Pyrene-Functionalized Fluorescent Nanojars: Synthesis, Mass Spectrometric, and Photophysical Studies

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ABSTRACT: Nanojars are a class of supramolecular coordination complexes based on pyrazolate, CuII, and OH− ions that self-assemble around highly hydrophilic anions and serve as efficient anion binding and extraction agents. In this work, the synthesis, characterization, and photophysical properties of pyrene-functionalized fluorescent nanojars are presented. Three pyrene derivatives, 4-(pyren-1-yl)pyrazole (HL1), 4-[(pyren-1-yl)pent-4-yn-1-yl]-pyrazole (HL2), and 4-(3-(pyrazol-4-yl)propyl)-1-(pyren-1-yl)-1,2,3-triazole (HL3), and the corresponding nanojars were synthesized and characterized using nuclear magnetic resonance spectroscopy and mass spectrometry. Electronic absorption, steady-state, and time-resolved fluorescence measurements were carried out to understand the interaction between the pyrene fluorophore and copper nanojars. Optical absorption measurements have shown minor ground state interaction between the fluorophore and nanojars. The fluorescence of pyrene is significantly quenched when attached to nanojars, suggesting strong contribution from the paramagnetic CuII+ ions. Significant static quenching is observed in the case of L1, when pyrene is directly bound to the nanojar, whereas in the case of L2 and L3, when pyrene is attached to the nanojars using flexible tethers, both static and dynamic quenching are observed.

INTRODUCTION

Nanojars (NJs) constitute an interesting class of cyclic coordination oligomers based on the neutral \{cis-Cu(μ-OH)(μ-pz)\} (pz = pyrazolate anion or a derivative) repeating unit (Figure 1).1 Highly hydrophilic oxyanions, such as carbonate, sulfate, phosphate, and arsenate, template the self-assembly of NJs of the formula \{anionCu(μ-OH)(μ-pz)\}m (Cu:anion = 26−33).2−7 Each member of this series of oligomerization isomers is composed of three \{[Cu(μ-OH)(μ-pz)]m\} (m = 6−14, except 11) metallamacrocycles, strongly held together by an intricate network of hydrogen bonds and Cu⋯O interactions. As the NJs self-assemble, a hydrophilic cavity lined by a multitude (26−33) of O−H hydrogen bond donors forms at their center, which incarcerates the templating oxyanion. Consequently, NJs have been developed into highly efficient anion-extracting agents,8 capable of binding trace amounts of oxyanions and transferring even the most hydrophilic ones from water into long-chain aliphatic hydrocarbon solvents.9 Selective binding of oxyanions has recently been achieved by tethering pairs of pyrazolate ligands together.10,11 Aiming at detecting NJs using fluorescence and ultimately developing NJ-based sensors for oxyanions, we pursued the synthesis and characterization of fluorescent NJs. Various polar substituents (including nitro, amine, aldehyde, carboxylate) as well as acidic groups (phenol, thiol, carboxylic acid) interfere with NJ formation. Therefore, we opted for pyrene as the fluorescent label, an aromatic hydrocarbon with no polar or acidic groups yet a very versatile fluorescent moiety. Indeed, pyrene-based fluorescent tags have been used in various coordination complexes,12 metal−organic frameworks,13,14 and self-assembled nanomaterials,15 in fluorescent sensors for the detection of explosives16 or transition metal ions such as CuII,17 ZnII,18 HgII,19 and FeIII20 in luminous molecular liquids,21 surfactant aggregates,22,23 and dendrimers.24 Pyrene-based materials are also valuable in organic electronics,25−28 photoswitchable nonlinear optical29 and electroluminescent materials,30 light-harvesting antenna systems,31−33 photoinitiators of polymerization34 and other light-induced reactions,35,36 molecular receptors,37−40 and electrocatalysis.41 Pyrene is also used for imaging biological systems42 and for photodynamic therapy.43 The methods of functionalization of pyrene to prepare luminescent materials have been reviewed.44 However, very few studies have focused on the

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excited-state interactions between fluorescent dyes and supramolecular inorganic nanomaterials.48

Herein, we present the synthesis and characterization of three novel pyrene-functionalized pyrazole ligands, 4-(pyren-1-yl)pyrazole (HL1), 4-(5-(pyren-1-yl)pent-4-yn-1-yl)pyrazole (HL2), and 4-(3-(pyrazol-4-yl)propyl)-1-(pyren-1-yl)-1,2,3-triazole (HL3) (Scheme 1). In HL1, the fluorescent tag is directly bound to the pyrazole moiety; therefore, the fluorophore is conjugated with the Cu^{2+} centers in NJs via the pyrazole unit, and its position relative to the NJ is rigid. In contrast, in HL2 and HL3, the fluorescent tag is attached to the pyrazole moiety via a propylene chain (and a −C≡C− or triazole group in HL2 and HL3, respectively), which allows for flexibility of the fluorophore relative to the NJ and disrupts conjugation between the fluorophore and the Cu^{2+} centers. Three different ways of synthesizing fluorescent NJs based on these ligands are described. Electrospray-ionization mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR), optical absorption, steady-state and time-resolved fluorescence measurements were carried out to characterize the novel NJs and to monitor their interaction with the attached pyrene dye.

