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TMIO-PyrImid Hybrids are Profluorescent, Site-Directed Spin Labels for Nucleic Acids

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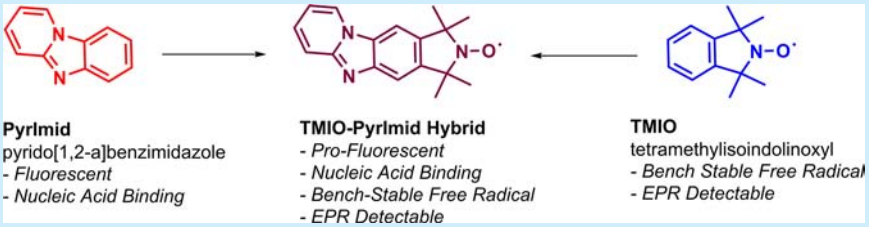
Supporting Information



ABSTRACT: We report the synthesis of a new class of molecules which are hybrids of long-lived tetramethylisoindolinoxyl (TMIO) radicals and the pyrido[1,2-*a*]benzimidazole (PyrImid) scaffold. These compounds represent a new lead for noncovalently binding nucleic acid probes, as they interact with nucleic acids with previously unreported C (DNA) and C/U (RNA) complementarity, which can be detected by electron paramagnetic resonance (EPR) techniques. They also have promising properties for fluorimetric analysis, as their fluorescent spin-quenched derivatives exhibit a significant Stokes shift.

Research has established nitroxides[1] as the most prominent class of bench-stable free radicals,[2] and they have therefore been adapted to a diverse array of applications.[3] Ongoing efforts have capitalized upon opportunities for the application of structurally diverse nitroxide compounds as antioxidants (free-radical scavengers of superoxides),[4] stabilizers in the materials industry,[5] and both chemical (radical clocks[6]) and biological (cellular redox[7]) probes. Recent examples of these include biologically active agents, including roles that can be considered as both metabolically "active" (e.g., chemotherapeutic antioxidant[8]) and "passive" (e.g., electron paramagnetic resonance probes[9]). A prime example of the latter is the use of nitroxides as site-directed spin labels in the analysis of the structure and dynamics of nucleic acids,[10] thereby providing information about their function.[11] This involves EPR studies upon a probe molecule bound to RNA or DNA. As the spin label becomes more constrained (by binding to a nucleotide), the EPR spectrum becomes increasingly anisotropic,[12,11b] which gives information about local structure and orientations in distance measurements.[5] Binding is also flanking-sequence dependent, providing information about neighboring nucleotides.[13]

Introduction of the requisite spin label is usually achieved by either covalent incorporation of a nitroxide-bearing nucleobase analogue into the nucleic acid sequence[14] or by postsynthetic covalent linkage of the spin label to a functionality of the nucleic acid.[15] More recently, Sigurdsson and co-workers have pioneered noncovalent binding of ç ("c-spin"), which is the nucleobase of the nucleoside Ç (Figure 1).[16] This site-directed spin label is a nucleobase isostere of cytidine. It forms stable Watson−Crick pairing with guanine residues and $\pi-\pi$ interactions with adjacent base pairs, enabling it to be added to a variety of preformed nucleic acid polymers. A contrasting method for analysis of nucleic acids is with the use of fluorescent noncovalent binders for spectrophotometric titration. In a recent example, Ihmels and co-workers have described the application of quinizolinium scaffolds such as "**F**" as new probes for abasic DNA.[17] Despite these recent advances in nucleic acid probe technology, noncovalently binding spin labels with complementarity for cytidine (C), thymine (T), adenine (A), and uracil (U) await discovery.

