Synthesis of 9-arylalkynyl- and 9-aryl-substituted benzo[b]quinolizinum derivatives by Palladium-mediated cross-coupling reactions

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Abstract
9-Arylbenzo[b]quinolizinum derivatives were prepared with base-free Suzuki–Miyaura coupling reactions between benzo[b]quinolizinum-9-trifluoroborate and selected benzenediazonium salts. In addition, the Sonogashira coupling reaction between 9-iodobenzo[b]quinolizinum and the arylalkyne derivatives yielded four novel 9-(arylethynyl)benzo[b]quinolizinum derivatives under relatively mild reaction conditions. The 9-(N,N-dimethylaminophenylethynyl)benzo[b]quinolizinum is only very weakly emitting, but the emission intensity increases by a factor >200 upon protonation, so that this derivative may operate as pH-sensitive light-up probe. Photometric and fluorimetric titrations of duplex and quadruplex DNA to 9-(arylethynyl)benzo[b]quinolizinum derivatives revealed a significant binding affinity of these compounds towards both DNA forms with binding constants of $K_b = 0.2–2.2 \times 10^5$ M$^{-1}$.

Introduction
Polycyclic cationic heterarenes are a paradigm of DNA-binding ligands whose association with the nucleic acid may affect the biological activities of the DNA [1-4]. For example, a DNA-bound heterocyclic ligand may interfere with DNA–enzyme recognition events, which are essential for DNA-based cellular processes, e.g., gene replication or transcription [1]. To this end, it was shown that DNA-binding ligands may operate as chemotherapeutic anticancer, antiviral or antibacterial drugs, for example as topoisomerase inhibitors [5]. More recently, much interest in this research area is focused on the non-canonical quadruplex DNA (G4-DNA) [6-8]. Mostly based on the principles and requirements of ligands that bind to duplex DNA, nu-
merous G4-DNA ligands have been developed to study their selectivity and binding properties towards G4-DNA because of the biological importance of G4-DNA [9-13]. Along these lines, we and others have established the class of annelated quinolizinium derivatives as versatile ligands that bind to duplex, triplex and quadruplex DNA depending on their shape and size [14-18] and whose interaction with the nucleic acid may be used for fluorimetric detection of the latter [19,20].

To further exploit the DNA-binding properties of this specific class of cationic hetarenes, synthetic routes to novel derivatives with the desired substitution pattern and functionalization are necessary. In this context, Palladium-mediated cross-coupling reactions provide a powerful tool [21-27]; specifically, as these C–C coupling reactions have been demonstrated to be very useful for the introduction of various substituents to quinolizinium [28-33], benzo[b]quinolizinium [34,35] and naphthoquinolizinium [36] derivatives.

Unfortunately, in the case of benzo[b]quinolizinium substrates, the presence of strong nucleophiles, and for that matter bases in general, often interferes with the Pd-mediated reaction because of the competing addition of the nucleophile at the 6-position of the substrate and subsequent ring-opening reaction [37,38]. Considering this impediment and the additional difficulties that may occur during purification of these cationic hetarenes the reaction and work-up conditions of Pd-mediated coupling reactions have to be optimized [34,35]. Accordingly, we extended our studies to improve the conditions of the Suzuki–Miyaura coupling reaction towards biaryl-type benzo[b]quinolizinium derivatives 1a–d (Figure 1), namely to apply the alternative base-free Suzuki–Miyaura coupling reaction [39-42] between the benzo[b]quinolizinium-9-trifluoroborate (3b) and aryldiazonium salts. We focused our attention on derivatives 1a–d because in these cases a direct comparison with the already reported synthesis with a Suzuki–Miyaura reaction is possible. As we are particularly interested in benzo[b]quinolizinium derivatives with a large extension of the π-system, which should provide promising properties as G4-DNA ligands, we also focused our attention on the Sonogashira reaction as synthetic route to arylalkynyl-substituted derivatives. In this case, we aimed at donor-substituted derivatives such as 2b–d since they were proposed to have ideal photophysical and DNA-binding properties. Herein, we present the successful Suzuki–Miyaura and Sonogashira coupling reactions of benzo[b]quinolizinium substrates. In addition, the absorption and emission properties of the novel arylalkynylenbenzo[h]quinolizinium derivatives 2a–d are reported (Figure 1), along with preliminary studies of their duplex and quadruplex DNA-binding properties.

