Nitrogen-Doped Carbon Nanodots Produced by Femtosecond Laser Synthesis for Effective Fluorophores

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**ABSTRACT:** Understanding the effect of heteroatom doping is crucial for the design of carbon nanodots (CNDs) with enhanced luminescent properties for fluorescence imaging and light-emitting devices. Here, we study the effect and mechanisms of luminescence enhancement through nitrogen doping in nanodots synthesized by the bottom-up route in an intense femtosecond laser field using the comparative analysis of CNDs obtained from benzene and pyridine. We demonstrate that laser irradiation of aromatic compounds produces hybrid nanoparticles consisting of a nanocrystalline core with a shell of surface-bonded aromatic rings. These nanoparticles exhibit excitation-dependent visible photoluminescence typical for CNDs. Incorporation of nitrogen into pyridine-derived CNDs enhances their luminescence characteristics through the formation of small pyridine-based fluorophores peripherally bonded to the nanoparticles. We identify oxidation of surface pyridine rings as a mechanism of formation of several distinct blue- and green-emitting fluorophores in nanodots, containing pyridine moieties. These findings shed additional light on the nature and formation mechanism of effective fluorophores in nitrogen-doped carbon nanodots produced by the bottom-up route.

1. INTRODUCTION

Nanoscale luminescent carbonaceous particles or carbon nanodots have attracted enormous attention due to their bright, stable, and tunable luminescence, biocompatibility, and ease of production from benign and inexpensive sources, making them an attractive material for fluorescence bioimaging, fluorescence sensing, light-emitting devices, photocatalysis, and other applications.1–6 The structure of CNDs is complex and highly variable and is usually described as a carbon core with varying proportions of crystalline (usually graphitic), amorphous, and polymeric domains and an outer shell of functional groups, many of them are polar groups comprising heteroatoms.7 Despite a variety of structures and compositions, carbon nanodots in most cases demonstrate common luminescent features, which are different from most organic molecular fluorophores, namely, (a) a large Stokes shift, (b) excitation-dependent8 or even multicolor9 luminescence, and (c) wavelength-dependent fluorescence lifetime.10 The mechanism of CND luminescence as well as the reasons underlying their specific luminescent properties evoked much debate with many, frequently conflicting, explanations.11–14 While the discussion about the luminescence mechanism is still ongoing; it was found that doping with heteroatoms can considerably enhance CND luminescent properties,15,16 the most widely used dopant atom being nitrogen.17,18 Given the complex and variable structure of CNDs, many options exist for nitrogen doping. It can be incorporated into sp²-hybridized aromatic domains as graphitic, pyridinic, or pyrrolic nitrogen, can enter sp³-hybridized polymeric and amorphous domains of the CND core, or can be a part of surface moieties. Many experimental works demonstrated that the effect of nitrogen doping strongly depends on the position of nitrogen in the CND structure as well as on the type of the nitrogen source.9,19–25 A complete understanding of these nitrogen doping effects is still lacking and the pathways for incorporation of nitrogen from the precursors into the CNDs are difficult to predict and control. Even more confusing is the finding of a series of experimental studies that for the most popular synthetic way for production of nitrogen-doped CNDs, that is the reaction between citric acid with nitrogen donors, the luminescence of the products depends on the position of nitrogen in the CND structure as well as on the type of the nitrogen source.9,19–24 In fact, it was found that CND samples obtained by solvatothermal synthesis are strongly contami-
nated by nitrogen-containing molecular fluorophores, which can yield a stronger luminescence than CNDs themselves.28 Naturally, this complicates the analysis of the effect of nitrogen doping and challenges the validity of many earlier studies. Also, a question arises as to whether the same or similar molecular fluorophores can underlie the photoluminescence of CNDs obtained by other synthetic methods. All these recent findings inspire a closer look at the effects of nitrogen doping on the optical properties of CNDs. For this re-examination, we decided to choose a simple model precursor that allows a straightforward way to distinguish the effect of nitrogen. Simple aromatic molecules are especially attractive in this context since their aromatic ring is a natural building block for constructing nanoparticles with a graphitic crystalline structure. A number of works demonstrated that intense optical fields of pulsed lasers are an effective instrument for the assembly of aromatic molecules into CNDs with characteristic excitation-dependent visible fluorescence.29−31 Besides, recent interest in unconventional strategies for carbon nanodot fabrication attracted attention to this synthetic method.32 To date, pulsed laser synthesis was only applied to homocyclic aromatic compounds, benzene or toluene, and produced nitrogen-free nanodots, hence the understanding of the nitrogen effect on the properties of CNDs produced by this method was lacking. In this work, we for the first time produced carbon nanodots by laser synthesis from a heterocyclic aromatic compound (pyridine), and also, to the best of our knowledge, synthesized nitrogen-doped CNDs from a compound where all carbon and nitrogen atoms belonged to the aromatic system. Pyridine is the simplest model compound for realization of nitrogen doping, and the fact that it is only different from benzene by substitution of one carbon atom with nitrogen allows us to analyze the effect of nitrogen by comparing the optical properties of benzene- and pyridine-derived carbon dots. Also, unlike many other precursors, pyridine already has (pyridinic) nitrogen as a part of the aromatic ring, which makes it an interesting system to trace the pathways of nitrogen derived from precursor molecules and the place where it resides in the structure of CNDs in the context of its effect on CND’s optical properties.

2. RESULTS AND DISCUSSION

Already after 15 min of exposure to femtosecond laser pulses, the originally colorless pyridine liquid became red-colored, indicating the formation of blue-absorbing irradiation products. Upon further irradiation, the solution became dark red or almost black and a precipitate formed at the bottom of the vial. Laser irradiation of benzene produced similar colored products. After the removal of the unreacted precursor by drying, the irradiation products obtained from pyridine and benzene could be dissolved in ethanol, yielding a transparent solution with a strong blue luminescence. The solution remained luminescent after dialysis, which removed small-
molecular-weight compounds; thus, the visible luminescence was partly attributed to products with high molecular weights (>2 kDa) formed during irradiation. AFM imaging of the dried dialyzed solution revealed nanoscale particles lying on a glass substrate (Figure 1a). Observed transverse sizes of the particles were limited by the spatial resolution of the AFM probe, whereas the heights above the substrate were measured with subnanometer resolution. Typical heights of pyridine-derived nanoparticles were within the range of several nanometers (Figure 1b). The statistical analysis of 122 individual particles yielded a median diameter of 4.35 nm. A fit with a Gaussian distribution yielded a similar median height of 4.3 nm and a full width at half-maximum (FWHM) of the statistical distribution of ca. 2.9 nm. Thus, nanoparticles were polydisperse with a broad size distribution. Similar measurements for nanoparticles obtained from irradiated benzene gave a median height of 3.1 nm and an FWHM of 1.07 nm (Figure S1).

