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Study of a Convenient Method for the Preparation of Hydrosoluble Fluorescent Triazatruxene Derivatives

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Triazatruxene derivatives find many applications as lipophilic compounds, but few examples of hydrosoluble derivatives have been reported so far. In this paper, we compare different synthetic routes for the preparation of hydrophilic triazatruxene derivatives and define the most versatile pathway. The synthesized compounds are fluorescent not only in organic solvents, as reported for other lipophilic derivatives, but also in water, making them particularly suitable for biological applications.

Introduction

Triazatruxene derivatives have been of great interest in supramolecular chemistry and in particular in organic electronics, where they are studied as potential light-emitting diodes,[1] batteries and capacitors.[2] For their intrinsic photophysical and redox properties and their π-stacking capability,[3] these molecules can behave as electroactive discotic liquid crystals.[4] More recently, a triazafullerene was synthesized, using the triazatruxene core as a precursor.[5] Nevertheless, so far few examples of hydrosoluble triazatruxene derivatives have been reported,[6] whereas the simultaneous presence of a large hydrophobic core and flexible hydrophilic chains can give rise to very interesting properties. In particular, these two molecular features are essential in determining the interactions between small organic molecules and G-quadruplex DNA structures, which are unusual DNA secondary structures involved in several important biological processes, such as cell transformation.[7] We have recently reported a hydrophilic three side-chained triazatruxene as a new strong and selective G-quadruplex ligand, and thus potential anticancer drug.[8] Here, we consider different routes to obtain hydrosoluble triazatruxene derivatives in order to rationalize the different approaches present in the literature and define the most efficient method for the preparation of several compounds of this series and to analyze their spectroscopic properties.

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Results and Discussion

Synthesis

The triazatruxene core consists of a C3 symmetric cyclotrimer of indoles, which presents a wide aromatic surface with three facile points for the attachment of side chains: the three indolic NH groups in the 5-, 10- and 15-positions (Figure 1). The most common synthetic approach to obtain this kind of derivative is the cyclocondensation of either substituted 2-oxindole derivatives,[9] or indole itself,[10] through halogenation. In both cases, only the symmetric cycle-trimer can be obtained, as the reaction proceeds through a series of 2,3-couplings of monomeric units (Figure 2):[11] probably, in the first case the reacting intermediate is 2-chloroindole and in the second 3-bromoindole.

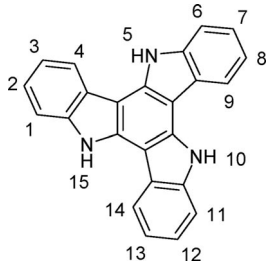


Figure 1. Chemical structure of the triazatruxene core, showing its complete numbering.

As for the reaction of indole with bromine, although in principle one equivalent of bromine would be sufficient to complete the cyclotrimerization, as a matter of fact, under these conditions, only the 2,3-dimer was isolated, whereas