Results

Synthesis

Synthesis of 9-aryl-substituted benzo[b]quinolizinium derivatives 1a–d

The 9-aryl-substituted benzo[b]quinolizinium derivatives 1a–d were prepared under base-free conditions by the Pd-catalyzed Suzuki–Miyaura reaction of the aryldiazonium salts 4a–d with benzo[b]quinolizinium-9-trifluoroborate (3b). The latter substrate was obtained as analytically pure product in moderate yield by the reaction of benzo[b]quinolizinium-9-boronic acid (3a) [34] with NaBF₄ (Scheme 1).

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To identify appropriate reaction conditions for the base-free synthesis of derivatives 1a–d, different catalysts and solvents were tested for the cross-coupling reaction of benzo[b]quinolizinium-9-trifluoroborate (3b) and benzenediazonium salt 4a (Scheme 1, Table 1). With Pd(dppf)Cl₂·CH₂Cl₂ or Pd(PPh₃)₄ as catalyst, no conversion was observed, whereas the reaction could be achieved with Pd(OAc)₂ as a catalyst and water as a solvent (Table 1, entries 1–5). Thus, the latter reaction conditions were used for the synthesis of 9-arylbenzo[b]quinolizinium derivatives 1b–d (Scheme 1, Table 1). The methoxyphenyl- and dimethylaminophenyl-substituted derivatives 1b and 1c were obtained in a moderate to good yield, but only trace amounts of the pyridyl-substituted derivative 1d were formed as
Scheme 1: Synthesis of benzo[b]quinolizinium-9-trifluoroborate (3b) and 9-arylbenzo[b]quinolizinium derivatives 1a–d (see Table 1 for assignment of indices a–d and reaction conditions).

Table 1: Reaction conditions for the synthesis of 9-arylbenzo[b]quinolizinium derivatives 1a–d according to Scheme 1.

| Entry | Solvent       | Catalyst                  | t (h) | Product         | Yield (%) |
|-------|---------------|---------------------------|-------|-----------------|-----------|
| 1     | H₂O           | Pd(OAc)₂                  | 48    | 1a, R = Ph      | 43        |
| 2     | DME/H₂O/MeOH  | Pd(dppf)₂Cl₂·CH₂Cl₂³       | 24    | 1a, R = Ph      | n.r.⁵      |
| 3     | DMF           | Pd(dppf)₂Cl₂·CH₂Cl₂³       | 24    | 1a, R = Ph      | n.r.⁵      |
| 4     | CH₃CN         | Pd(OAc)₂                  | 24    | 1a, R = Ph      | n.r.⁵      |
| 5     | CH₃CN         | Pd(PPh₃)₄                 | 24    | 1a, R = Ph      | n.r.⁵      |
| 6     | H₂O           | Pd(OAc)₂                  | 168   | 1b, R = 4-MeO(C₆H₅) | 95        |
| 7     | H₂O           | Pd(OAc)₂                  | 144   | 1c, R = 4-PhN(C₆H₅) | 44        |
| 8     | H₂O           | Pd(OAc)₂                  | 168   | 1d, 4-pyridyl   | <2        |
| 9     | DMF           | Pd(PPh₃)₄                 | 168   | 1d, 4-pyridyl   | 16        |

³dppf = 1,1'-bis(diphenylphosphino)ferrocene. ⁵No reaction.

shown by the ¹H NMR spectroscopic analysis of the reaction mixture (Table 1, entries 6–8). Nevertheless, the 9-pyridinyl derivative 1d was obtained in low yield by the reaction of the trifluoroborate 3b with the diazonium salt 4d at 80 °C in DMF with Pd(PPh₃)₄ as a catalyst (Table 1, entry 9). It should be noted that some of these Suzuki–Miyaura coupling reactions require relatively long reaction times (Table 1, entries 6–9), which is a disadvantage considering the competing decomposition of the aryldiazonium ions under the reaction conditions. Thus, the corresponding diazonium salt was added in portions in intervals of 24 h until all of the substrate was consumed.

