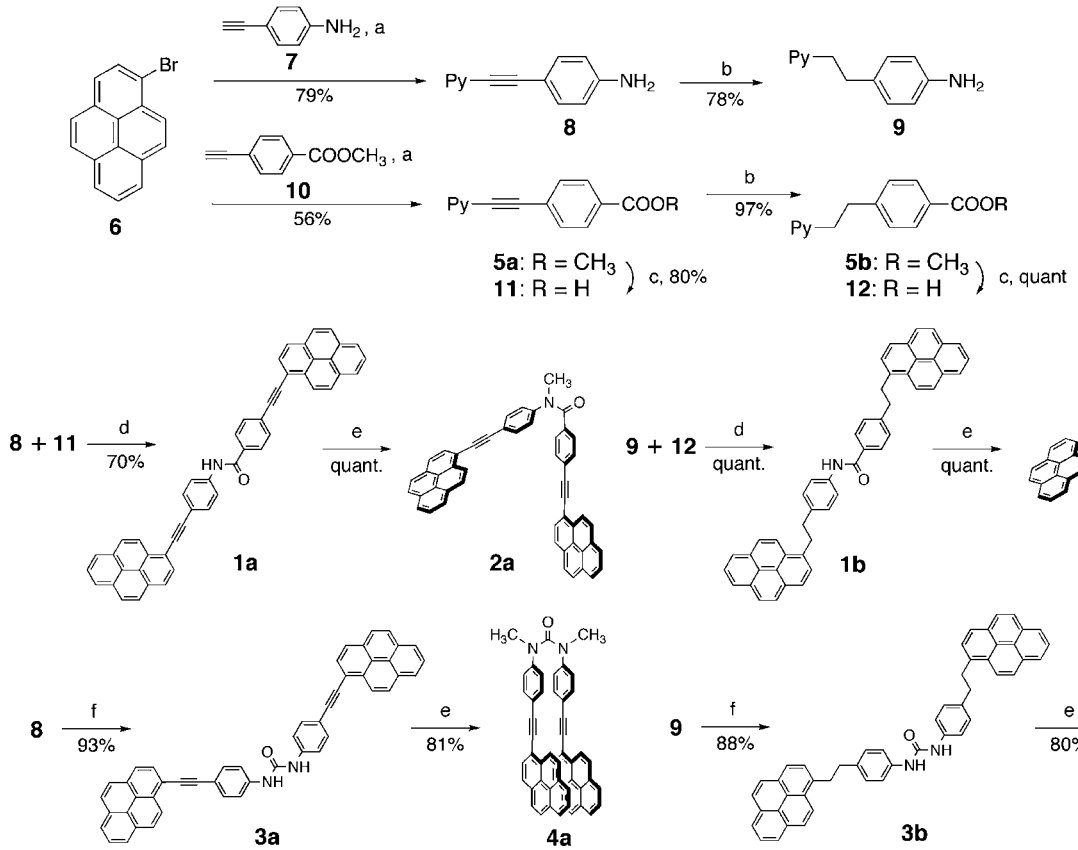


Figure 2. Structures of pyrene (Py)-substituted aromatic amides and ureas.

The absorption spectra and fluorescence spectra are shown in Figure 3. In the case of the compounds with the ethynylene groups (**1**–**5**, **a** series), the absorption spectra were broad and red-shifted, compared to those of the corresponding compounds with the ethylene groups (**1**–**5**, **b** series). For example, the monomeric compound **5b** showed typical pyrene monomer absorbance spectrum with sharp absorbance peaks at 300–350 nm, while **5a** had an absorption in the region of 350–450 nm. The absorption pattern is essentially similar between the compounds in the **b** series (Fig. 3c and d). On the other hand, there are small differences in the absorption wavelength between each set of monomeric compounds **5a**, secondary (**1a** or **3a**), and N-methylated compounds (**2a** or **4a**) (Fig. 3a and b). For example, the maximum absorption wavelength of **5a** (377 and 400 nm) was blue- and red-shifted in **1a** (382 and 404 nm) and **2a** (370 and 397 nm), respectively.