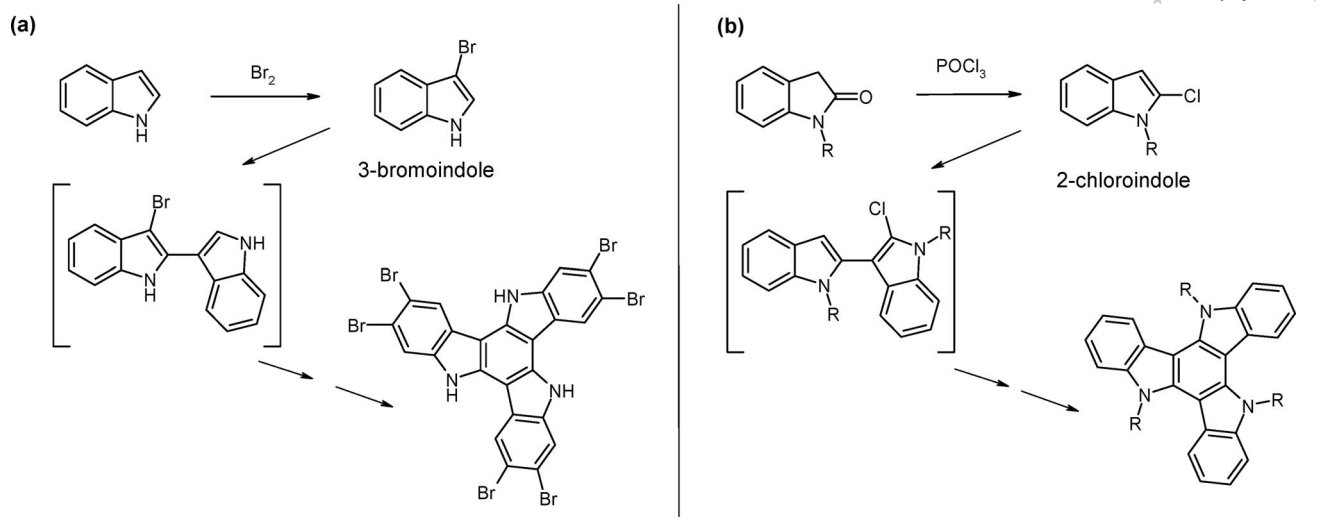
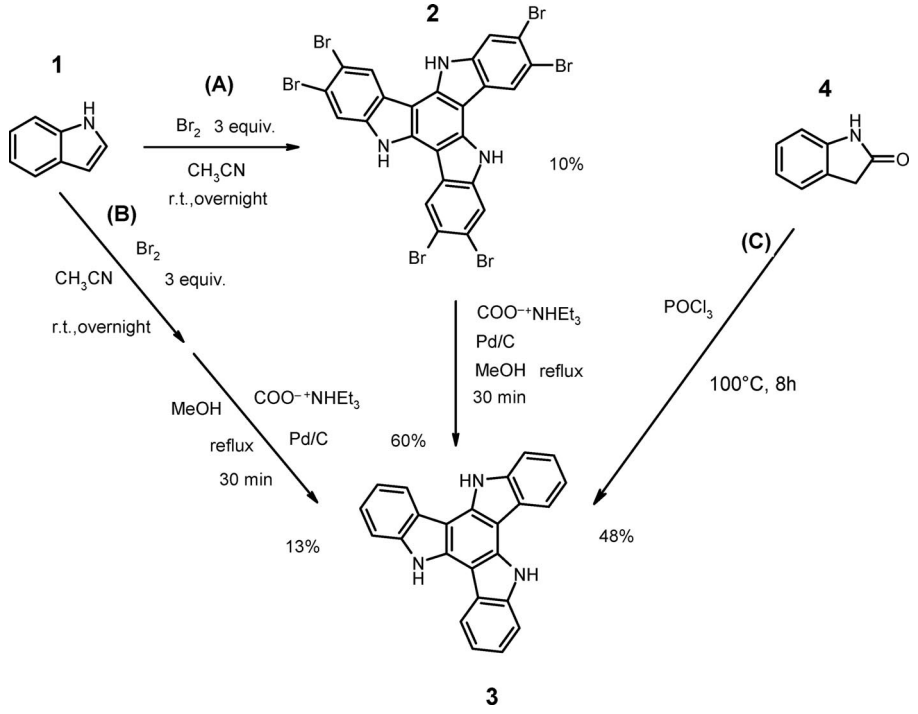


Figure 2. Hypothesized mechanisms for the 2,3-couplings of indole (a) and *N*-substituted-2-indolones (b), leading to the cyclocondensation of the triazatruxene core.

three equivalents were necessary to obtain the brominated symmetric indole trimers.[12] In particular, the synthesis of unsubstituted triazatruxene core **3** has been reported by isolation of hexabrominated trimer **2** and subsequent dehalogenation.[12,13] As a first synthetic approach, a mixture of **1** and Br_2 in acetonitrile was stirred overnight at room temperature. Obtained product **2** was washed and recrystallized, taking advantage of the low solubility of this intermediate compound. The dehalogenation was realized by reduction with formic acid in triethylamine, catalyzed by Pd/C, obtaining desired compound **3** in quite a scarce total yield (Scheme 1A). Because, as expected, the mass spectrum of the crude product of the first reaction step showed a mixture of brominated indole trimers (Figure 3), whose main component was not the hexabrominated trimer, we wondered if its isolation was really necessary.