Synthesis of 9-(arylethynyl)benzo[b]quinolizinium derivatives 2a–d

The 9-(arylethynyl)benzo[b]quinolizinium derivatives 2a–d were prepared by Pd-mediated Sonogashira coupling reactions of 9-iodobenzo[b]quinolizinium bromide (5) with aryldiazonium salts (Scheme 2). To suppress the ring opening of the benzo[b]quinolizinium ring by nucleophilic attack at the 6-position [34,35] two methods were used that avoid the addition or formation of strong nucleophiles during the reaction. In the first approach, (phenylethynyl)copper (6) [44] was prepared separately and subsequently made to react with the substrate 5 to provide derivative 2a as hexafluorophosphate salt in moderate yield (Scheme 2). During the preparation of derivatives 2b–d with this method a crude product was isolated that contains the desired compound along with unidentified impurities, as shown by ¹H NMR spectroscopic analysis of the product. Unfortunately, the product could not be further purified.

In the second approach, the copper acetylide was formed in situ by the reaction of the acetylene derivative with triethyl-
amine in the presence of Cu$^+$ salts. Hence, the reaction of iodo[9]-[b]quinolizinium 5 with ary lacetylenes 7b–d in the presence of one equivalent of triethylamine and CuI under anhydrous conditions gave (arylethynyl)[b]quinolizinium derivatives 2b–d in moderate to good yield (Scheme 2).

Several attempts to purify the derivatives 2a–d by column chromatography failed. Apparently, these compounds decompose when in contact with the silica or alumina of the column, so that the pure products were only available by crystallization from appropriate solvents, which resulted in lower yields of these products.

**Single crystal X-ray diffraction analysis of 9-(arylethynyl)[b]quinolizinium derivatives 2a and 2b**

Single crystals of derivatives 2a and 2b were obtained by crystallization from acetone and CHCl$_3$/MeOH, respectively (Figure 2 and Figure S1, Supporting Information File 1). Derivative 2a crystallizes in the triclinic space group $P\overline{1}$ with two molecules in the unit cell. The crystals were twinned and the compound shows some considerable disorder. The chemical composition, however, was unanimously proven by the data. Derivative 2b crystallizes with one molecule of CHCl$_3$ as lattice solvent in the highly symmetric orthorhombic space group $I\overline{2}a$ with 8 molecules in the unit cell. Both cations are essentially planar and π-stacked in an anti-head-to-tail (ht) arrangement in the solid state. A preference of such anti-ht arrangement was observed before in the crystal structures of two series of 9-substituted benzo[b]quinolizinium salts with halides or small alkyl substituents [45,46]. Stratford et al. attributed this observation to repulsion forces between the positively charged nitrogen atoms and π···π donor–acceptor attractions between the phenyl and pyridinium moieties. In our case, the situation is somehow more complex as the novel compounds bear aromatic substituents (via alkyne spacer) in the benzo[b]quinolizinium 9 position. These aromatic substituents now engage in π···π donor–acceptor attractions with the pyridinium moiety (outer most ring of the tricyclic moiety) and the two positively charged nitrogen atoms are per se much further apart due to the larger intramolecular separation between the intermolecularly interacting π-systems. In addition, the aromatic character of the substituent and its engagement in the π···π interaction also brings the two phenyl rings of adjacent benzo[b]quinolizinium moieties in close proximity, which can now also interact in an off-set π···π fashion. The contribution of the charge repulsion has, hence, to be less significant here and the preference for the anti-ht arrangement must be dominated by the π···π attractions.

Centroid distances between the aromatic 9-substituent and the pyridinium moiety are 3.619 Å for 2a (C16 → C21; C5 → C9, N1) and 3.676 Å for 2b (C16 → C21; C1 → C5, N1), respectively (Figure S1, Supporting Information File 1). These separations are comparably short as the reported ones range from 3.69 Å to 3.99 Å [46]. The π-system separation between the centroids of the two benzo[b]quinolizinium phenyl rings are 3.803 Å for 2a (C1, C2, C3, C11, C12, C13) and 3.599 Å for 2b (C7 → C12), respectively. Notably, for 2b the phenyl to phenyl π···π interaction of one molecule is not with the same neighbor.
as the π−π donor–acceptor attraction between the phenyl and pyridinium rings. Therefore, these two distinct π-system-based attractions alternate and form infinite chains of molecules roughly protruding along the a axis. In 2a both interactions are with the same neighbor leading to distinct dimeric associates.