More drastic differences were observed in their fluorescence spectra. Compound **5b** showed fluorescence spectrum with fine vibrational structure at 370–390 nm (Fig. 3g), while compound **5a** showed broad and red-shifted fluorescence in the region of 400–550 nm (Fig. 3e). These data suggested that the 1-phenyl-2-pyrenylacetylene moiety would no longer show pyrene-like fluorescence, probably due to their conjugation properties. Interestingly, the amides **1a** and **2a** did not show any significant fluorescence (Fig. 3e). In the case of urea derivatives, both **3a** and **4a** showed fluorescence in the region of 400–550 nm, and the fluorescence intensity and wavelength of *N*,*N*′-dimethylated urea **4a** (emission maximum at 481 nm) were smaller and a little red-shifted than those of **3a** (emission maximum at 447 nm) and **5a** (Fig. 3f). Among the compounds of **b** series, **1b** and **3b** showed fine vibrational structure at 370–390 nm like monomeric compound **5b**. In contrast, **2b** and **4b** showed broad fluorescence spectra with its maximum of 477 nm that could be identified as pyrene excimer spectra, accompanied with a corresponding decrease of monomer emission (Fig. 3g and h). The spectra of **b** series compounds did not show any significant change by the concentration (1–50 μM) and temperature (273–353 K). Taken together with ^{1}H NMR spectra, these results suggest that in the extended trans-structures of **1b** and **3b**, the pyrene moieties exhibit monomer-type fluorescence spectra, whereas in the cis-structures of the corresponding N-methylated derivatives **2b** and **4b**, they exhibit intramolecular excimer-type fluorescence spectra. The degree of fluorescent change of the ureas (**3b** vs **4b**) was greater than that of the amides (**1b** vs **2b**), probably due to the difference in the distance and orientation of two phenyl rings between N-methylated amide **2b** and *N*,*N*′-dimethylated urea **4b**.[11]

Our present results showed that the conformational change could be visualized by the excimer fluorescence of the two pyrenes linked to the aromatic amides and ureas through the ethylene spacers. When the spacer structure is ethynylene group, both the absorption and fluorescence spectra did not show pyrene-like monomer or excimer spectra. Previously, similar observation was reported in the fluorescence spectra of *N*,*N*′-dimethyl-*N*,*N*′-dipyrenylurea, in which two pyrenes were directly connected to nitrogen atoms of *N*,*N*′-dimethylurea with the folded (cis, cis) conformation.[12,13] In these reports, both absorption and fluorescence spectra of this compound were different from those of the corresponding monomeric compound, and depended on the temperature and viscosity. However, in this system, the difference in fluorescence between extended trans-structure and folded cis-structure seems unclear. For this reason, the changes in the fluorescence spectra



Scheme 1. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, triethylamine, 70 °C; (b) $Pd(OH)_2/C$, H_2 (1 atm), AcOH (for **8**) or CH_2Cl_2/AcOH(for **5a**); (c) 2 M NaOH aq, EtOH; (d) (1) $SOCl_2$, DMF, (2) **8** or **9**, pyridine, DMAP; (e) MeI, NaH, DMF, 60 °C; (f) triphosgene, triethylamine, THF.

of urea structure directly connected with two pyrenes are supposed not to reflect the conformational changes of the molecular structures, such as our ethynylene-spacered series (**1a**–**4a**), and therefore these systems seem not suitable for scaffolds of fluorescent sensor candidates. In contrast, our ethylene-spacered systems (**1b**–**4b**) are expected to be good scaffolds of fluorescent sensor candidates because the conformational preference of aromatic amide or urea structure certainly reflects the fluorescence