So, we used this mixture, without further purification, and under the reaction conditions for the Pd-catalyzed dehalogenation (Scheme 1B) triazatruxene core **3** was obtained in a yield higher than before, but still comparable to that reported in the literature.[12] The low yields of these two synthetic routes do not allow us to consider the opportunity of inserting the side chains on the indole nitrogen atom before the cyclization step.



Scheme 1.

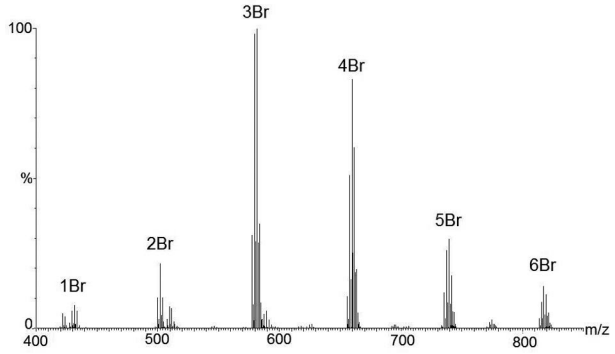


Figure 3. Mass spectrum of the crude product of the first reaction step of Scheme 1A, showing a mixture of brominated indole trimers, whose number of bromine atoms is indicated by the labels.

In the alternative synthetic approach (Figure 2b), the starting compounds are represented by *N*-substituted-2-indolones, which were treated with phosphoryl chloride to give the corresponding tris-*N*-susbstituted-triazatruxenes.[6,14] Following an analogous strategy, we performed the symmetric cyclotrimerization of 2-indolone (**4**) in $POCl_3$ at 100 °C,[15] leading to unsubstituted triazatruxene core **3** in 48% yield (Scheme 1C). To the best of our knowledge, this way represents the best synthetic approach to obtain unsubstituted triazatruxene core **3** in terms of time and yield.

At this point, the insertion of the desired side chains could be performed in two steps: by N-alkylation under basic conditions (KOH) with an excess amount of dihalides **5a**–**d** to give the corresponding tris-*N*-substituted derivatives **6a**–**d**, followed by substitution of the halogen at the end of the chain with an excess amount of piperidine to get final compounds **7a**–**d** (Scheme 2). Both steps were carried out in refluxing THF and easily worked up. The first step is surely the most limiting, with yields not over 40%, as several side reactions are possible, such as polymerization or undesired substitutions by OH^-. Unfortunately, the use of different bases to avoid these problems (such as triethylamine) was not successful, probably because of the very weak acidity of the indole, which requires a strong base for efficient deprotonation.

It is worth noting that the four synthesized compounds (Figure 4) represent just a few examples of many hydrophilic triazatruxene derivatives that can be easily prepared through this synthetic pathway. In fact, the length and the basicity of the side chains can be efficiently modulated by choosing appropriate and, if desired, substituted dihalides (such as **5c**), as well as different amines for the final substi-