In summary, laser irradiation of benzene and pyridine produced luminescent nanoparticles, which are hereafter referred to as benzene and pyridine carbon nanodots (b- and p-CNDs). HRTEM images revealed an obvious crystalline structure of larger (5–10 nm in diameter) nanoparticles, such as onion-type concentric layers with a period of 0.35–0.4 nm (Figure 1d) or parallel sheets with a period of \( \sim 0.25 \) nm (Figure 1e). These lattice parameters are close to the interlayer spacing (0.335 nm) and the in-layer period (0.246 nm) of the crystalline lattice of graphite. Thus, at least a part of nanoparticles formed through laser synthesis had a graphene-like crystalline structure. The presence of crystalline ordering was confirmed by the XRD pattern, which demonstrates a broad diffraction peak (Figure 1c). The peak maximum at \( 2\theta = 20.7^\circ \) corresponds to a crystalline period of 0.43 nm (Figure 1d), which is close the period observed on the HRTEM images of p-CNDs (0.36–0.4 nm). This period is within the range of values typically reported for carbon dots \(^{33,34}\) and likely corresponds to the spacing between graphenic layers in graphite-like crystallites. This spacing is larger than the interlayer spacing in the crystalline lattice of graphite (002 lattice fringe of \( -0.34 \) nm), probably due to defects and interlayer groups. The XRD peak was much broader than the XRD peak of macroscopic graphite, which is attributed to the small size of the crystallites. The average transverse crystallite width can be estimated using the Debye–Schererr equation

\[
D = \frac{0.9 \lambda}{\beta \cos(\theta)}
\]

where \( \lambda \) is the X-ray wavelength (0.1541 nm), \( \beta \) is the full width at half-maximum of the diffraction peak and \( \theta \) is the Bragg angle. From the diffraction pattern shown in Figure 1c, we arrive at \( D \approx 1.0 \) nm, meaning that most crystallites consist of just a few graphenic layers. A broad and barely recognizable shoulder at \( 2\theta = 44^\circ \) probably corresponds to the (101) lattice fringe of graphite, which further confirms the presence of a graphite-like crystalline lattice. Partial graphitization of CNDs was confirmed by Raman spectra, which showed typical G and D bands at 1590 and 1310 cm\(^{-1}\), respectively, attributed to sp\(^2\)-hybridized carbon and fused hexagonal aromatic rings in graphitic crystallites (Figure S2).

In summary, nanodots produced by laser synthesis include crystalline domains consisting of stacked graphenic layers and thus can be described as graphene dots. Unsurprisingly, the EDS elemental analysis demonstrated that carbon was the most abundant element of aromatics-derived CNDs; also, they included heteroatoms (nitrogen and oxygen), the proportion of which varied depending on the precursor. For the benzene dots, the elemental composition is described with the empirical formula \( \text{C}_{0.71}\text{O}_{0.22}\text{N}_{0.07} \) and for the pyridine dots, with \( \text{C}_{0.61}\text{O}_{0.20}\text{N}_{0.19} \). A large proportion of oxygen evidences oxidation during the laser synthesis or even after the synthesis in the presence of ambient air, which produced a shell of polar oxygen-containing groups on the surface of carbon nanoparticles. Higher nitrogen content in p-CNDs was a result of nitrogen derived from pyridine, while a small amount of nitrogen detected in b-CNDs was probably due to adventitious nitrogen. Curiously, the oxygen content was similar for both types of dots and the ratio of carbon content in b- and p-CNDs was approximately equal to the ratio of the number of carbon atoms in benzene and pyridine (6:5).

FTIR spectra of aromatics-derived carbon nanodots exhibited characteristic features of aromatic moieties: a series of sharp aromatic ring stretching peaks between 1400 and 1600 cm\(^{-1}\), out-of-plane CH bending peaks at 730–780 cm\(^{-1}\), an aromatic ring deformation peak near 700 cm\(^{-1}\), and aromatic CH stretching peaks between 3000 and 3100 cm\(^{-1}\) (Figure S3).
p-CNDs also showed a fingerprint peak of the pyridine ring breathing vibration at 995 cm$^{-1}$. The number and position of aromatic-related FTIR bands, especially the CH bending and ring bending peaks within the range of 650–900 cm$^{-1}$, are indicative of the substitution pattern and demonstrate that benzene and pyridine rings in b-CNDs and p-CNDs, respectively, are mostly monosubstituted (2-monosubstituted in the case of p-CNDs, Tables S1 and S2). The fact that benzene and pyridine moieties were not removed by dialysis suggests that they are bonded to products of higher molecular weights, so it can be assumed that their one substitution is a covalent bond connecting them with a nanoparticle. Thus, CNDs obtained by laser synthesis can be visualized as a partly graphitized core particle decorated with a shell of benzene (pyridine) rings and additional oxygen groups produced by oxidation (Figure 2c). Additionally, some oligomer chains decorating the carbon core might be present.

In addition to aromatic bands, b-CNDs exhibited strong alkyl CH stretching peaks at 2800–3000 cm$^{-1}$, demonstrating that the benzene precursor was partly converted to aliphatic products. Other strong bands in the FTIR spectra are indicative of the presence of polar oxygen and nitrogen-containing groups: carbonyl and hydroxyl groups in the case of b-CNDs and amide carbonyl groups in the case of p-CNDs. Importantly, no C–N stretching peaks were observed on the FTIR spectrum of p-CNDs, which suggests that nitrogen mostly belonged to isolated or fused aromatic rings. The presence of aromatic rings decorating CNDs was confirmed by TOF-SIMS: the strongest signal in the mass spectrum of p-CNDs except a sodium cation peak was a peak at $m/z = 78.04$ attributed to deprotonated pyridine ring [M – H]$^+$ (C$_5$H$_4$N$^+$). The spectra also exhibited intense neighbor peaks at $m/z = 77.04$, 79.04, and 80.05 corresponding to the [M – 2H]$^+$, M$^+$, and [M + H]$^+$ ions, respectively (Figure 2b). Similarly, TOF-SIMS spectrum of b-CNDs exhibited strong peaks at $m/z = 77.04$ and 91.05 corresponding to the deprotonated benzene ring (C$_6$H$_5$)$^+$ and the same ring with the attached methylene group (C$_7$H$_7$)$^+$.