In the individual molecules, the C–C bond lengths of the alkyne unit are 1.24 Å (C14–C15) for the triple bond and 1.41 Å (C13–C14 and C15–C16) for the single bonds in compound 2a, while in derivative 2b they are 1.18 Å (C14–C15) and 1.45 Å (C9–C14 and C15–C16), respectively. Moreover, the π-surface of derivative 2b deviates slightly more from the mean plane as compared with 2a, i.e., as the torsion angle C8–C9–C16–C17 is −12.0° whereas it is 5.6° (C12–C13–C16–C21) in 2a. These data indicate a slightly more pronounced delocalization of π-electrons within the diarylalkyne unit of compound 2a, at least in the solid state.

Absorption and emission properties of 9-(arylethynyl)benzo[b]quinolizinium derivatives 2a–d

In general, compounds 2a–d have a low solubility in water and derivative 2d is moderately soluble in DMSO. The absorption spectra of 9-(arylethynyl)benzo[b]quinolizinium derivatives 2a,b,d show two low-energy maxima between 380 nm and 450 nm which resemble the ones of similar aryl-substituted benzo[b]quinolizinium [34] and naphthoquinolizinium [36] derivatives (Figure 3, Table 2). As a notable exception, the derivative 2c has a broad absorption band with maximum wavelength depending on the solvent, namely at 470 nm in MeOH, 515 nm in CH2Cl2 and 505 nm in CHCl3 (Figure 3C). At low pH, the broad long wavelength absorption band of 2c disappeared and a new absorption band was formed (λmax = 418 nm) that is similar to that of the parent compound 2a (Figure 3C). It should be noted that the benzo[b]quinolizinium derivatives 2a–d have

Figure 3: Absorption spectra of derivatives 2a (A), 2b (B), 2c (C) and, 2d (D); c = 20 μM; solvents: H2O (magenta), MeOH (black), CHCl3 (blue), CH2Cl2 (red), DMSO (green) and 1 N HCl (orange).
Table 2: Absorption and emission properties of benzo[b]quinolizinium derivatives 2a–d.

| Solvent | 2a | 2b | 2c | 2d |
|---------|----|----|----|----|
|         | $\lambda_{\text{abs}}^b$ / nm | $\lambda_{\text{flu}}^c$ / nm | $\Phi_{\text{F}}^d$ / 10$^{-2}$ | $\lambda_{\text{abs}}^b$ / nm | $\lambda_{\text{flu}}^c$ / nm | $\Phi_{\text{F}}^d$ / 10$^{-2}$ | $\lambda_{\text{abs}}^b$ / nm | $\lambda_{\text{flu}}^c$ / nm | $\Phi_{\text{F}}^d$ / 10$^{-2}$ |
| H$_2$O  | 419 | 460 | < 1 | 422 | 562 | 434 | n.d. | 422 | 572 | <1 |
| MeOH    | 418 | 462 | 64 | 426 | 545 | 470 | n.d. | 428 | 571 | 6 |
| EtOH    | 420 | 462 | 39 | 428 | 558 | 481 | n.d. | 430 | 570 | 6 |
| MeCN    | 418 | 460 | 40 | 423 | n.d. | 472 | n.d. | 428 | 578 | 5 |
| DMSO    | 423 | n.d. | n.d. | 428 | n.d. | 472 | n.d. | 432 | 570 | <1 |
| aceton  | 419 | 460 | 34 | 424 | n.d. | 472 | n.d. | 429 | 580 | 2 |
| CH$_2$Cl$_2$ | 428 | 470 | 44 | 435 | 554 | 515 | 497 | 439 | 560 | 4 |
| CHCl$_3$ | 427 | 470 | 43 | 438 | 485 | 505 | 501 | 443 | 460 | 2 |

*a Solvents in order of decreasing $E_T$ values [47]. b Long-wavelength absorption maximum; $c = 20 \mu$M. c Fluorescence emission maximum (Abs. = 0.10 at excitation wavelength); $\lambda_{\text{ex}} = 375$ nm. d Fluorescence quantum yield relative to coumarin 1 [47,48]; estimated error for $\Phi_{\text{F}}$: ± 10%. e Not determined.

lower absorbance and significantly broadened spectra in less polar solvents, presumably due to their low solubility and the resulting aggregation in these media.