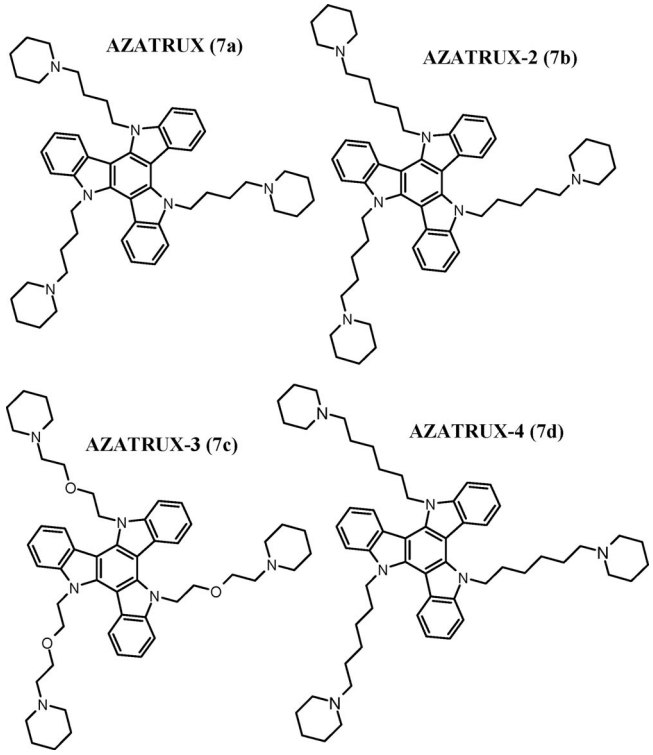
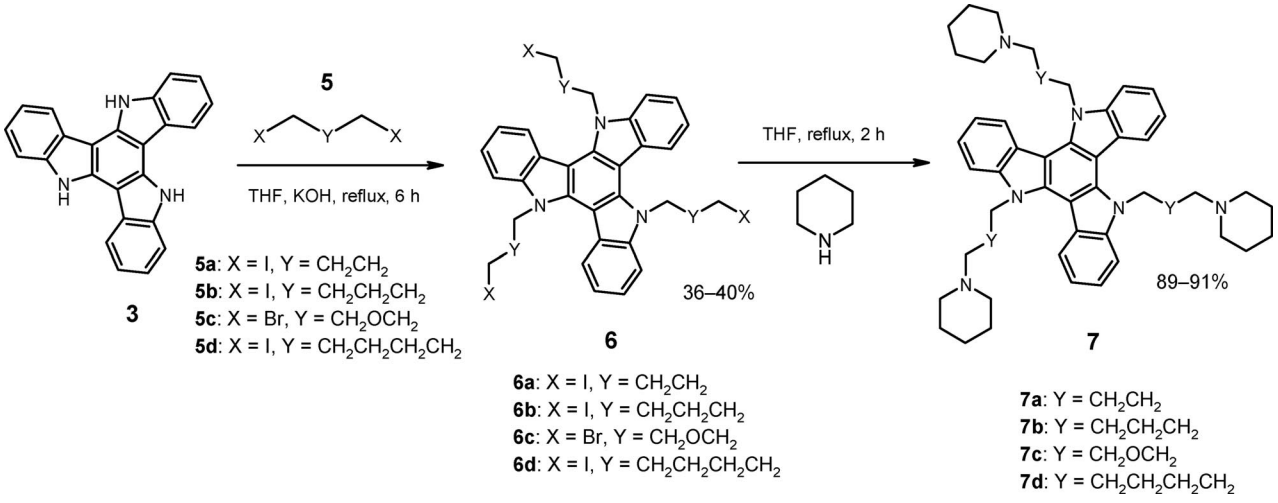
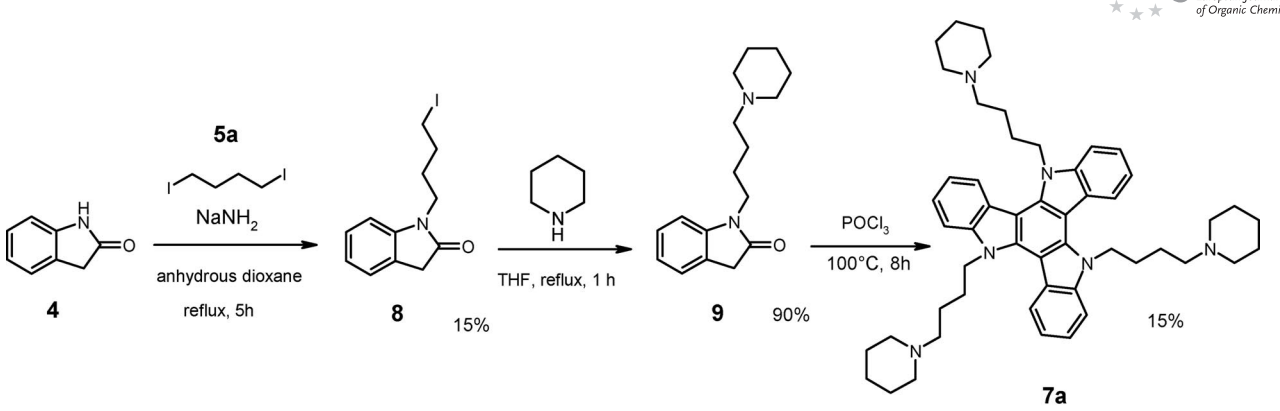