**RESULTS AND DISCUSSION**

**Synthesis of the Fluorescent Pyrazole Ligands.** Our previous investigations had established that steric influences from bulky substituents at the 3-position of pyrazole ligands can hinder the self-assembly of NJs, whereas functionalization at the 4-position does not interfere with NJ formation.9 Thus, the simplest fluorescent pyrazole ligand was synthesized by directly attaching pyrene to the 4-position of the pyrazole moiety (Scheme 1). 4-Pyrazoleboronic acid pinacol ester was first protected with a tetrahydropyranyl (THP) group (Figures S1 and S2), followed by a Suzuki−Miyaura coupling with 1-bromopyrene. The resulting intermediate was deprotected and the product was purified by flash chromatography to give HL1 in 50% overall yield (Figures S3 and S4).

We were also interested in developing appropriately functionalized pyrazole ligands as an avenue to constructing NJs that have potential to be functionalized both pre- and post-synthetically. We identified 4-(pent-4-yn-1-yl)pyrazole as a suitable starting point for our studies, which combines several desired features: (1) the reactive terminal alkyne functionality allows for the installation of pyrene moieties; (2) the alkyne group is tolerated during NJ self-assembly;49 (3) the propylene unit between pyrazole and the alkyne prevents π-electron...
conjugation between the pyrazole and the pyrene moieties, which might lead to diminishing of fluorescence intensity. This compound has been previously prepared from 4-(3-hydroxypropyl)pyrazole by first converting the hydroxyl group to the primary bromide using phosphorus tribromide and subsequently performing an nucleophilic substitution reaction with sodium acetylide to give the alkyne.49

A terminal alkyne can be directly coupled to pyrene using a Sonogashira reaction. Thus, a coupling reaction was carried out using the THP-protected 4-(pent-4-yn-1-yl)pyrazole and 1-bromopyrene (Scheme 1). Using Pd(PPh₃)₄ as a catalyst produced the desired product along with ∼16% of Glaser-type homocoupled di-alkyne. Replacing the catalyst with Pd-(PPh₃)₅Cl reduced the amount of the side product to ∼4%. Deprotection and purification by flash chromatography afforded HL2 in 22% yield over two steps (Figures S5 and S6).

The copper catalyzed azide–alkyne cycloaddition “click” reaction is also a convenient method to install pyrene-based fluorophores on larger molecular structures containing an alkyne functionality. Coupling of THP-protected 4-(pent-4-yn-1-yl)pyrazole with 1-azidopyrene was carried out using click chemistry (Scheme 1). The Cu⁺ catalyst was prepared in situ by the reduction of Cu²⁺ using sodium ascorbate. The resulting crude product was deprotected using concentrated sulfuric acid in ethanol and the product was purified by dissolving in minimal amount of chloroform and precipitating with hexanes.
Filtration gave the pure HL3 in 47% yield over two steps (Figures S7 and S8).

