A pyridinium cation–π interaction sensor for the fluorescent detection of alkyl halides†‡

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Received 15th May 2010, Accepted 10th June 2010
DOI: 10.1039/c0cc01420f

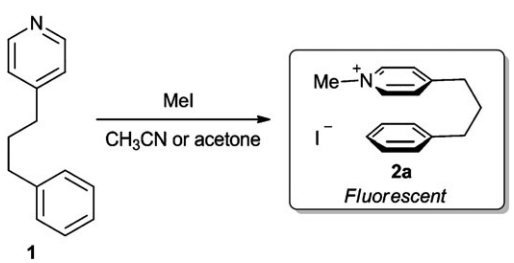
***N*-Alkylation of a novel pyridine sensor results in pyridinium salts whose conformations are stabilised by pyridinium cation–π interactions resulting in a fluorescent response that can be used to sense the presence of alkylating agents in solution at low concentration.**

Potent alkylating agents such as methyl iodide or benzyl bromide are extremely useful reagents for organic synthesis that have been used widely for the synthesis of many drug molecules. However, their inherent reactivity means they are potentially toxic/mutagenic due to their ability to react with numerous biological nucleophiles present within the human body.[1] Consequently, their use requires rigorous detection regimes to ensure that no alkylating agent is present in any pharmaceutical agent that is used for human consumption.[2] Alkylating agents are also present in trace amounts in the environment due to their use as soil sterilizers,[3] cancer drugs,[4] chemical reagents and chemical weapon agents.[5] Therefore, there is significant demand for the development of reliable assays that allow the presence of alkylating agents to be detected in a wide range of scenarios.[6]

Cation–π interactions have been widely exploited in supramolecular chemistry, where they play pivotal roles in the design of host–guest assemblies[7] and synthetic receptors.[8] These type of interactions have also proven useful for synthesis,[9] where their presence has been invoked to explain the selectivity of nucleophilic catalysts,[10] cyclopropanations,[11] cycloadditions[12] and various nucleophilic additions.[13] In the biological arena cation–π interactions have been implicated in many biomolecular recognition events,[14] whilst they also play an important role in stabilising the secondary and tertiary structures of proteins.[15] We have previously used NOEsy and fluorescence spectroscopy to show that conversion of conformationally flexible 4-(phenylpropyl)-pyridine **1** into its corresponding *N*-methyl-pyridinium iodide **2a** (Scheme 1) results in a large change in fluorescence response, due to the presence of intramolecular cation–π interactions in **2a**.[16] Consequently, we now report that the increased fluorescent response of pyridinium salts **2** may be exploited to enable pyridine **1** to be used to sense the presence of low concentrations of alkylating agents in solution (Scheme 2).[17,18]

We reasoned that the contrasting fluorescent properties of pyridine **1** and its corresponding *N*-alkyl-pyridinium salts **2** might enable it to be used as a sensor to detect the presence of alkylating agents in solution. In order to test this hypothesis, aliquots of methyl iodide (0–0.6 mM) were sequentially added to a solution of pyridine **1** in CH_2Cl_2 (1.0 mM) and the fluorescent spectra of the solution recorded 5 minutes after each addition. This revealed an eleven fold fluorescence enhancement (F/F_o) over the concentration range (0–0.6 mM) of methyl iodide assayed (see Fig. 1).[19] In order to determine the detection limits of this sensor we reduced the concentration of pyridine **1** to 0.01 mM in CH_2Cl_2 and assayed its fluorescent response to the presence of low concentrations of methyl iodide (0–400 nM). This resulted in a three fold increase in fluorescence over a concentration range of 0–400 nM, with a lower detection limit for methyl iodide of 1 nM (see Fig. 2 and ESI†).

In order to demonstrate the potential of this cation–π stacking sensor towards other types of alkylating agent, we then treated pyridine **1** (1.0 mM, CH_2Cl_2) with a range of commonly used electrophiles **3a–k** (0.6 mM) under reflux for 3 h to ensure complete consumption of electrophile, and determined the maximum fluorescence of each solution. In each case a fluorescence enhancement was observed (29–114 fold increase), indicating that pyridine **1** has the potential to be used as a sensor for a wide range of alkylating agents. It is



Scheme 1 Alkylation of pyridine **1** with methyl iodide forms pyridinium **2** which exhibits characteristic cation–π fluorescence.