Figure 4. Chemical structures of the synthesized triazatruxene derivatives.



Scheme 2.



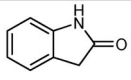
Scheme 3.

tution of the remaining halogen. On the contrary, the use of commercially available halogen amines as hydrophilic chains to be directly inserted into the triazatruxene core can strongly limit the variability of the molecular features of possible substituents.

Similarly, the preparation of N-substituted precursors with hydrophilic chains, necessary to follow the synthetic pathway reported in Figure 2b, using such halogen amines suffers from analogous limitations.[16] As an alternative approach, we prepared the *N*-substituted-2-indolone (**9**) suitable for the preparation of AZATRUX (**7a**) by following a two-step process similar to that reported in Scheme 2. The weaker reactivity of the NH group of 2-indolone (**4**) with respect to that of indole (**1**), due to the amidic nature of the former, resulted in a very scarce yield when trying to prepare such a precursor (Scheme 3). The low yield is surely also related to the necessary use of a dihalide instead of a monohalide, as in the case of simple lipophilic chains. Moreover, different from what was reported in the literature for lipophilic derivatives,[14] the cyclocondensation step also showed a very low yield, probably because of the different basicity of the precursor.

For a complete comparison of the different synthetic routes, the total yields for the preparation of AZATRUX (**7a**) in the different cases are reported in Table 1. In the first three lines, the limiting step is the formation of unsubstituted triazatruxene core **3**, whose yields are also reported.

Table 1. Comparison of the total yields of the final compound AZATRUX (**7a**) for the different synthetic routes with respect to the two starting compounds. When present as an intermediate compound, the yield of unsubstituted triazatruxene core **3** is also reported.

Starting compound	Scheme	Yield of triazatruxene core (**3**) [%]	Yield of AZATRUX (**7a**) [%]
 **1**	1A and 2	6	0.02
	1B and 2	13	0.05
 **4**	1C and 2	48	17
	3	–	2

Spectroscopic Properties

As suggested by UV/Vis spectra registered at different temperatures[8] (also reproduced in the Supporting Information, Figure S1), these hydrophilic triazatruxene derivatives showed a poor tendency to aggregate in water solution, despite the large aromatic core. A confirmation of this behaviour was obtained by the observed linearity of absorbance versus concentration (Figure 5), both in MES/KCl buffer (pH 6.5) and in a 0.1 M aqueous solution of HCl. Because at this very low pH we can be reasonably sure that any possible aggregates are broken up, we can conclude that no aggregates are present also at physiological pH, at least in the considered (micromolar) concentration range. As further and independent evidence of this, we also performed an NMR DOSY (diffusion-ordered spectroscopy) experiment (Supporting Information, Figure S2): the experiment clearly indicates that only one molecular species is present even in the mM concentration range. As a result of the high