Except for the derivative 2a the arylethynylbenzoquinolizinum derivatives have low emission quantum yields (Table 2, Figure 4). The derivative 2a has a moderate to high fluorescence intensity with slight deviations of the emission maxima in different solvents (Table 2, Figure 4A). In chloroform, it has two emission maxima at 446 and 470 nm. The derivative 2d has a weak fluorescence intensity in different solvents ($\Phi_{\text{F}}$: 0.02–0.06). In chloroform, it shows an emission maximum at 460 nm, while in other solvents it has emission maxima between 560 and 580 nm with a shoulder at 430 nm (Figure 4C).
On the other hand, derivatives 2b and 2c exhibit very weak fluorescence intensity in different solvents ($\Phi_f < 0.02$). Derivative 2c shows only a weak emission ($\Phi_f = 0.02$) in 1 N HCl with significantly blue-shifted emission maxima at 427 and 454 nm (Figure 4B).

To further assess the effect of the pH on the absorption and emission properties of derivative 2c, photometric and fluorimetric acid–base titrations of 2c were performed (Figure 5). With decreasing pH of the solution (pH 7.3–1.1), new absorption bands developed at $\lambda_{\text{max}} = 418$ nm, 395 nm and 322 nm, along with the disappearance of the initial broad long wavelength absorption (Figure 5A). The emission intensity of derivative 2c increased by a factor of 250 with decreasing pH value (Figure 5B). The p$K_a$ value of the protonated amine 2c in water was determined from the titration curve to be 3.1 which is in the same range as the ones of 9-(p-amino)phenylacridinium ions (p$K_a$ = 2.5–3.5) [49,50] and the dimethylaminophenyl-substituted benzob[4]quinolizinium ion [34] (p$K_a$ = 3.8).

Photometric and fluorimetric DNA titrations of 9-(arylethynyl)benzo[b]quinolizinium derivatives 2a–d

The interactions of the arylethynylbenzoquinolizinium derivatives 2a–d with ct DNA and G4-DNA 22AG [d(GT3T2AG3T2AG3T2AG3)] were investigated with photometric and fluorimetric titrations (Figures 6–9, Table 3). In general, a hypochromic effect and a bathochromic shift were observed by the addition of DNA. For example, the addition of ct DNA and G4-DNA 22AG to derivative 2a led to the evolution of a new maximum at 437 nm and 423 nm, respectively, with an isosbestic point at 325 nm. However, during the titration of DNA to the derivatives 2b–d isosbestic points were not formed. In the case of 2d, only a hypochromic effect was observed upon the addition of ct DNA (Figure 6D). In contrast, the addition of 22AG to 2d resulted in a red shift with $\Delta \lambda_{\text{abs}} = 16$ nm. Notably, the addition of DNA to derivative 2c led to the largest bathochromic shifts and hypochromic effect (ct DNA: $\Delta \lambda_{\text{abs}} = 42$ nm; 22AG: $\Delta \lambda_{\text{abs}} = 58$ nm). Only the data extracted from the photometric titration of 22AG to derivatives 2b and 2c could be used to deduce the binding constant $K_b$ (Figure S2, Table 3).

The addition of ct DNA to the derivative 2a led to quenching of the emission intensity (Figure 8A). In contrast, a light-up effect with a factor of 3 was observed upon the addition of ct DNA to derivative 2b (Figure 8B, Table 2). Notably, the emission intensity of derivative 2d at $\lambda_{\text{fl}} = 572$ nm decreased at the beginning of the titration with ct DNA at a ligand–DNA ratio ($LDR > 8$). With further addition of ct DNA, however, the emission intensity increased slightly at the same emission wavelength (Figure 8C). The binding constants, $K_b$, between ct DNA and derivatives 2a ($1.4 \times 10^5$ M$^{-1}$) and 2b ($1.5 \times 10^4$ M$^{-1}$) were determined from the fluorimetric titrations by fitting the resulting binding isotherms to the theoretical model (insets in Figure 8, Table 3) [51]. Unfortunately, the data obtained from the fluorimetric titration of 2d with ct DNA could not be fitted to the theoretical model. The low emission intensity of the derivative 2c was not affected by the addition of ct DNA or 22AG.

The emission intensity of 2a was quenched upon addition of G4-DNA 22AG (Figure 9A). Remarkably, the addition of 22AG to derivative 2d resulted in a decrease of the emission intensity at $\lambda_{\text{fl}} = 572$ nm and a new weak emission band evolved...
Figure 6: Photometric titration of 2a (A), 2b (B), 2c (C), and 2d (D) with ct DNA in BPE buffer (16 mM Na\textsuperscript{+}; 5% DMSO; pH 7.0); c\textsubscript{L} = 20.0 \mu M. Arrows indicate the development of bands with increasing DNA concentration. Inset: Plot of the absorption at long wavelength versus DNA concentration.