A survey XPS spectrum of p-CNDs revealed three distinct peaks attributed to C, N, and O 1s photoelectron emission, from which the elemental content was estimated as 79.8:12.4:7.8% (Figure 3a and Table S3). According to decomposition of high-resolution spectra, the largest contribution to the C 1s peak was made by sp$^2$- and sp$^3$-hybridized carbon.
carbon bonded to C and sp²-hybridized carbon bonded to N atoms (Figure 3b). The ratio between the intensity of C- and N-bonded components was close to 3:2, as expected for pyridine. Other contributions came from oxygen-bonded carbon in oxidized moieties (C–O and C=O) and a π*–π satellite peak, the last confirmed aromatic character of carbon moieties. In agreement with the FTIR spectrum, no carbon belonging to carboxylic groups was detected. The bulk of the N signal in XPS spectra came from two components with binding energies of 398.7 eV (N1) and 399.4 eV (N2), which are within the range typical for pyridinic nitrogen and multiple graphitic defects (i.e., several graphitic nitrogen atoms per one aromatic ring), respectively, in carbon nanomaterials (Figure 3c). We assign the N1 component to pyridinic nitrogen in surface aromatic groups and the carbon core and N2 to graphitic nitrogen in the graphitized core of the CND. This assignment is supported by the presence of the π*-π satellite peak shifted by 6.9 eV from the combined N1 and N2 signals. Since pyridinic nitrogen is bonded to two carbon atoms and graphitic nitrogen to three atoms, the total number of sp²-hybridized carbon bonded to both types of nitrogen is 2N1 + 3N2. Experimentally, a linear combination of N1 and N2 intensities 2N1 + 3N2 is 26.2% of the total XPS signal, while the intensity of the C2 component in the C 1s spectrum (N-bonded sp²-hybridized carbon) is 27.6% (Table S2), this fit the intensity of the C2 component in the C 1s spectrum (N-bonded components was close to 3:2, as expected for pyridine. Other contributions came from oxygen-bonded carbon in oxidized moieties (C–O and C=O) and a π*-π satellite peak, the last confirmed aromatic character of carbon moieties. In agreement with the FTIR spectrum, no carbon belonging to carboxylic groups was detected. The bulk of the N signal in XPS spectra came from two components with binding energies of 398.7 eV (N1) and 399.4 eV (N2), which are within the range typical for pyridinic nitrogen and multiple graphitic defects (i.e., several graphitic nitrogen atoms per one aromatic ring), respectively, in carbon nanomaterials (Figure 3c). We assign the N1 component to pyridinic nitrogen in surface aromatic groups and the carbon core and N2 to graphitic nitrogen in the graphitized core of the CND. This assignment is supported by the presence of the π*-π satellite peak shifted by 6.9 eV from the combined N1 and N2 signals. Since pyridinic nitrogen is bonded to two carbon atoms and graphitic nitrogen to three atoms, the total number of sp²-hybridized carbon bonded to both types of nitrogen is 2N1 + 3N2. Experimentally, a linear combination of N1 and N2 intensities 2N1 + 3N2 is 26.2% of the total XPS signal, while the intensity of the C2 component in the C 1s spectrum (N-bonded sp²-hybridized carbon) is 27.6% (Table S2), this correspondence also supports our interpretation. A smaller contribution to the N signal was made by a peak at 400.6 eV, probably pyridone nitrogen or pyrrolic nitrogen produced by the pyrolysis of pyridine. Finally, the O peak was decomposed into components corresponding to aromatic and aliphatic C–O and C–O groups (Figure 3d). Thus, XPS analysis confirmed the presence of surface pyridine functionalities and the graphitized carbon core in p-CNDs and revealed that the bulk of nitrogen belonged to these functionalities or pyridinic and/graphitic defects within the carbon core. The most important distinction of XPS spectra of b-CNDs from p-CNDs was the absence of nitrogen (Figure S3 and Table S4). In agreement with FTIR spectra, decomposition of the C 1s peak revealed that a large proportion of carbon was converted to sp³-hybridized carbon as a result of benzene ring opening. Unlike p-CNDs, the presence of carboxylic groups was detected, while the aromatic C–O component of the O 1s peak was negligible.

Based on the presence of graphitized domains and separate aromatic rings revealed by structural and chemical analyses, we propose the following mechanism of CND formation under laser irradiation (Figure 3e). Firstly, aromatic molecules combine into polyphenil (polypyridine) clusters, and then the central part of these clusters undergoes graphitization producing a carbon core with multilayer graphene domains. Some of the aromatic (phenyl or pyridine) rings remain bonded to the surface of the carbon core. Oligomeric fragments left by incomplete graphitization can also be expected. The further growth of this hybrid nanoparticle is possible through binding of additional aromatic molecules and expansion of the crystalline core through continuing graphitization. Additionally, oxidation in the presence of atmospheric oxygen produces a shell of polar groups on the surface as well as oxygen defects in the graphitic core and can oxidize some of the surface-bonded aromatic rings. Previously, nanosecond laser synthesis from benzene and toluene produced graphene dots, which did not exhibit a crystalline core–aromatic shell structure. We surmise that such a structure can be specific to synthesis with femtosecond laser pulses.

As a next step, we examined the optical properties of laser-synthesized nanodots. The UV–vis absorption spectra of both benzene and pyridine CNDs exhibited a strong absorption peak in the UV spectral region at ca. 250 nm and a weaker emission tail in the near-UV and visible regions (Figure 4a). This general character of absorption spectra is typical for many CNDs obtained from various precursors. Usually, the UV absorption peak is attributed to intrinsic π–π* absorption of sp²-hybridized carbon domains in the CD carbon core and a weaker absorption tail in the near-UV and visible region attributed to extrinsic π–π* transitions of surface and edge groups. In our case, the CNDs contain many aromatic rings, which are not part of the carbon core, so the UV absorption should be considered the sum of carbon core π–π* absorption and π–π* absorption in peripheral aromatic groups. The near-UV–visible absorption tail lacks a clear structure and conspicuous peaks, which indicates its heterogeneous nature. It can be assumed that it is formed by an overlap of n–π* absorption of various CND surface/edge groups and a red edge of π–π* absorption of graphitized carbon core domains with a possible contribution of absorption of oxidized peripheral aromatic groups. Impor-
Figure 5. (a) Emission anisotropy decay of b-CNDs in ethanol and p-CNDs in ethanol and the ethanol–glycerol mixture measured at 450 nm emission wavelength excited at 370 nm. Anisotropy decay of Rhodamine 6G fluorescent dye in ethanol (excitation at 480 nm and emission at 550 nm) is presented on the same graph for comparison. (b) Average rotation time of b- and p-CNDs in ethanol as a function of emission wavelength excited at 370 nm. Anisotropy decay of Rhodamine 6G fluorescent dye in ethanol (excitation at 480 nm and emission at 550 nm) is presented on the same graph for comparison. (c) Concept of rotational motion in a carbon nanodot bearing peripheral and rigidly bonded fluorophores.

tantly, compared with benzene CNDs, pyridine CNDs had higher values of absorbance in the near-UV and visible range on the normalized absorption spectrum (Table S4), indicating that nitrogen doping enriched them with moieties having a LUMO−HOMO gap smaller than ~4 eV.