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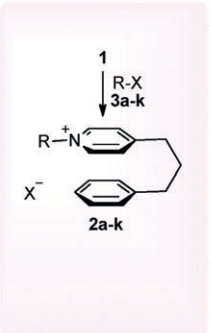
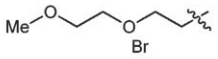
† Electronic supplementary information (ESI) available: A representative synthetic procedure, corresponding analysis and X-ray crystallographic data. CCDC 775629. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc01420f

‡ This article is part of the 'Emerging Investigators' themed issue for ChemComm.



	R	X	F/F_0 Enhancement$_{(max)}$
a	Me	I	49
b	Me	OTf	114
c	Et	I	29
d	Et	Br	89
e	Bn	Br	89
f	*p*-OMe Bn	Br	81
g	*p*-NO_2 Bn	Br	36
h	CH_2CH_2Ph	Br	99
i	$CH_2CH_2CH_2Ph$	Br	46
j	propargyl	Br	61
k			45

Scheme 2 Screening of fluorescent response of *N*-alkyl-pyridinium salts **2a–k** derived from different alkylating agents.

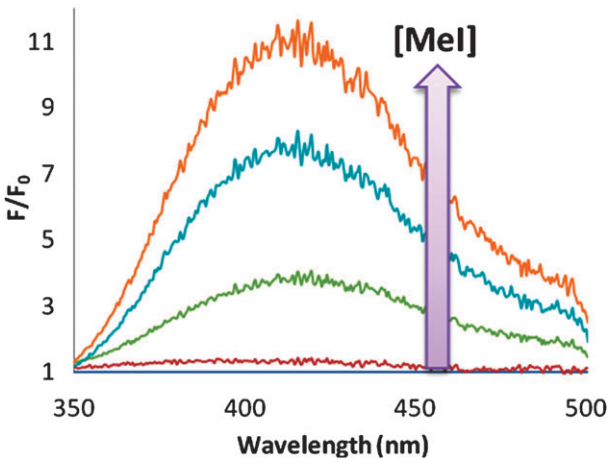


Fig. 1 Fluorescence increase on addition of aliquots of MeI (0 to 0.6 mM) to a solution of pyridine **1** (1.0 mM) in CH_2Cl_2 (λ_{ex} = 260 nm); F/F_0 *versus* wavelength (nm) for increasing [MeI].

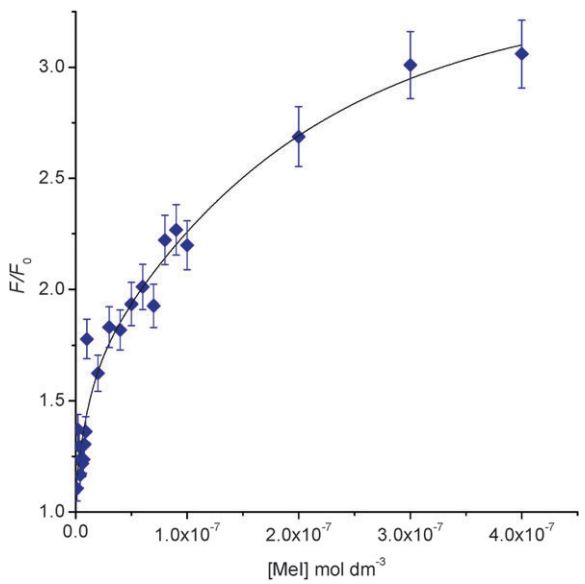


Fig. 2 Fluorescence F/F_0 at 400 nm for addition of MeI (0–400 nM) to pyridine **1** (0.01 mM) in CH_2Cl_2 (λ_{ex} = 260 nm).