Table 3: Absorption and emission properties of ligands 2a–d upon the addition of DNA, and binding constants $K_b$.

| Ligand | ct DNA | 22AG |
|--------|--------|------|
|        | $\lambda_{abs}^a$ / nm | $\Delta \lambda_{abs}^b$ / nm | $I/I_0^c$ | $K_b^d$ / $10^4$ M\textsuperscript{-1} | $\lambda_{abs}^a$ / nm | $\Delta \lambda_{abs}^b$ / nm | $I/I_0^c$ | $K_b^d$ / $10^4$ M\textsuperscript{-1} |
| 2a     | 437    | 18   | 0.14 | 14 | 432 | 19 | 0.05 | 22 |
| 2b     | 443    | 21   | 3    | 1.5 | 440 | 18 | n.d.\textsuperscript{e} | 2.6\textsuperscript{f} |
| 2c     | 476    | 42   | n.d.\textsuperscript{e} | n.d.\textsuperscript{e} | 492 | 58 | n.d.\textsuperscript{e} | 1.6\textsuperscript{f} |
| 2d     | 422    | 0    | 0.38 | n.d.\textsuperscript{e} | 438 | 16 | 0.19 | 3.0 |

\textsuperscript{a}Long-wavelength absorption maximum of the DNA-bound ligand. \textsuperscript{b}Shift of the long-wavelength absorption maximum between free and bound ligand. \textsuperscript{c}Relative emission intensity, $I/I_0$ ($I$ = emission intensity of DNA-bound ligand at saturation, $I_0$ = emission of unbound ligand). \textsuperscript{d}Binding constant of ligand–DNA complex, $K_b$, determined from fluorimetric titrations. \textsuperscript{e}Not determined. \textsuperscript{f}$K_b$ determined from photometric titrations; DNA concentration in base pairs for ct DNA and in oligonucleotide for 22AG.

Discussion

Pd-mediated coupling reactions of halogenbenzo[b]quinolizinium derivatives

Although it was shown in this work that in particular cases appropriately substituted benzo[b]quinolizinium substrates can be functionalized as aryl- or alkynyl-substituted derivatives by...
Sonogashira and base-free Suzuki–Miyaura coupling reactions, it is obvious that this synthetic approach has its limitations. As compared with the corresponding quinolizinium substrates, that can be used for a variety of metal-mediated coupling reactions [28-32], the benzo[h]quinolizininium core appears to be very sensitive towards the reaction conditions, leading to serious side or secondary reactions. All experimental results indicate that the “usual” experimental protocols cannot be applied due to the high susceptibility of the benzo[h]quinolizininium ring towards nucleophilic attack at 6-position that leads to ring opening [37,38]. Thus, the Sonogashira reaction of 5 requires either the separate generation of copper acetylide or strict water-free conditions to avoid the formation of hydroxide ions. To avoid the potential interference of bases, we attempted to improve of the conditions for the Suzuki–Miyaura coupling in the base-free variant using aryldiazonium reagents [39]. Although the coupling reactions between aryldiazonium salts and arylboronic acids or esters with base-free conditions are known [39,42], in our hands the reaction of benzo[h]quinolizininium-9-boronic acid (3a) with benzenediazonium tetrafluoroborate 4a only resulted in the formation of the benzo[h]quinolizininium-9-trifluoroborate (3b). Consequently, we used the latter substrate for subsequent synthesis, as it has been reported that organotrifluoroborates may also be employed as starting materials in Suzuki–Miyaura coupling reactions of aryl halides [52,53]. Indeed, starting from benzo[h]quinolizininium-9-trifluoroborate (3b) and the corresponding aryldiazonium ions the 9-arylbenzo[h]quinolizininium derivatives 1a–d were available in yields that are comparable, or even slightly higher, than the ones obtained with the Suzuki–Miyaura reaction of benzo[h]quinolizininium-9-boronic acid (3a) with bromoarenes [34].