Although pyridine is virtually nonluminescent and benzene emits in the deep-UV region, the carbon nanodots obtained from them demonstrated appreciable visible photoluminescence (PL). Additionally, both types of CNDs, especially b-CNDs demonstrated strong PL in the near-UV region, which will be not addressed further. Typically for carbon nanodots, the photoluminescence emission (PL) spectra were excitation-dependent and exhibited a change of the peak position with an increase of the excitation wavelength above 350 nm (Figure 3b). The analysis of normalized PL spectra shows that the visible emission peak and its peak wavelength continuously shifted to higher wavelengths when the excitation wavelength was increased (Figure S4). This shift was accompanied by attenuation of emission intensity. The rate of this attenuation, however, was different for p- and b-CNDs. For benzene CNDs the integral emission intensity decreased by 11 times when the excitation wavelength increased from 350 to 450 nm, whereas for pyridine CNDs, this decrease was only 5.5 times, giving p-CNDs relatively stronger green and yellow photoluminescence (Figure 3c). The excitation dependence of PL spectra can be viewed as a result of CNDs having a mixture of fluorophores with different HOMO−LUMO gaps. In this way, a stronger green emission of p-CNDs indicated that nitrogen doping shifts this distribution of the HOMO−LUMO gap to the smaller energy side. In other words, p-CNDs tend to contain more fluorophores with emission in green and yellow regions than b-CNDs.

Finally, b- and p-CNDs were different in their apparent luminescence quantum yields of 1.8 and 5.5%, respectively, when excited at 356 nm (Table S5). The term “apparent yield” indicates that the yield is estimated relative to the total absorption corresponding to the sum of CND chromophores. Some of the absorbing chromophores can be nonfluorescent or weakly fluorescent, thus masking the quantum yield of fluorescent moieties, which can be considerably larger than the average value. The measurement of fluorescence decay kinetics demonstrates that p-CNDs have a consistently larger fluorescence lifetime than b-CNDs across the entire PL spectrum in agreement with their higher quantum yield (Figure S5). This suggests that b- and p-CNDs contain different types of fluorescent moieties, and p-CNDs have fluorophores with higher luminescence yields and a longer fluorescence lifetime due to the presence of nitrogen in their structure. The higher luminescence yield of p-CNDs is combined with larger absorption in the near-UV and visible range, and the multiplication of these two effects makes them much more efficient emitters than b-CNDs.

We also examined the photostability of CNDs in ethanol solution when subjected to continuous irradiation by the 350 nm light of a spectrofluorometer (Figure S6). The results indicate that p-CNDs are somewhat more photostable, as their emission intensity decreased by ~10% after 5 h irradiation versus an ~16% decrease for b-CNDs. Importantly, the UV–vis and PL spectra of CND samples purified using dialysis with a higher cutoff membrane (10k MWCO) were similar to those presented above, albeit the luminescence quantum yield was somewhat smaller (Figure S7). This confirms that absorption and PL mostly belonged to nanometer-sized particles rather than to smaller-sized molecules or clusters.

In conclusion, both pyridine and benzene nanodots demonstrate absorption and photoluminescence properties typical for carbon nanodots. Nitrogen doping of pyridine CNDs considerably enhances their optical properties in three ways: it gives them a stronger near-UV and visible absorption, a higher quantum yield of blue luminescence, and a stronger green luminescence.