noteworthy that the fluorescent response of the *N*-alkyl-pyridinium triflate salt **2b** was 2.3 fold greater than the corresponding iodide salt **2a**, which we ascribed to the known heavy atom quenching effect of the iodide counter ion.[19] Whilst alkylation of pyridine **1** with the potent triflate/iodide alkylating agents **3a–c** gave an almost instantaneous increase in fluorescence (seconds), use of alkyl bromides **3d–k** required a longer time (hours) to react before solutions with stable fluorescence maxima were obtained. The corresponding control experiments where pyridine and benzene with and without added methyl iodide had previously confirmed an intramolecular interaction is responsible for the observed fluorescence.[16a]

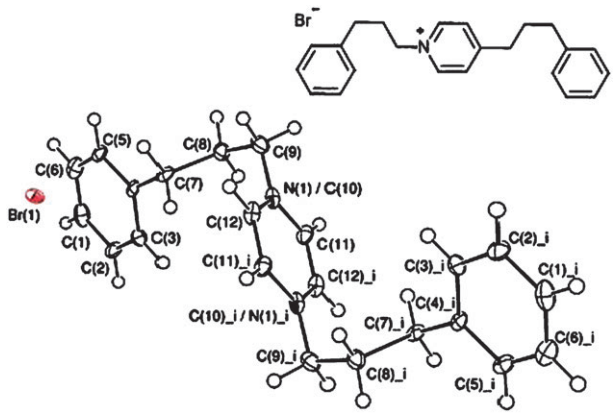


Fig. 3 ORTEP plot of **2i** with ellipsoids drawn at the 50% probability level. Atoms N(1) and C(10) lie on the same site at 50% occupancy each. Selected bond lengths (Å) and angles (°): C(9)–C(10)/N(1) = 1.490 (9), C(10)/N(1)–C(11) = 1.371 (8), C(10)/N(1)–C(12) = 1.379 (8), C(4)–C(7)–C(8) = 111.9 (5), C(7)–C(8)–C(9) = 112.5 (5), C(8)–C(9)–C(10)/N(1) = 111.5 (5), C(11)–C(10)/N(1)–C(12) = 118.9 (6), C(4)–C(7)–C(8)–C(9) = −171.0 (5), and C(7)–C(8)–C(9)–C(10) = 62.2 (7).

A range of *N*-alkyl-pyridinium salts **2a–k** were then prepared in order to confirm that these species were responsible for the fluorescent response observed in these assays. Isolated yields of each fluorescent salt were generally high (> 90%).[20] The structure of compound **2i** was determined by X-ray crystallography (see Fig. 3),[21] which revealed that electrostatic cation–anion interactions dominate in the solid state, with no intramolecular cation–π stacking interactions being observed.[16b]

In conclusion, we have reported an operationally simple, robust and cheap pyridine sensor **1** for the fluorescent detection of a range of commonly employed alkylating agents. We are currently working on the design of new pyridine architectures with higher fluorescent output and improved response time that we envisage will enable even lower concentrations of alkylating agent to be detected.

The Leverhulme Trust, Royal Society, Universities of Birmingham and Bath, EPSRC, Birmingham AWM II and the JSPS are thanked for support. SAE acknowledges Cairo University (NILES-LAMPA). The EPSRC National Crystallography Service is thanked for the collection of X-ray diffraction data on **2i**.

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20 Pyridinium salt **2j** was not isolated as a single compound, rather as a mixture of **2j** and its corresponding allene, Dr Richard Grainger (University of Birmingham) is thanked for helpful discussion.
21 X-Ray crystal structure data for **2i**: single crystals of **2i** were recrystallised from acetone, mounted in inert oil and transferred to the cold gas stream of the diffractometer. $C_{23}H_{26}N{\cdot}Br$, M = 396.36, orthorhombic, a = 7.3748 (2) Å, b = 10.0622 (2) Å, c = 25.6743 (8) Å, U = 1905.21 (9) Å^3, T = 120 (2) K, space group *Pbca* (no. 61), Z = 4, 22 489 reflections measured, 2183 unique (R_{int} = 0.0468) which were used in all calculations. The final R-indices were: R_1 = 0.0950 (observed data) and wR_2 = 0.2020 (all data). CCDC 775629.