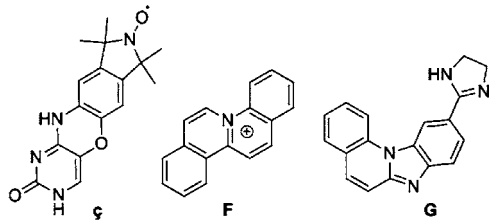


Figure 1. Nitroxide ç (EPR detection),[16] quinizolinium **F** (spectrophotometric titration),[17] and DNA intercalator **G** (anticancer).[21]

A compound class that may provide an opportunity to achieve this goal are pyrido[1,2-*a*]benzimadoles, or "PyrImids".

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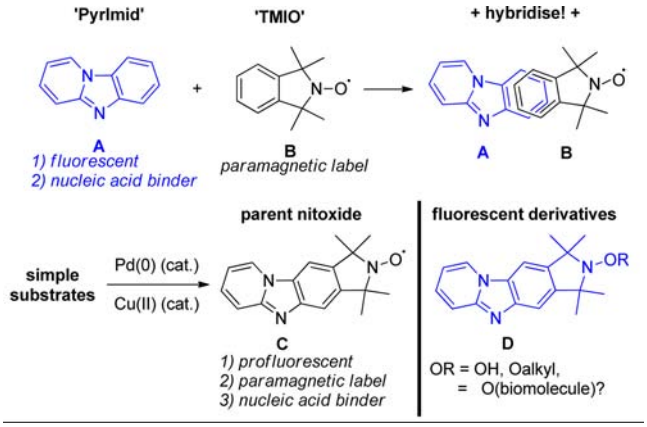
These interesting imidazole-containing heterocycles are known to possess biological activities, including antipyretic,[18] antibiotic,[19] and anticancer effects.[20] The latter property arises through the isosterism of PyrImid derivatives such as benzimidazo[1,2-*a*]quinazoline, **G**, with purines, which enables their nucleic acid intercalation. Hranjec, Kralj, Zamola, and co-workers found[21] that derivatives such as **G** (Figure 1) arrest cellular mitosis through their interaction with topoisomerase II, thereby inhibiting growth of human colorectal and other cancer cells. PyrImids are furthermore highly fluorescent[22] and possess a remarkable Stokes shift.

Isoindolinoxyl radicals based upon "TMIO", or 1,1,3,3-tetramethylisoindolin-2-oxyl, are one of the most rigid and stable nitroxide classes commonly prepared. Importantly, these nitroxides have a moiety that strongly suppresses fluorescence[23] when in conjugation with a fluorophore; the fluorescence can be (re)generated by "spin-deletion" via one of several methods. It seemed that the marriage of the key features of both PyrImid "**A**" and TMIO "**B**" cores may result from simply superimposing the C_6 aryl ring of the core in each to create a hybrid molecular probe, "**C**" (Scheme 1). It was hypothesized that the

Scheme 1. Fusion of TMIO and PyrImid

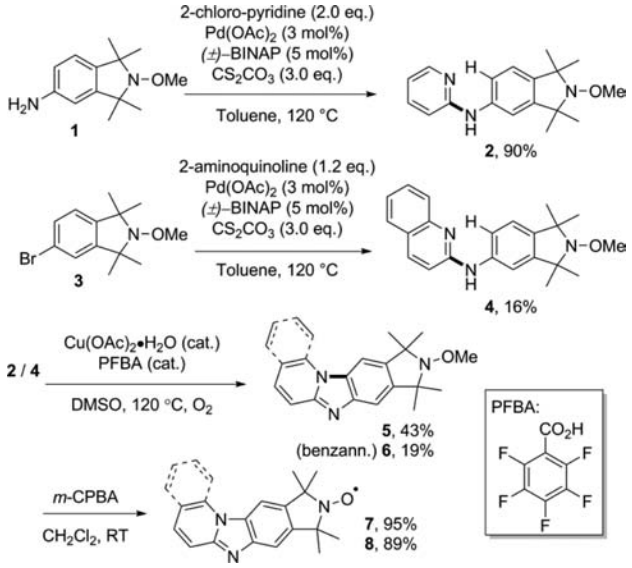


probe would retain the features of each of the individual progenitor compounds: the (pro)fluorescence, notable Stokes shift, nucleobase isosterism/intercalation, and detection by electron paramagnetic resonance (EPR). To facilitate the development of this project, direct and modular methods for the synthesis of the "PyrImid" core via C−H fuctionalization have been developed by the research groups of Zhu[24] and Maes.[25]