Further information on the luminescence properties of CNDs was obtained by the analysis of luminescence anisotropy and its decay kinetics in ethanol solution. The luminescence of both b- and p-CNDs in the blue spectral region was strongly anisotropic with an initial value of emission anisotropy $r_0$ of ca. 0.3 (Figure S8), which indicates that luminescence is emitted by dipole-like emitters similar to molecular fluorophores, and their emission dipoles were nearly parallel with excitation dipoles: the angle between them, $\beta$, estimated from $r(t) = (3 \cos^2(\beta) - 1)/5$ gives 25° or less. The emission anisotropy of b- and p-CNDs decayed on nanosecond and subnanosecond time scales, respectively. Curiously, the rate of depolarization was different: for p-CNDs, the averaged depolarization time measured at various parts of the emission spectrum was 0.4–0.45 ns and was virtually independent of the emission wavelength, whereas for b-CNDs, it varied from 0.6 to 0.9 ns and increased with the emission wavelength (Figure 5b).
Depolarization of emission can be explained by rotational diffusion of nanodots in solution; however, the measured depolarization times were too short compared with nanoparticle dimensions. For example, for p-CNDs from the average depolarization time, we arrive at the characteristic hydrodynamic volume of the luminescent particle equal to only 1.3−1.5 nm³. Such particles are much smaller than the average size established by the AFM measurements and they are expected to be removed from the solution by dialysis since particles with volumes smaller than approximately 2.5 nm³ can pass through the pores of the dialysis membrane. Moreover, the anisotropy decay kinetics of p-CNDs was multiexponential, with a short decay time as small as 0.1−0.15 ns, which corresponds to even smaller hydrodynamic volumes. A comparison with Rhodamine 6G demonstrates that the initial depolarization rate was similar to this relatively small fluorescent molecule, although both b- and p-CNDs exhibited appreciably slower depolarization than Rhodamine at times more than ~0.5 ns (Figure 5a). Also, the shorter depolarization time of p-CNDs compared with b-CNDs contradicts to their larger sizes established by the AFM measurements. Similar anisotropy decay kinetics, which was anomalously fast compared with carbon nanodot size, was observed in a number of studies and was attributed to either rotation of small subunits of nanodots or resonance energy transfer between fluorophores.10,39,40 To verify that depolarization results from rotation rather than from other physical effects, we carried out the measurement of p-CND anisotropy decay kinetics in an ethanol−glycerin mixture with a viscosity more than 15 times higher than the viscosity of ethanol (21 mPa·s). As demonstrated in Figure 5a, increased viscosity resulted in much longer emission anisotropy decay than that in ethanol, thus confirming that depolarization is mostly caused by rotational motion. Typical values of the fast component of the multieponential fit in Figure 5a were about 0.7−1 ns, corresponding to very small fluorescent moieties with characteristic volumes equal to 0.15−0.2 nm³. We attribute anomalously fast rotational emission depolarization to the segmented motion of CNDs, having fluorophores, which are not rigidly connected to nanoparticles, but form small separate units connected with the nanoparticle by covalent bonds and can freely rotate around these bonds (Figure 5c). The possibility that such so-called peripherally bond fluorophores can be responsible for CND fluorescence was demonstrated in earlier works.41 Rotation of these peripheral fluorophores changes the orientation of the emission dipole and results in partial depolarization of emission, which corresponds to a fast component of the anisotropy decay kinetics. A slower rotation of the nanoparticle as a whole further depolarizes the emission and corresponds to a slow component of the anisotropy kinetics, which is of the nanosecond scale in ethanol. In principle, “associated” fluorophores rigidly bonded to the nanoparticle structure can also contribute to the photoluminescence emission. Since they cannot rotate separately from the nanoparticle, their luminescence depolarization kinetics has only a slow decay component. Thus, slower depolarization kinetics of b-CNDs compared with p-CNDs can be explained by a larger contribution of associated fluorophores to their photoluminescence. Moreover, the dependence of the average rotation time of b-CNDs on the emission wavelength suggests that the contribution of “associated” fluorophores is larger in the green and yellow parts of the emission spectrum. To verify this, we measured the anisotropy kinetics of b-CNDs when excited with a longer wavelength (420 nm instead of 370 nm) and found that this wavelength shift led to a considerable increase in the measured average rotation time, which was now 0.9 ns or larger (Figure S8). This result indicates that with the increase of the excitation wavelength, absorption by “associated” green fluorophores increases, while absorption of peripheral blue fluorophores decreases. As a result, the PL of b-CNDs at 420 nm excitation wavelength is dominated by slowly rotating associated fluorophores, leading to a larger rotation time.
By contrast, the rotation time of p-CNDs exhibits little dependence on the excitation wavelength, indicating that their PL is in all cases dominated by peripheral fluorophores. Our model of small peripheral fluorophores is in good agreement with the results of FTIR analysis, which demonstrates that CNDs bear a shell of benzene or pyridine aromatic rings decorating the nanoparticle. Fluorescent moieties can be derived from these peripheral aromatic groups. For example, whereas pyridine is virtually nonluminescent, already its simplest oxidized product hydroxypyridine exhibits appreciable photoluminescence in the near-UV range. Further oxidation, formation of bipyridine and polypyridine units, interaction with aromatic structures of CNDs core, and interactions between closely situated fluorophores can shift the emission further into the visible region. Thus, we hypothesize that peripheral fluorophores of p-CNDs are formed by oxidized pyridine derivatives produced during nanodot synthesis. To verify this assumption, we examined the properties of benzene and pyridine nanodots, which were oxidized in ethanol solution in the presence of atmospheric oxygen under exposure to UVC light of the mercury lamp. Whereas in other works, it was reported that UV irradiation of CNDs resulted in luminescence photobleaching, in our case, visible photoluminescence of both b- and p-CNDs became stronger after UV exposure (Figure 6a). This increase in emission intensity was accompanied by stronger absorption in the near-UV and visible spectral range, which is indicative of further oxidation and formation of additional oxygen-containing moieties on the surface of CNDs (Figure S9). Interestingly, the increase in absorption as a result of oxidation was larger than the increase in luminescence intensity. This observation confirms that absorption of CNDs is heterogeneous, and in addition to fluorophores, oxidation produces nonluminescent chromophores.

FTIR spectra measurement confirmed the oxidation of p-CNDs under UV exposure, in particular p-CNDs-UV had a considerably higher content of OH and amide C=O (Figure 6b). Less conspicuous changes included changes of the aromatic CH out-of-plane bending peaks at 780 and 750 cm$^{-1}$ and changes of the relative intensity of pyridine ring stretching modes at 1570, 1468, and 1434 cm$^{-1}$, indicating changes in the substitution pattern of the pyridine ring, and a stronger NH bending peak at 1570 cm$^{-1}$. Amide C=O and NH groups likely originate from the oxidation of the pyridine rings producing pyridone-like structures (Figure 6c). Hydroxypyridine, which exists in tautomeric equilibrium with pyridone, is a known product of pyridine biodegradation or UV photolysis, which makes it a likely intermediate in the formation of fluorophores under UV exposure. Oxidation of pyridine was corroborated by mass spectra, which demonstrated an increase of the output of the C$_5$N$_4$NO$^+$ cation with m/z = 94.03 (attribution according to the SurfaceLab database), derived by fragmentation of the pyridone-like moiety (Figure S10). Significantly for further discussion, we observed no increase of the carboxylic peak around 1700 cm$^{-1}$, which is characteristic of the formation of citrazinic acid or its derivatives.

In summary, these observations confirm that UV irradiation oxidizes peripheral pyridine rings of b-CNDs, producing C=O and OH groups and converting pyridine to pyridone-based moieties. This oxidation is a likely mechanism of new fluorophore formation under UV exposure. The analysis of
the FTIR spectra of b-CNDs-UV revealed redistribution of OH groups in favor of phenolic moieties, decrease of aromatic CH stretching peaks, and changes of the benzene ring substitution pattern (Figure S11). Similar to p-CNDs, these changes also support oxidation of benzene rings decorating b-CNDs under UV exposure.

Importantly, such luminescent characteristics of p-CNDs as normalized emission spectra, emission kinetics, and emission anisotropy kinetics underwent a slight change after UV irradiation (Figures 5b, S12, and S13). This means that oxidation produced the same fluorophores that were present before UV irradiation, and the only characteristics that changed were their number. It can be assumed that oxidation of pyridine during the laser synthesis of p-CNDs converted part of surface pyridine rings to fluorescent moieties. In the case of b-CNDs, changes were more complex. After oxidation, luminescence decay kinetics became longer, while anisotropy decay kinetics became shorter and characteristic depolarization time was now comparable with that of p-CNDs (0.45–0.5 ns, Figure 5b). This indicates that the fluorophore content of b-CND was altered by UV irradiation, and oxidation preferentially produced peripheral fluorophores, probably by oxidation of pendant benzene rings. As explained above, before oxidation, the luminescence of b-CNDs had a large contribution from “associated” fluorophores. After oxidation, this situation changed and now the number of peripheral fluorophores increased and their contribution in photoluminescence became larger, resulting in the shortening of effective rotation time.