In our previous attempts to synthesize the corresponding benzo[h]quinolizininium-9-trifluoroborate (3b), the reaction of benzo[h]quinolizininium-9-boronic acid (3a) with KHF₂ only resulted in a partly contaminated product [34]. In this work, we used NaBF₄ as reagent, as we have rather accidentally observed that it can be used for the synthesis of the trifluoroborate
Figure 8: Fluorimetric titration of 2a (A), 2b (B) and 2d (C) with ct DNA in potassium phosphate buffer (95 mM K\(^+\); 5% DMSO; pH 7.0); \(c_{\text{Ligand}} = 20.0 \mu\text{M}\). Arrows indicate the development of the bands with increasing DNA concentration. Inset: Plot of the relative emission intensity, \(I_0\) versus \(c_{\text{DNA}}/c_{\text{L}}\). Lines denote the best fit of experimental data to the theoretical model; \(\lambda_{\text{ex}} = 335\) nm (A), 420 nm (B) and 380 nm (C).

Figure 9: Fluorimetric titration of 2a (A) and 2d (B) with 22AG in potassium phosphate buffer (95 mM K\(^+\); 5% DMSO; pH 7.0); \(c_{\text{Ligand}} = 20.0 \mu\text{M}\). Arrows indicate the development of the bands with increasing DNA concentration. Inset: Plot of the relative emission intensity, \(I_0\) versus \(c_{\text{DNA}}/c_{\text{L}}\). Lines denote the best fit of experimental data to the theoretical model; \(\lambda_{\text{ex}} = 335\) nm (A) and 380 nm (B).
Scheme 3: Photoinduced charge transfer upon the excitation of derivative 2d.
were studied. It was demonstrated that derivatives 2a–d bind to DNA in a similar binding mode. Notably, a pronounced decrease of the long-wavelength absorption followed by the development of new band at longer wavelength was observed during the photometric titrations (Figure 6 and Figure 7), and only in the titration of the phenylethynyl-substituted derivative 2a an isosbestic point was formed. These observations clearly show that the ligands bind in at least two different binding modes to DNA. Considering the low solubility of these compounds in water it is assumed that at the beginning of the titration, i.e., with large ligand–DNA ratio and a paucity of DNA binding sites, the ligand forms aggregates along the DNA backbone. With increasing DNA concentration more binding sites are available such that the ligands can intercalate. In the case of quadruplex DNA, the derivatives 2a–d show a typical titration signature for ligands that bind to the quadruplex by terminal π-stacking [14]; however, in analogy to the binding to duplex DNA the derivatives 2b–d form aggregates along the DNA backbone at large ligand–DNA ratio, i.e., at the beginning of the titration.

The fluorescence intensity of the derivatives 2a and 2d is significantly quenched by the addition of DNA, respectively (Figure 8 and Figure 9). This observation usually indicates a photoinduced electron transfer between the excited molecules and the DNA bases [69]. By contrast, the association of ct DNA with the methoxy-substituted derivative 2b led to an increase of the low emission intensity by a factor of 3 (Figure 8B). Although this effect is rather small, it indicates the suppression of a deactivation pathway in the excited state upon the accommodation of 2b in a constrained binding site of ct DNA, presumably due to the restriction of the conformational flexibility inside the binding site [65].

Conclusion
In summary, different synthetic approaches toward the Pd-mediated coupling reactions of benzo[h]quinolizinium derivatives were assessed that enable the functionalization and further development of this useful class of compounds. In particular, we demonstrated that optimized base-free Suzuki–Miyaura and Sonogashira coupling reactions can be used for the synthesis of aryl- and arylalkynyl-substituted benzo[h]quinolizinium derivatives in moderate to good yields. Therefore, the optimized protocol for Pd-mediated reactions may be employed for other base-sensitive substrates as well.

The photophysical properties as well as the DNA-binding properties of the (arylethynyl)benzo[h]quinolizinium derivatives were studied. It was demonstrated that derivatives 2a–d bind to duplex and quadruplex DNA with binding constants $K_b$ of 0.2–2.2 × 10$^5$ M$^{-1}$. Unfortunately, a differentiation between duplex and quadruplex DNA by derivatives 2a–d was not observed. Therefore, future work has to focus on further functionalizations that lead to selective binding of the ligands to particular DNA forms, e.g., by fine tuning of the stereoelectronic or steric properties of substituents.

Supporting Information
Supporting Information File 1
Additional spectral data, detailed description of the experiments performed, $^1$H NMR of the derivatives 2a–d and crystallographic data.

[https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-14-161-S1.pdf]

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