The synthesis of an array of π-extended and rigid nitroxides may be readily achieved through the application of Buchwald−Hartwig coupling to form *N*-arylamidines[26] and copper-catalyzed C−H amination to cyclize the subsequent methyl-protected TMIO derivatives; these has recently benefitted from the use of a methoxyamine protecting group strategy.[27] Beginning from known TMIO derivatives, the methoxyamines **1**[23] and **3**[27] (Scheme 2) can be generated in high yields via Fenton chemistry. Subsequent coupling of either 2-chloropyridine (with **1**) or 2-aminoquinoline (with **3**) can be achieved through Buchwald−Hartwig amination under the conditions of Maes and co-workers[26b,c] to yield amidines **2** and **4** in yields which varied depending upon the substrates (90−16%, with 2-chloroquinoline failing to deliver **4**).

Cyclizations of *N*-aryl-*N*-pyridylamine **2** and *N*-aryl-*N*-quinylamine **4** were performed with conditions developed by

Scheme 2. Synthesis of PyrImid-TMIO Hybrids 7 and 8



Maes and co-workers.[25] Catalytic amounts of cupric acetate monohydrate and a fluorinated benzoic acid ligand, 2,3,4,5,6-pentafluorobenzoic acid (PFBA, Scheme 2), were applied to the amidines in DMSO under an atmosphere of oxygen to effect Cu-mediated C−H amination/oxidative cyclization to the aromatic products **5** and **6**, which were isolated in workable yields (19−43%) following purification. The existing procedure was notably improved by the use of diethyl ether as eluent to quickly deliver the TMIOMe−PyrImid derivative as a sharp band (cf. NH_3 in methanol/CH_2Cl_2 in the reported method). The subsequent *m*-CPBA deprotection, which proceeds via N-oxidation and Cope-like elimination,[27] delivered the spin-labeled targets in high yields (89−95%).

In line with previously characterized PyrImid derivatives, TMIOMe−Pyrimid hybrid **5** was discovered to produce a significant Stokes shift (165 nm, Figure 2). In the case of π-

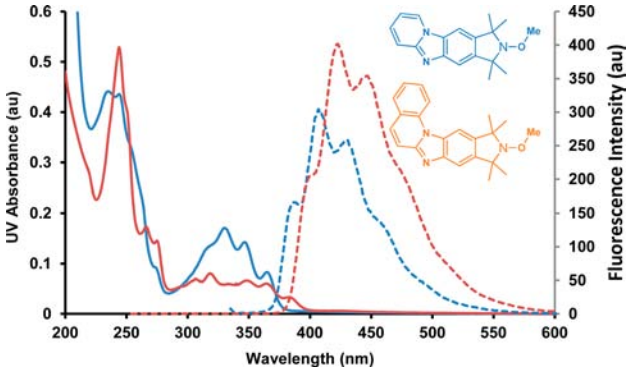


Figure 2. UV (solid line) and fluorescence (dashed line) spectra for Compounds **5** and **6**.

extended **6**, this was even greater (179 nm). Alkoxyamines **5** and **6**, once deprotected to the nitroxide radicals **7** and **8**, exhibited a decreased fluorescence, particularly in the case of **8** (see the Supporting Information). These properties provide supporting evidence for the potential application of compounds related to **7** and **8** as a profluorescent nitroxide (PFN) probes. Structural confirmation and crystal packing analysis for the

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novel structures of **5** and **6** were achieved by X-ray diffractometry (see the Supporting Information).