Additional insights into chemical nature of fluorophores were provided by the analysis of the photoluminescence reaction to the pH of aqueous solution. In all cases, in alkaline solution, the photoluminescence of p-CNDs was weaker than at that neutral or acidic pH (Figure 7a). The reaction to acidic media was more nuanced. When excited at 350 nm, the PL strongly increased at acidic pH, whereas at 370 nm excitation, the intensity of the emission peak at ca. 450 nm was roughly equal at neutral and acidic pH, but the luminescence spectrum in acidic solution had stronger wings on the blue and red sides of the main emission peak. Finally, when excited at longer wavelengths (e.g., 450 nm), PL in acidic media was somewhat stronger than that in neutral. The increase and decrease of PL components are well observed as recession and humps on the differential emissions spectra (i.e., difference of PL spectra at acidic and neutral pH, Figure S14). The analysis of the pH dependence reveals at least three components of photoluminescence: (1) a component with maximum at 420–430 nm, increasing at acidic pH, (2) a component with maximum at 450 nm, decreasing at acidic and alkaline pH, and (3) a component with maximum at ca. 500–520 nm, increasing in acidic medium. We attribute the decrease of PL intensity in alkaline solution to deprotonation of phenolic OH (typical pK_a ~ 8–10), while a reaction to acidic pH is probably caused by protonation of pyridine oxygen or nitrogen in the lactim form of fluorophores. It should be noted that pH dependence of the p-CND emission was different from commonly described citrubic acid-based fluorophores of CNDs, which have the strongest PL at neutral pH values.45 The dependence of b-CND PL on pH values was simpler: at all excitation wavelengths, the luminescence strongly increased in alkaline solution (Figure S15). This indicates that the fluorophores of b-CNDs contain phenolic OH groups either belonging to surface-bonded aromatic groups or belonging to the surface layer of the aromatic domains of the CND’s core. These phenol groups are deprotonated in alkaline medium, which increases their electron-withdrawing strength and produces a stronger emission of b-CNDs.

The analysis of pH dependence as well as the excitation-dependent character of CND photoluminescence suggests that their emission is heterogeneous. This heterogeneity can result either from the presence of several types of chemically distinct fluorophores with different excitation and emission spectra or from the interaction of fluorophores with the local environment, such as the solvent CND core or surface groups and neighbor fluorophores. Effects described in the literature, such as formation of fluorophore aggregates,46,47 dependence of emission spectra on the carbon dot size, or slow solvent relaxation,48 can produce heterogeneous and wavelength-dependent emission even from chemically identical fluorescent moieties. Since the magnitude of interactions with the environment is tunable, these effects are in most cases equivalent to a continuum of emitters with different emission spectra and luminescence lifetimes, instead of a limited number of distinct emitters in the case of chemical heterogeneity. To clarify the mechanism behind the excitation dependence, we employed the time-resolved area-normalized emission spectroscopy (TRANES) technique, which is a standard tool for the analysis of multicomponent fluorescence.49 TRANES spectra of p-CNDs, excited by femtosecond laser pulses centered at 370 nm exhibited considerable evolution on a nanosecond time scale and a shift of emission intensity to the red side of the spectra (Figure 7c). A clear isoemissive point is seen at 470 nm. The number of isoemissive points in TRANES spectra is indicative of the number of different emitters.49 Here, one isoemissive point means that the photoluminescence of p-CNDs at this excitation wavelength is dominated by two fluorescent species: one with emission maximum in the blue region below 470 nm and another with emission maximum in the green region above 470 nm. These components most probably correspond to components (2) and (3) established from the analysis of the luminescence pH dependence. It has been already demonstrated from anisotropy decay kinetics that both these species belong to the peripheral type, i.e., they are small molecular fluorophores decorating the surface of the carbon nanodot, and both types of fluorophores were arguably produced by oxidation of pyridine. Such effects as slow solvent relaxation, aggregation, or polydispersity of CNDs do not play a notable role in the excitation dependence of their fluorescence—these effects would not produce an isoemissive point on TRANES spectra. In conclusion, based on anisotropy kinetics, pH dependence and TRANES measurements attribute the excitation dependence of p-CND luminescence to the presence of at least three distinct peripheral fluorophores decorating nanodots with emission maximum at 420–430, 450, and 500–520 nm (Figure 7b).

In summary, the main contribution to the luminescence of both b- and p-CNDs is made by small fluorescent segments (peripheral fluorophores) decorating the nanoparticle core. These segments are formed by oxidation of aromatic rings of the CND shell in the process of laser synthesis. The difference in the luminescence properties of b- and p-CNDs is caused by the presence of pyridinic nitrogen in the fluorophores of p-CNDs, which gives them higher quantum yield and luminescence lifetimes. p-CNDs posses a number of fluorophores with emission in blue, green, and yellow spectral regions, making their emission tunable by excitation wave-
length. At the same time, b-CNDs contain only blue-emitting peripheral fluorophores, and compared with p-CNDs, their green emission is weaker and is caused by electronic levels associated with the core. As a result, their photoluminescence is less tunable and is attenuated more sharply with the increase of the excitation wavelength.

Our analysis of the luminescence characteristic of aromatic-derived nanodots provides an interesting perspective on the structure–property relationship of fluorescent carbon nanoparticles and their emission mechanisms. First, we confirmed an already established notion that fluorescence does not originate from the nanodot as a whole but rather from some smaller chromophores within the nanoparticle. Different authors considered these fluorescent elements as polycyclic aromatic domains within the carbon core, heteroatom groups bonded to conjugated structures of the carbon core, molecular fluorophores comprised within the nanoparticles or decorating their surface. We have found that the position of fluorescent moieties is actually versatile: they can be comprised within the rigid nanoparticle structure or they can form separate freely rotating units connected with the nanoparticle by covalent bonds. Even nanodots of the same type can contain fluorescent moieties with different types of positioning, such as b-CNDs in the present work, and moreover, the proportion between them can be changed by chemical modification of the nanoparticle. Yet, we have discovered that the fluorescence of emitters belonging to the rigid structure is weak, whereas the strongest fluorescence is emitted by the fluorescent moieties of peripheral type, which are essentially small molecular fluorophores decorating carbon nanoparticles. This finding also sheds light on the mechanism of nitrogen doping and its effect on the optical properties of CNDs. We found that nitrogen from the pyridine precursor is partly converted to the pyridinic nitrogen of p-CNDs, which enhances the absorption and luminescence of aromatic-derived CNDs and this enhancement effect is related to the formation of molecular fluorophores containing oxidized pyridine rings. The carbon nanoparticle itself and polycyclic aromatic structures of the nanoparticle core evidently play a limited role in the photoluminescence mechanism: in the case of pyridine CNDs, the core merely provides a scaffold for binding molecular fluorophores. At the same time, polar groups formed on the surface of aromatic domains of the carbon core are probably responsible for weak green emission of benzene-derived CNDs, which agrees with the interpretation of Zhang who ascribed weak green photoluminescence in graphene dots to defect states produced by oxidation.