**Synthesis of Fluorescent NJs by Self-Assembly Using Pyrene-Labeled Pyrazole Ligands.**

**NJ Based on L1.** The synthesis of homoleptic NJs based solely on a given pyrene-labeled ligand was carried out by self-assembly using a 1:1:2:1 molar mixture of ligand {[(HL1), (HL2), or (HL3), Cu(NO3)2, NaOH, and Na2CO3] in tetrahydrofuran (THF) (Scheme 2, Method A). With HL1, Cu27 and Cu29 NJs of the formula [CO3Cu(Cu(OH)(HL1))]2− (n = 27, m/z 4726; n = 29, m/z 5074) are observed by ESI-MS(−) (Figure S9). Compared to unsubstituted NJs, the signal intensity of ESI-MS(−) is very weak indicating poor ionization efficiency, attributable to the large size and hydrophobicity of the pyrene-decorated NJs. The 1H NMR spectrum of the NJ mixture displays peaks in the 20–40 ppm window that are consistent with previous observations for NJs.4,5 The signals of the chemically equivalent pyrazole protons in the 3- and 5-positions of both Cu27 (Cu6+Cu12+Cu8) and Cu29 (Cu4+Cu11+Cu6) NJs are significantly downfield shifted compared to free pyrazole, due to the presence of paramagnetic Cu2+ ions. The extent of these shifts is temperature-dependent, as confirmed by variable temperature 1H NMR studies in the 25—140 °C range (Figure S10). At 25 °C, the spectrum shows four broad distinguishable peaks at 30.3 (Cu6-ring), 27.1 (Cu5-ring), 22.4 (Cu12-ring), and 21.2 ppm (Cu13-ring). Increasing temperatures lead to the sharpening of these peaks as the magnetic moment of the copper complex decreases and reveal a new peak at ~34.0 ppm (Cu8-ring) at 50 °C which was too broad to be observable at 25 °C. As in the [CO3Cu(Cu(OH)(pz))]2− (n = 27, 29) NJs studied before,4,5 the pyrazole protons in the Cu9-ring of the Cu27 NJ are the most sensitive to temperature changes as the corresponding peak shifts upfield by over 5 ppm units on going from 25 to 140 °C in a DMSO-d6 solution. Meanwhile, the peaks corresponding to the Cu6- and Cu12-rings of the Cu27 NJ, as well as the Cu5- and Cu13-rings of the Cu29 NJ experience much smaller shifts of less than 0.6 ppm units.

Heteroleptic NJs synthesized with a 1:1 molar mixture of pyrazole (Hpz) and fluorescent ligand HL1 display a statistical distribution of species consisting primarily of Cu27 NJs [CO3Cu27(OH)(pz)](L1)(pz)2− (x = 3–19, m/z 2323–3925), as shown by ESI-MS(−) (Figure S11). The ionization efficiency of these heteroleptic NJs is drastically improved compared to those containing only L1 as the ligand.

**NJ Based on L2.** The synthesis of homoleptic NJs based on L2 proceeded similarly to the one using L1. However, these very large and hydrophobic NJs do not ionize sufficiently to be properly characterized by ESI-MS. While some low-intensity peaks could be discerned from the background noise in the expected mass region for the corresponding homoleptic NJs, unequivocal assignments could not be performed. On the other hand, heteroleptic NJs {[CO3Cu(Cu(OH)(OH)(L2))(pz)2− (n = 27, x = 4–13, m/z 2556–3754; n = 29, x = 7–12, m/z 3103–3769; n = 31, x = 9–15, m/z 3517–4316) could be observed by ESI-MS(−) if a 1:1 molar mixture of pyrazole and HL2 was used for the synthesis. In the NJ mixture obtained, Cu25 species are the major components; smaller amounts of Cu29 and Cu31 NJs are also observed (Figure S12). Although the ionization efficiency of these assemblies is significantly less than that of NJs containing only unsubstituted pyrazole, the mass spectrum has a sufficient signal-to-noise ratio to clearly distinguish and identify these species.

**NJ Based on L3.** Attempts to obtain homoleptic NJs based on L3 yielded brown, insoluble solids instead of blue-green NJs. The triazole functionality in L3 is also a possible coordination site for copper which can lead to the formation of coordination polymers. This assumption is consistent with previous studies which showed that NJs do not form with pyrazole ligands containing additional coordinating functional groups.4,5 Contrary to L1 and L2, no NJs could be obtained with pyrazole/L3 mixtures in 1:1 or 6:1 molar ratios. Instead, unidentified copper-containing species with m/z < 1000 were observed in the ESI-MS in both cases. A mixture of pyrazole and L3 in a 19:1 molar ratio, however, did allow the formation of NJs (Figure S13). Thus, the [CO3Cu6(OH)12(L3)](pz)2− (n = 27, x = 1–4, m/z 2178–2642; n = 31, x = 1–4, m/z 2473–2937) species observed in the corresponding ESI-MS(−) spectrum indicate that L3 is tolerated on NJs in small numbers (up to four L3 ligands per NJ). According to the statistical distribution6,49 of the pyrene-functionalized ligand, unsubstituted NJs are also observed in the mixture.

**Synthesis of Fluorescent NJs by “Click” Reaction between Alkyne-Functionalized NJs and 1-Azidopyr- azole.** Post-synthetic functionalization was performed on alkyne-substituted NJs using the Cu+ catalyzed azide–alkyne Huisgen cycloaddition (“click”) reaction.60,61 In most click reactions, the Cu+ catalyst is generated in situ by reducing Cu2+ with an excess of sodium ascorbate. This prevents Glaser-type oxidative homocoupling products of alkynes. To prevent the possible reduction of Cu2+ ions in NJs by ascorbate, we opted for the use of CuI as the catalyst. Furthermore, because we had observed that the coupled product (L3) is only tolerated on NJs in small amounts, NJs prepared with a 90:10 molar ratio of pyrazole and 4-(pent-4-ynyl-1-yl)pyrazole were used for this study (Figure S14).