Using EPR spectroscopy, nitroxide radicals **7** and **8** were evaluated as spin labels for noncovalent binding (see the Supporting Information) to an abasic site in DNA and RNA duplexes at temperatures ranging from 0 to −30 °C (Figure 3

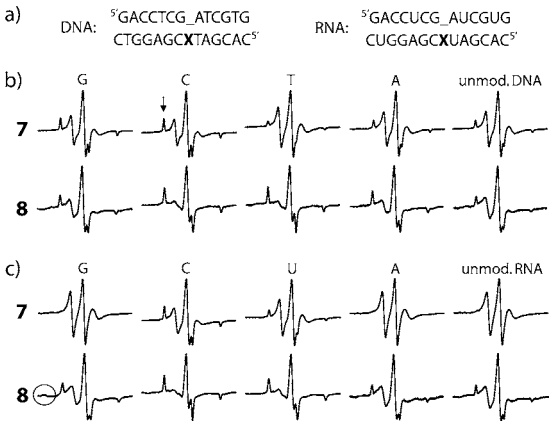

Figure 3. (a) DNA and RNA duplexes used for EPR binding studies where "_" denotes an abasic site and **X** the complementary base. EPR spectra showing the extent of binding of **7** and **8** to DNA (b) and RNA (c), respectively, at −30 °C. Letters above each spectrum denote the base opposite to the abasic site. For unmodified duplexes, "_" and **X** stand for **C** and **G**, respectively. Binding studies were performed in phosphate buffer (pH = 7.0) containing 2% DMSO and 30% ethylene glycol at −30 °C. The arrow indicates the slow moving (bound) component and the circle shows signs of aggregation.

and Figures SI1−SI4, Supporting Information). A spin label bound to a nucleic acid duplex moves slower and results in a wider EPR spectrum that reflects a shorter rotational correlation time.[16b] Prior to recording EPR data, it was confirmed by thermal denaturation experiments and circular dichroism (CD) that the DNA and RNA oligonucleotides were in duplex form under the conditions used for ligand binding (see the Supporting Information). The EPR data revealed partial binding of nitroxide **7** to DNA as judged by the emergence of a slow moving component at low temperatures (denoted by an arrow in Figure 3b), which was also observed in the control experiment, indicating some nonspecific binding. However, there was slight, but noticeable, specific binding (ca. 10%) observed at −30 °C when G or C was the orphan base opposite to the abasic site. More substantial binding was observed when **7** was incubated with an RNA duplex containing an abasic site, in particular opposite C (ca. 40%) and U (ca. 25%). In addition, the binding was clearly specific since the unmodified RNA duplex showed no indication of binding to nitroxide **7** (Figure 3c).

In contrast to **7**, the EPR data for nitroxide **8** revealed significant and substantially increased binding to both DNA and RNA, presumably due to the extra aromatic ring that facilitates additional stacking interactions. For DNA, the highest affinity was observed for an abasic site complementary to C or T, where nearly all of the label was bound at −30 °C; however, some nonspecific binding was also observed (Figure 3b). Although the extra aromatic character of **8** led to better binding, it also showed signs of aggregation in the EPR spectra (denoted by a circle in Figure 3c), especially for the sequences that had limited affinity for the spin label. The EPR data of nitroxide **8** in the presence of RNA duplexes surprisingly resembled those for DNA (Figure 3c). However, close observation revealed less RNA binding, compared with DNA, when the abasic site was placed opposite to A and slightly more binding to C, the latter of which showed complete binding.

In summary, we have developed an expedient route for the synthesis of a promising new class of hybrid PyrImid−TMIO probes while retaining the useful properties of each of the parent compounds and may provide a mechanism for fluorescence/EPR detection. Nitroxide **8** provided complete binding to C as an orphan base in an abasic DNA or RNA duplex, previously inaccessible, target site. Further structural refinement of this exciting new class of probes in terms of nucleobase specificity, optical properties, and solubility under biological conditions is in progress, and the results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, NMR spectra and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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