The molecular origin of nitrogen-doped carbon nanodot fluorescence was discussed in a number of recent works, which identified a family of fluorescent molecules responsible for the bright luminescence of some CNDs, in particular of those synthesized by the reaction between citric acid and amines. What most of these fluorescent molecules have in common is a chemical motif of citrazinic acid, which includes a pyridone ring bearing a carboxylic group and is formed by cyclization of citric acid. We demonstrated that CNDs can contain pyridone-based molecular fluorophores, produced by a different mechanism, that is oxidation of pyridine functionalities, and unlike citrazinic-type fluorophores, they do not seem to contain carboxylic groups, which were not revealed by either XPS or FTIR. The luminescence quantum yield of these pyridine-derived fluorescent moieties was modest compared with the brightest citrazinic-type species but still large enough to give CNDs appreciable visible photoluminescence. It seems probable that similar pyridone-based fluorophores can contribute to the fluorescence of nitrogen-doped CNDs obtained from other compounds or by other bottom-up synthetic procedures, e.g., solvothermal or microwave synthesis.

3. CONCLUSIONS

We demonstrated that femtosecond laser irradiation of aromatic benzene and pyridine compounds produces carbon nanodots with a partly crystalline structure and excitation-dependent visible luminescence with potential applications in fluorescence imaging and light-emitting devices. In contrast to the previously reported results of the nanosecond laser synthesis of carbon nanodots from aromatic molecules, femtosecond laser-synthesized CNDs have a characteristic hybrid structure of carbon nanoparticles decorated with pyridine or benzene aromatic rings. Nitrogen doping of pyridine-derived CNDs enhances their optical properties compared with benzene nanodots in three aspects: it gives them stronger near-UV and visible absorption, increases their luminescence quantum yield, and gives them a relatively stronger luminescence in green and yellow spectral regions.

The nitrogen doping effect is attributed to several (at least three) luminescent moieties formed by oxidation of the surface-bonded pyridine rings. These oxidized pyridine derivatives form small and freely rotating fluorophores connected with the carbon nanoparticle by covalent bonds. By contrast, the luminescence of benzene-derived CNDs comes from a combination of peripheral fluorophores and fluorescent moieties rigidly bonded to the carbon nanodot. Our results suggest that oxidation of pyridine rings producing similar pyridine-based fluorophores could be one of the mechanisms responsible for the fluorescence of carbon nanodots synthesized by bottom-up methods from nitrogen-containing precursors.

4. EXPERIMENTAL SECTION

4.1. Synthesis, Purification, and UV Modification of CNDs (Scheme 1). Two milliliters of liquid aromatic precursors (pyridine or benzene) were placed in a serum vial and irradiated at room temperature with trains of focused femtosecond laser pulses amplified using a regenerative amplifier (Spitfire, Spectra-Physics) and focused by a spherical lens with NA = 0.3. The pulse central wavelength, repetition rate, duration, and energy were 800 nm, 1 kHz, 50 fs, and 1.4 mJ, respectively. Irradiated samples were dried in serum vials at room temperature to remove unreacted precursors or their volatile products, leaving a precipitate that was dissolved in ethanol, sonicated for 10 min, and centrifuged at 14 000 rpm to remove large aggregates, resulting in a transparent yellow or yellow reddish liquid. For further purification and removal of small-molecular-weight products, the ethanol solution was dissolved in water in a 1:5 volume ratio. This water solution was then dialyzed for 72 h in 2k MWCO dialysis units in a water container constantly stirred by a magnetic stirrer. Dialysis resulted in a solution of irradiation products with a mass larger than ca. 2 kDa, which was then analyzed by various methods (Scheme 1).

In experiments with UV irradiation, about 3 mL of ethanol solution of pyridine- and benzene-derived nanodots was subjected to UVC light of a low-pressure mercury lamp for
30–60 min. UV–vis absorption and luminescence spectra of the solution were measured in the same cuvette before and after the irradiation.

4.2. Atomic Force Microscopy (AFM) and High-Resolution Transmission Electron Microscopy (HRTEM). One microliters of the dialyzed water solution of irradiation products was dissolved in 50 μL of HPLC-grade absolute ethanol. A drop of the solution was dried on a borosilicate glass coverslip, which was pretreated with UV light from a quartz mercury lamp for 10 min to clean the surface. The sample was then scanned with an AFM unit (SMENA-B, NT-MDT) in an intermittent contact mode.

HRTEM micrographs of the samples were recorded on a JEM 2100F high-resolution transmission electron microscope (JEOL Co., Ltd., Japan) equipped with a corrector of spherical chromatic aberration, an energy-dispersive X-ray spectral analyzer, and an electron energy loss spectrometer (Gatan), measured at an accelerating voltage of 200 kV. Before measurement, the samples were placed on a copper grid.

4.3. Elemental Analysis. Two microliters of the sample solution was dried on a silicon plate, and then elemental analysis was performed with energy-dispersive X-ray spectrometry (EDS) using a Prisma E electron microscope (Thermo Fisher, Czech Republic). A copy (EDS) using a Prisma E electron microscope (Thermo Fisher Scientific) was glued to a borosilicate glass coverslip, which was pretreated with UV light from a quartz mercury lamp for 10 min to clean the surface. The sample was then scanned with an AFM unit (SMENA-B, NT-MDT) in an intermittent contact mode.

JRTEM micrographs of the samples were recorded on a JEM 2100F high-resolution transmission electron microscope (JEOL Co., Ltd., Japan) equipped with a corrector of spherical chromatic aberration, an energy-dispersive X-ray spectral analyzer, and an electron energy loss spectrometer (Gatan), measured at an accelerating voltage of 200 kV. Before measurement, the samples were placed on a copper grid.

4.4. X-ray Powder Diffraction (XRD). XRD was performed on a DRON-7 X-ray diffractometer. The lattice spacing was calculated from the diffraction maximum \( 2θ_{\text{max}} \) using Bragg’s law \( \lambda = 2d \sin(θ) \), where \( \lambda \) is the copper wavelength equal to 0.15406 nm.