Using THF as a solvent, NJs containing up to three groups were observed: [CO3Cu6(OH)12(L3)](4-pentynylpyr- azole)2− (n = 27, x = 1, y = 0–2, m/z 2178–2244; n = 31, x = 1–3, y = 0–2, m/z 2473–2848) (Figure S14b). A significant amount of NJs containing unreacted alkyne moieties remained. To test whether this is due to the poor solubility of CuI in THF, we carried out the reaction in acetonitrile (ACN) which is a good solvent for the catalyst. In this case, a dark-blue precipitate formed in the blue reaction mixture. Its blue color and its solubility in THF suggest that this product consists of NJs with multiple pyrenyl-triazole substituents. The increasing hydrophobicity of the assembly with increasing number of coupled ligands makes them gradually less soluble in ACN. Concomitantly, the mother liquor contained uncoupled NJs and those containing only one L3 ligand (Figure S14c). In an attempt to prevent the precipitation of the larger assemblies as well as to dissolve the catalyst, we also conducted the reaction in a 1:1 mixture of THF and ACN. Most of the alkyne functionalities have reacted in this case; however, the abundances of the NJs containing pyrene-coupled ligands is lower than expected in the corresponding ESI-MS(−) spectrum, likely due to decreased ionization efficiency of the complexes containing multiple L3 ligands (Figure S14d). Nevertheless, these experiments clearly demonstrate that NJs can be functionalized post-synthetically.

**Synthesis of NJs for Photophysical Studies.** Fluorescence studies of NJs were hampered by trace amounts of residual, free fluorescent ligand. Because purification of NJs containing large numbers of fluorescent ligands proved to be
difficult, we employed a mixture of pyrazole (Hpz) and fluorescent ligand (H1, H2 or H3) in a 19:1 molar ratio (5 mol % fluorescent ligand) to obtain NJ mixtures free of unbound ligand, suitable for photophysical studies. Because only a small amount of fluorescent dye was employed relative to pyrazole, it was expected that only one or two dye molecules would be present per NJ. The corresponding ESI-MS(−) spectra, shown in Figures S15−S17, confirm the expected results. Some of the NJs in the mixture do not contain any dye, which is typical for mixtures obtained by statistical distribution of ligands. Also, in the case of L2 and L3, small amounts of NJs with an additional allyl substituent are observed. This is due to the presence of trace amounts of 4-allylpyrazole byproduct in the 4-(pent-4-yn-1-yl)pyrazole precursor used in the synthesis of L2 and L3. This impurity is very difficult to remove completely, and as shown before, even trace amounts are drastically amplified upon incorporation into large assemblies, such as NJs. Fortunately, the presence of traces of allyl-substituted NJs is not a concern for studying the fluorescent properties of the synthesized NJ samples, used for the photophysical measurements described below, are denoted L1-NJ, L2-NJ, and L3-NJ.

**Photophysical Studies.** The main focus of the photophysical studies described below is understanding how the spacing between the fluorophore and the NJ influences the optical properties of the assembly. To that end, photophysical properties of the synthesized fluorophores and NJ-bound fluorophores were studied using electronic absorption, steady-state and time-resolved fluorescence measurements. Figure 2 shows the normalized absorbance and fluorescence spectra of L1, L2 and L3 in ACN (8.4 × 10⁻⁶, 6.0 × 10⁻⁶ and 6.4 × 10⁻⁶ M, respectively).

| system | λ\text{abs} | λ\text{em} | quantum yield |
|---|---|---|---|
| H1 | 349 | 388 | 0.26 |
| H2 | 359 | 381 | 0.08 |
| H3 | 341 | 385 | 0.085 |

Table 1. Photophysical Properties of the Synthesized Pyrene Derivatives
systems in THF solution with increasing amounts of acid. Our previous studies have shown that in the presence of 100 equivalents of acid, pz-NJs are completely broken down. As expected, the fluorescence of the dyes is restored close to the value of the free dye in the presence of 100 equiv of acid (Figure S19).