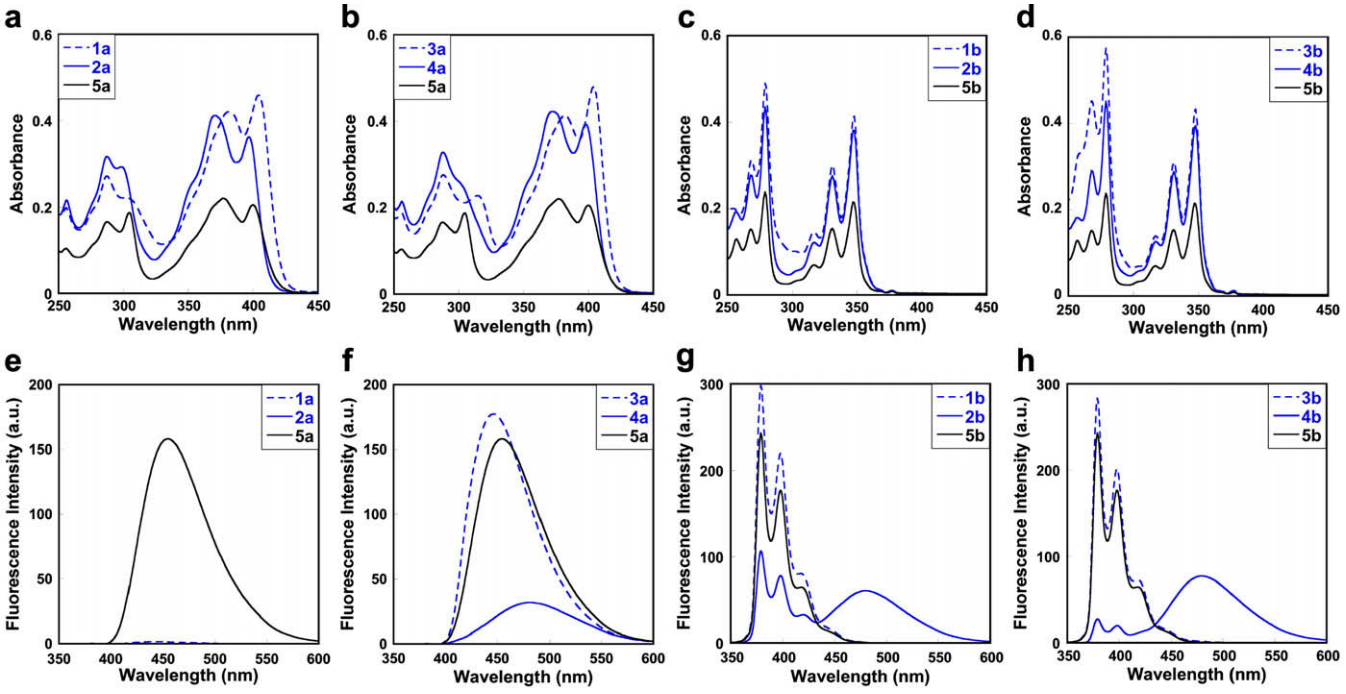


Figure 3. Absorption (a–d) and fluorescence (e–h) spectra of **1**–**5** in DMSO at 303 K. Excitation wavelength is 380 nm (for e and f) or 345 nm (for g and h). Concentration of each compound was 5 μM.

properties. In the case of pyrene derivatives connected directly or conjugated via ethynylene spacer systems to the amide or urea structures, some further derivatizations for obtaining fluorescent sensors might unexpectedly influence these fluorescence properties, as observed in **1a** and **2a**, whose fluorescence quenched, while ethylene-spacered systems (**1b**–**4b**) probably will not.

In conclusion, we synthesized the pyrenyl derivatives of aromatic amides and ureas. The fluorescence from the compounds substituted with two pyrenes via ethylene group depended on the skeletal conformation, that is, extended trans-form or folded cis-form, of the amides and the ureas. Because the conformational preference of aromatic urea or amide structure can be changed by suitable derivatization,[3–5] these structures are considered to be potential platforms for fluorescent sensors of various stimuli, and the studies to develop such sensors are in progress.

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Supplementary data

Experimental procedure and NMR spectral data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.11.044.

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