4.5. X-ray Photoelectron Spectroscopy (XPS). XPS was performed using a Kratos Axis Ultra DLD X-ray photoelectron spectrometer with a monochromatic Al Kα X-ray source. The sample (a powder of pyridine nanodots) was glued to a dielectric scotch tape. Transmission energy was 160 eV for survey and 40 eV for high-resolution XPS spectra.

4.6. Fourier Transform Infrared (FTIR) Spectroscopy. FTIR infrared spectra were recorded using a Lumos I FTIR microscope spectrometer. For spectral measurements, a drop of dialyzed solution was dried on an aluminum mirror and then its FTIR spectrum was measured in the reflection mode with averaging over 1000 scans.

4.7. Raman Spectroscopy. Raman spectra were registered using a SENTERRA Raman microscope spectrometer (Bruker) with excitation at 785 nm. A drop of sample solution was dried on a silica glass surface and its Raman spectra were recorded with averaging over 200 scans.

4.8. Time-of-Flight Secondary Ion Mass Spectroscopy (TOF-SIMS). TOF-SIMS analysis of dialyzed CND solution dried on glass coverslips was performed in positive and negative ions with a TOF-SIMS 5 mass spectrometer (IONTOF, Germany) using a drop of sample dried on a borosilicate glass coverslip; 30 keV Bi₃⁺ clusters were used as primary ions. Mass spectra were recorded in spectroscopy mode with a pixel size of \( \sim 5 \mu m \). Primary ion dose density did not exceed \( 2 \times 10^{11} \) ions/cm². A low-energy electron flood gun was activated to avoid charging effects.

4.9. Optical Spectroscopy. Absorption spectra of sample solutions in ethanol were measured in a 3.5 mL quartz cuvette using a UV–vis spectrometer (UV-3600, Shimadzu) and photoluminescence emission (PL) and excitation (PLE) spectra in the same cuvette using a spectrophotometer (RF-5031PC, Shimadzu). The luminescence quantum yield of blue emission at 356 nm excitation wavelength was determined using the slope method relative to the reference fluorophore, which was an ethanol solution of anthracene (Φ = 27%), using a series of sample solutions of varying concentrations. The quantum yield was calculated by the formula \( \Phi = \frac{G \cdot n^2}{G_{\text{ref}} \cdot n_{\text{ref}}^2} \), where \( \Phi \) is the quantum yield, \( G \) is the slope of the dependence of the integral luminescence intensity on the value of absorption in solution, \( n \) is the refractive index, and subscripts \( x \) and \( s \) refer to the sample and reference, respectively.

The luminescence lifetime was measured using excitation by frequency-doubled pulses of a femtosecond titanium–sapphire oscillator (Tsunami, Spectra-Physics) with a central wavelength of 370 nm, a repetition rate of 80 MHz, a duration of 100 fs, and a pulse energy of 10 pJ. After reflecting from a FESH0750 dielectric filter (Thorlabs) mounted at an angle of 45°, femtosecond laser pulses were coupled into an objective lens (Olympus, 20×, 0.5 NA) and focused into a serum vial with a sample. Luminescence, excited by laser absorption was collected by the same lens and directed to a monochromator (Acton SP300i), where it was detected by the photomultiplier tube of a time-correlated photon counting system (SPC-150N, Becker & Hickl GmbH), which recorded the luminescence decay kinetics in a time range of 0–12.5 ns with a resolution of 50 ps. The emission wavelength was selected by the monochromator and was tunable in the range from 430 to 620 nm with a spectral width of about 5 nm. Luminescence decay kinetics were fitted with a three-exponential decay function \( I(t) = A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2) + A_3 \exp(-t/T_3) \) using deconvolution with instrument response function performed with a SPCImage program (Becker & Hickl GmbH). Coefficients \( A_1, A_2, \) and \( A_3 \) were normalized so that \( A_1 + A_2 + A_3 = 1 \). Amplitude-weighted lifetime was calculated using parameters of the three-exponential fit as \( T_w = A_1T_1 + A_2T_2 + A_3T_3 \).
A, T, C. Time-resolved area-normalized emission spectra were built using a standard technique. Briefly, luminescence decay curves were collected as a function of emission wavelength with a wavelength interval of 5 nm. For each kinetic wavelength-dependent parameters of the multiexponential fit \( A_i(\lambda), T_i(\lambda) \) were determined from deconvolution with the instrument response function. Time-resolved emission spectra (TRES) at time \( t_0, I(\lambda, t_0) \) were calculated using the steady-state photoluminescence spectrum \( I_{ss}(\lambda) \) of the same sample solution excited at 370 nm wavelength recorded with a spectrophotofluorometer and multiexponential parameters as

\[
I(\lambda, t_0) = I_{ss}(\lambda) \left[ \sum_{i=1}^{3} \exp\left(-A_i t_0 / T_i\right) \right] \left[ \sum_{i=1}^{3} A_i T_i \right]
\]

where time values were taken with an interval of 0.1 ns. Time-resolved area-normalized emission (TRANES) spectra were obtained by renormalization of TRES spectra so that the area under each spectrum was equal to a constant value.

For registration of the luminescence anisotropy kinetics, the decay kinetics of luminescence with parallel and perpendicular polarizations relative to the polarization of the excitation laser were measured using a linear polarizer installed in front of the monochromator entrance. The fluorescence anisotropy was calculated by the formula \( r(t) = (I_1 - \alpha (I_2 + I_3)) / (I_1 + 2\alpha (I_2 + I_3)) \), where \( \alpha \) is the empirically determined correction factor and \( I_1, I_2, I_3 \) are the intensities of the luminescence with parallel and perpendicular polarizations, respectively. Luminescence decay kinetics were fit with a three-exponential decay \( r(t) = B_1 \exp\left(-t / \tau_1\right) + B_2 \exp\left(-t / \tau_2\right) + B_3 \exp\left(-t / \tau_3\right) \) using Origin 9.0 software. Average rotation time was calculated from the three-exponential fit by the formula \( \tau_{rot} = (B_1 \tau_1 + B_2 \tau_2 + B_3 \tau_3) / (B_1 + B_2 + B_3) \) and the initial anisotropy value as \( r_0 = B_1 + B_2 + B_3 \). The average hydrodynamic volume of the luminescent moiety was estimated by the equation \( V = k_B \tau_{rot} / \eta \) where \( k_B \) is the Boltzmann constant, \( T \) is the temperature, and \( \eta \) is the dynamic viscosity of the solution.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/10.1021/acsomega.1c06413.

Additional TOF-SIMS, PL, FTIR, and UV–vis spectra of samples; and table of spectral decomposition of the XPS spectrum of pyridine CNDs (PDF)

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