To understand the mechanism of fluorescence quenching in pyrene-bound NJs, time-resolved fluorescence measurements were carried out using time-correlated single photon counting. The free dyes and NJ-bound dyes were excited with a 373 nm diode and fluorescence of pyrene was monitored at 400 nm. Parts A, B, and C of Figure 5 show the comparative decay traces for H_L1, H_L2, H_L3 and the corresponding NJs, respectively. The fluorescence decay of pyrene is faster when bound to NJs than in the free form in solution. A significantly faster decay was observed for L2 and L3 bound to the NJs, compared to L1. Fluorescence lifetimes were determined by fitting the decay traces to a multi-exponential decay function and the obtained lifetime data are provided in Table 2. Two decay components were obtained for H_L1 in ACN with lifetimes of 2.1 ns (3.3%) and 11.7 ns (96.7%). An average lifetime was determined using the equation \( \tau_{\text{avg}} = \frac{\sum a_i \tau_i}{\sum a_i} \) which provides a value of 11.4 ns for H_L1. For L1-NJ, the decay is again fit to a double exponential function providing an average lifetime of 10.9 ns. The decrease in fluorescence lifetime is marginal in the case of L1-NJ, suggesting that the observed steady-state quenching is mostly static in nature. In contrast, the fluorescence decay of L2 was fit with two lifetime components of 3.2 ns (19.6%) and 14.4 ns (80.4%) and an average lifetime of 12.2 ns was obtained. However, the decay of L2-NJ is much faster than that of H_L2 and triple exponential decay was needed to fit the decay. An average lifetime of 4.4 ns is determined for L2-NJ indicating that the fluorescence quenching has contributions from dynamic quenching. Similarly, a decreased average lifetime of 5.4 ns was obtained for L3-NJ when compared to H_L3 (13.3 ns). The varying photoluminescence quenching observed for the different pyrene derivatives bound to copper NJs is likely related to the distance between fluorophore and NJ, as well as to the orientation of the fluorophore on the NJs. Additionally, through-bond vs. through-space fluorescence quenching needs to be considered in these systems.
A 6.3-fold steady-state fluorescence quenching is observed for L1-NJ, which is much smaller than the corresponding quenching for L2-NJ (25-fold) and L3-NJ (19-fold). Due to the structure of the tether, through-bond fluorescence quenching is only possible in the case of L1-NJ. Surprisingly, L1-NJ shows the least fluorescence quenching among the fluorescent NJ systems studied, despite the fact that the pyrene moiety is the closest to the paramagnetic Cu²⁺ centers in L1-NJ. Time-resolved fluorescence measurements have shown little to no decrease in lifetime for L1-NJ, while 2.7- and 2.4-fold lifetime quenching is observed for L2-NJ and L3-NJ, respectively. The fact that the lifetime change is negligible for L1-NJ indicates that most of the fluorescence quenching in this system is static in nature, with increased inter-system crossing as the pyrene is closer to the paramagnetic copper centers in the NJ. Additional fluorescence lifetime quenching is observed for L2-NJ and L3-NJ indicating a contribution of dynamic quenching. The dynamic quenching contribution is likely arising from the fact that L2 and L3 possess flexible aliphatic tethers that allow for a closer contact between the pyrene chromophore and the NJ. Current time-resolved fluorescence measurements with limited time resolution (~1 ns) are unable to capture faster deactivation pathways that are apparent in pyrene-bound NJs. Therefore, ultrafast time-resolved measurements are needed on these systems, which will be addressed in upcoming studies.

**CONCLUSIONS**

In summary, three novel pyrene-functionalized pyrazole ligands (HL1, HL2, and HL3) as well as the corresponding NJs containing these fluorescent ligands were synthesized and characterized using NMR spectroscopy, mass spectrometry, and photophysical techniques. Steady-state fluorescence measurements indicate strong and varied fluorescence quenching for these ligands when bound to NJs. With L1, where the pyrene fluorophore is directly bound to the copper NJ, a 6.3-fold quenching was observed, whereas with L2 and L3 the quenching is 25- and 19-fold, respectively. Time-resolved fluorescence measurements have shown only static quenching for L1-NJ, while significant contribution from dynamic quenching is observed for L2-NJ and L3-NJ. The additional dynamic quenching has origins in the flexibility of L2 and L3 which allows for the pyrene moiety to fold back onto the NJs. Because Cu²⁺ is the only metal ion that can be used for the preparation of NJs, future studies will focus on the attachment of the fluorophore using rigid, non-conjugated tethers, which will minimize fluorescence quenching caused by the paramagnetic Cu²⁺ ions.
EXPERIMENTAL SECTION

General. All reagents used were purchased from commercial sources and were used as received, unless otherwise specified. Solvents were also used as received, except in the synthesis of Pd(PPh₃)₂Cl₂, for which THF was dried using sodium/benzophenone, and in the transformation of THP-protected 4-(3-bromopropyl)pyrazole to the corresponding alkyne, for which DMF was dried over 4 Å molecular sieves. Standard Schlenk techniques were used for reactions sensitive to air and/or moisture, and degassing of solvents was performed using the freeze–pump–thaw method. Water was deionized and solvents used for mass spectrometry were mass spectrometry-grade.

1-Bromopyrene, 52 1-azidopyrene, 53 Pd(PPh₃)₂Cl₂,54 (Bu₄N)₂[CO₃{Cu(OH)(pz)}ₙ] (n = 27, 29–31; pz-NJs),4 and a mixture of heteroleptic NJs based on a 9:1 molar ratio of pyrazole/4-(pent-4-yn-1-yl)pyrazole were synthesized according to literature procedures.49 Synthesis of the THP-protected 4-(4-pentynyl)pyrazole (in 5 steps) was also conducted according to referenced procedures,49 with additional details and adjustments explained herein. In the final step going from the THP-protected 4-(3-bromopropyl)pyrazole to the alkyne product, fresh sodium acetylide (NaC≡CH) slurry was used and 2.65 equiv (of pure sodium acetylide, i.e., not the slurry mixture) were used in the reaction instead of 1.5 equiv listed in the original procedure. All reagents for this final reaction were added under inert atmosphere via syringe. For column chromatography, a ratio of 2.5:1 hexanes/ethyl acetate was used instead of the 2:1 ratio to improve separation and reduce band overlap between the alkyne and the allyl side product within the column. The first fractions of organic material to leave the column contain mostly the allyl impurity, and latter

Figure 5. Fluorescence kinetic decay traces for the free dye and NJ-bound dye in ACN at 400 nm for (A) L1, (B) L2 and (C) L3 after excitation at 370 nm.

Table 2. Fluorescence Lifetimes of the Synthesized Pyrene Derivatives and the Corresponding NJs

| system | lifetimes (ns) | average lifetime (ns) |
|--------|----------------|-----------------------|
| HL1    | 2.1 ± 0.2 (33%), 11.70 ± 0.04 (96.7%) | 11.4 |
| L1-NJ  | 2.0 ± 0.1 (6.6%), 11.60 ± 0.06 (93.2%) | 10.9 |
| HL2    | 3.24 ± 0.08 (19.6%), 14.4 ± 0.1 (80.4%) | 12.2 |
| L2-NJ  | 0.34 ± 0.04 (49.0%), 3.5 ± 0.2 (22.9%), 12.1 ± 0.1 (28.1%) | 4.40 |
| HL3    | 3.4 ± 0.1 (12.0%), 14.60 ± 0.08 (88.0%) | 13.3 |
| L3-NJ  | 0.14 ± 0.04 (32.4%), 3.0 ± 0.1 (33.3%), 12.8 ± 0.1 (34.3%) | 5.4 |

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fractions contain the desired alkyne with increasing purity. The impure fractions are purified in a second column using the same solvent mixture listed here to yield THP-protected 4-(4-pentynyl)pyrazole (yield of crude product: 83.3%; yield of purified product: 45%).

Mass spectrometry was performed using a Waters Synapt G1 HDMS instrument with electrospray ionization (ESI). The settings used for mass spectrometry were as follows: capillary voltage of 2.5 kV, sampling cone voltage of 40 V, extraction cone voltage of 2.5 V, source temperature of 80 °C, desolvation temperature of 110 °C, cone N<sub>2</sub> flow rate of 45 L/h, desolvation N<sub>2</sub> flow rate of 470 L/h. Samples were infused at 0.4 μL/min. All samples analyzed with the mass spectrometer were dissolved in ACN. 1H NMR spectra were collected using a Jeol JNM-ECZS (400 MHz) spectrometer.

The walls of the flask were rinsed with the solvent. The walls of the flask were rinsed with the solvent. The walls of the flask were rinsed with the solvent.

The crude product was dissolved in a 1:50 mixture of dichloromethane and ethanol (100 mL). Concentrated sulfuric acid (5.5 mL) was added dropwise and the reaction was vigorously stirred for 24 h. The reaction was quenched with NaHCO<sub>3</sub> to a pH of ~8. The product was extracted with chloroform (80 mL × 2), and the organic phase was washed with water and brine followed by drying over Na<sub>2</sub>SO<sub>4</sub>, filtration and removal of solvent under reduced pressure. The crude product (2.63 g) was purified by flash chromatography on silica gel using a 2:1 mixture of ethyl acetate and hexanes (R<sub>f</sub> = 0.36) to yield 702 mg of a yellow solid (yield: 48%). Using a 1:1 molar ratio of the pinacol ester starting material and 1-bromopyrene produces a 50% overall yield. 1H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.44 (d, 1H), 8.26–8.31 (m, 4H), 8.16–8.20 (m, 3H), 7.90–8.10 (m, 3H) ppm. 13C NMR (101 MHz, DMSO-d<sub>6</sub>): δ 139.7, 131.6, 131.1, 130.0, 129.4, 128.9, 128.2, 128.1, 128.0, 128.0, 127.5, 126.9, 125.7, 125.3, 124.9, 124.7, 120.2 ppm. HRMS (ESI-TOF) m/z: [M – H]<sup>+</sup> calcd for C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>, 327.1292; found, 327.1295.

**Synthesis of 4-((4-Pyren-1-yl)pent-4-yn-1-yl)pyrazole (HL2).** The THP-protected pentynyl-pyrazole (320 mg, 1.47 mmol) was added to a 50 mL Schlenk flask. 1-Bromopyrene (414 mg, 1.47 mmol) was added, followed by PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (110 mg, 0.16 mmol). Triethylamine (12 mL) was added to the flask and the mixture was purged with nitrogen while stirring. Cul (3 mg, 0.016 mmol) was added to the flask and stirred at 65 °C overnight using an oil bath. The deep red/brown mixture was cooled to room temperature and filtered. The flask was rinsed with EtOAc, which was filtered and combined with the previous filtrate. Then, the solvent was removed under reduced pressure yielding 669 mg of crude product, which was dissolved with minimal methanol. p-Toluenesulfonic acid monohydrate (608 mg) was added to the solution until a pH of 3 and was stirred for two days. The mixture was quenched with saturated NaHCO<sub>3</sub> solution to a pH of ~8, then extracted with chloroform (100 mL × 3). The combined organic fractions were dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed under reduced pressure. The residue was dry loaded onto a silica column and purified with flash chromatography (3:2 EtOAc/hexanes) to yield 115 mg of an orange oil. A second column was performed on impure fractions using 49:1 CHCl<sub>3</sub>/MeOH to yield an additional 30 mg of the product (overall yield: 30%). 1H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.91 (s, 1H, 5-H-pz), 7.80 (s, 1H, 3-H-pz), 5.38 (dd, 1H, CH-THP, J = 9 Hz, 3 Hz), 4.02 (d, 1H, CH<sub>2</sub>O-THP, J = 10 Hz), 3.67 (td, 1H, CH<sub>2</sub>O-THP, J = 11 Hz, 3 Hz), 1.92–2.20 (m, 3H, CH<sub>2</sub>-TTHP), 1.52–1.77 (m, 3H, CH<sub>2</sub>-TTHP), 1.28 (s, 12H, CH<sub>3</sub>) ppm. 13C NMR (101 MHz, CDCl<sub>3</sub>): δ 145.5, 134.8, 87.4, 67.8, 30.6, 25.0, 24.9, 24.8, 22.4 ppm. HRMS (ESI-TOF) m/z: [M + Na]<sup>+</sup> calcd for C<sub>31</sub>H<sub>25</sub>N<sub>5</sub>O<sub>2</sub>Na, 463.1736; found, 463.1754.

**Synthesis of 4-(Pyren-1-yl)pyrazole (HL1).** In a round-bottom Schlenk flask fitted with a septum, 1-(tetrahydro-2H-pyran-2-yl)pyrazole-4-boronic acid pinacol ester (1.53 g, 5.5 mmol) and 1-bromopyrene (1.95 g, 6.95 mmol) were added. The flask was purged with N<sub>2</sub> (g) and a degassed mixture of dioxane and water (5:1, 15 mL) was added via syringe. K<sub>2</sub>CO<sub>3</sub> (2.93 g, 21.2 mmol) was added, followed by Pd(PPh<sub>3</sub>)<sub>2</sub> (0.460 g, 0.40 mmol). The walls of the flask were rinsed with the dioxane solution, and a condenser was fitted. The reaction mixture was refluxed under inert atmosphere overnight. Water was added to the red solution, and the product was extracted with ethyl acetate (80 mL × 2). The combined organics were washed with brine (40 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration, the solvent was removed under reduced pressure.
MgSO₄, then it was dissolved with H₂O (60 mL × 2) and brine (120 mL). The organic layer was dried over MgSO₄, then it was filtered and the solvent was removed by evaporation. The residue was dissolved in EtOH (50 mL) and concentrated sulfuric acid was added dropwise via pipette until the solution had a pH of 3. This reaction mixture was stirred overnight, then it was quenched with saturated NaHCO₃ solution to a pH of 8. The mixture was extracted with chloroform (60 mL × 3), and the combined organics were washed with H₂O (120 mL × 2) and brine (120 mL × 2). The organic layer was dried over Na₂SO₄, filtered, and concentrated. The resulting red/brown solid was dissolved in a minimal amount of chloroform, and excess diethyl ether was added while mixing to form a brown precipitate. The precipitate was filtered and washed with diethyl ether. Residual solvent was removed from the precipitate under reduced pressure to yield 241 mg of HL3. Evaporating the filtrate followed by dissolving the residue in chloroform and precipitating with ether yielded an additional 16 mg of HL3 (overall yield: 55%). ¹H NMR (400 MHz, DMSO-δ): δ 8.57 (s, 1H, triazole-H), 8.49–8.18 (m, 8H, pyrene-H), 7.80 (d, 1H, pyrene-H, J = 9 Hz), 7.51 (br, 2H, 3,5-H-pz), 2.85 (t, 2H, C pz-CH₂CH₂), J = 7 Hz), 2.60 (t, 2H, CH₃CH₂C, J = 7 Hz), 2.02 (m, 2H, CH₂CH₂CH₂) ppm. ¹³C NMR (101 MHz, DMSO-δ): δ 147.8, 132.1, 131.3, 131.2, 130.7, 130.1, 129.3, 127.7, 127.1, 126.7, 125.9, 125.7, 125.7, 124.6, 124.4, 123.9, 121.7, 119.8, 31.1, 25.2, 23.8 ppm. HRMS (ESI-TOF) m/z: [M + Na]+ calcd for C₃₉H₃₉N₃Na, 400.1538; found, 400.1533.

**Synthesis of Fluorescent NJs.** All NJs in this study were synthesized by standard procedures with details mentioned here. The fluorescent ligand (0.05 mmol) was combined with pyrazole (0.95 mmol) in a 1:19 ratio, respectively, to total 1 mmol in a round bottom flask. Cu(NO₃)₂·2.5H₂O (1 mmol) was added, followed by THF (10 mL). NaOH pellets (2.13 mmol) were ground with a mortar and pestle, then added to the THF solution. Na₂CO₃·H₂O (1 mmol) was added to the dissolved mixture, capped, and stirred at room temperature for three days. The blue solution was then filtered, and the solvent allowed to evaporate. The residue was redissolved in THF and filtered into a clean beaker to evaporate once more, yielding a blue solid.

The blue NJ solids were dissolved in minimal ACN and stirred for 1 h. This solution was added dropwise to isopropyl ether (threefold excess) and stirred for an additional 30 min. After filtration, a dark-colored residue remained on the filter medium. The solvent was removed under reduced pressure, yielding clean NJs without any free fluorescent dyes (verified with fluorescence spectroscopy).

Click coupling reactions were conducted in degassed solvent and were stirred for 5 days under N₂, 0.1155 g NJ and 1-azidopyrene (0.0359 g) were dissolved in either THF (15 mL), ACN (10 mL), or a mixture of THF/ACN (1:1 v/v, 20 mL). A stock solution of Cul was prepared with 0.0791 g of Cul dissolved in 10 mL of ACN. 710 μL of this solution was added to the reaction mixture and was stirred in the dark.

**Titration of 1-Bromopyrene with (Bu₄N)₂[CO₂(Cu-(OH))(pz)]₄** (n = 27, 29–31; pz-NJs). Solutions of 1-bromopyrene (3.20 μM) and pz-NJs (4.11 μM) were prepared in ACN and were both measured to have an absorbance of 0.12 at 345 nm. Before any titrant was added, the absorbance and emission spectra of the 1-bromopyrene solution (3 mL) was collected as a baseline. 50 μL of the NJ solution was then added to the cuvette containing the analyte, and the absorbance and emission spectra were collected again. This process was repeated in 50 μL additions until a total of 1 mL of NJ solution was added to the cuvette (1 cm path length). The emission spectra were obtained by exciting the solution at 345 nm and monitoring the fluorescence from 360 to 550 nm (using a 5 nm slit width setting).

**Titration of L1-NJ, L2-NJ and L3-NJ with Acid.** Nitric acid was selected as a titrant because the nitrate ion is known not to interfere with NJs even at high concentrations. Two separate solutions were prepared using a serial dilution method to yield 0.038 M and 0.001 M HNO₃ in THF. A 14 μM L1-NJ solution was prepared in THF (10 mL). The absorbance and emission spectra of the L1-NJ solution was collected. Two equivalents of HNO₃ (i.e., 279 μL of 0.001 M nitric acid solution) were then added to the L1-NJ solution, and the absorbance and emission spectra were collected. This step was repeated after adding additional amounts of acid. Thus, fluorescence spectra were collected for L1-NJs with 0, 2, 10, 20, and 100 equiv of HNO₃, indicating an increase in fluorescence intensity with increasing acid equivalents. Each solution was excited at 350 nm and the fluorescence was monitored from 365 to 550 nm (slit widths 5 and 2.5 nm for excitation and emission, respectively). Similar procedures were employed using L2-NJ (8.8 μM in THF) and L3-NJ (2.3 μM in THF).

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c05619.

¹H NMR, ESI-MS(−), absorbance and fluorescence spectra (PDF)

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**Notes**

The authors declare no competing financial interest.

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