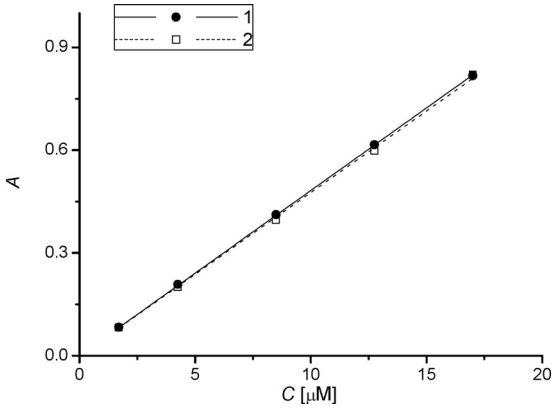


Figure 5. Absorbance vs. concentration of AZATRUX (**7a**) in aqueous 0.1 M HCl (1) and MES/KCl buffer (2), showing linearity in the considered concentration range.

resolution of the ^{1}H NMR spectrum in water (Supporting Information, Figure S3), and combined with the previous experiments, it is reasonable to conclude that the unique species in solution is a monomeric form.

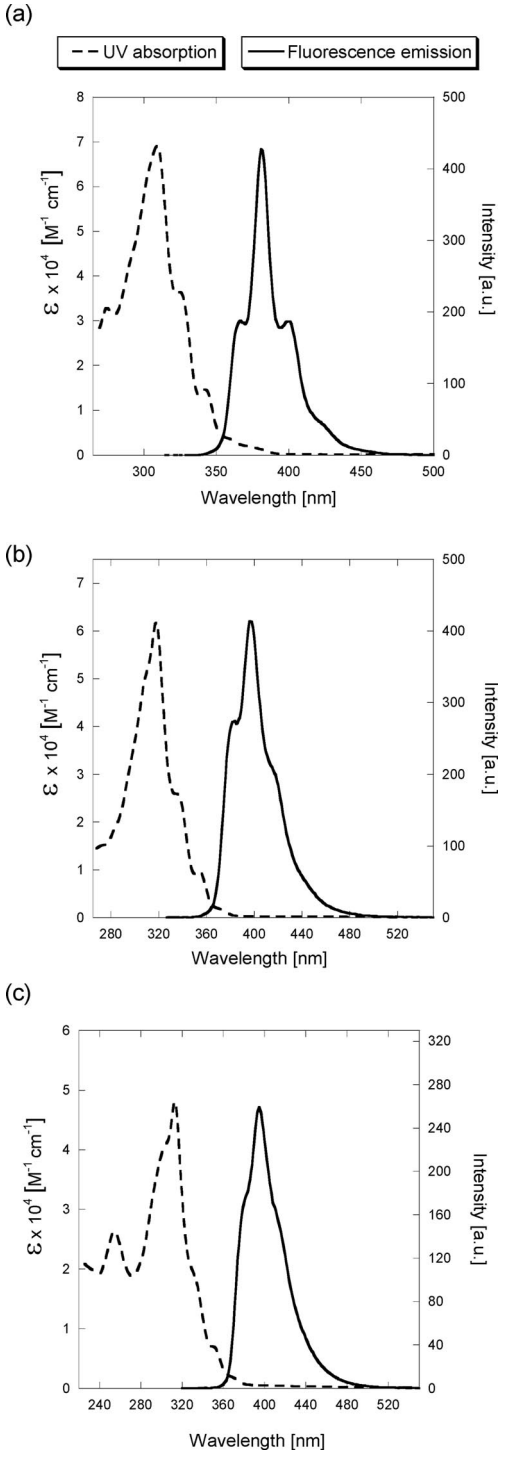


Figure 6. UV/Vis absorption (dotted line) and emission (continuous line) spectra of **3** in DMSO (a) and AZATRUX (**7a**) in DMSO (b) and in aqueous MES/KCl buffer (c). For the fluorescence spectra the following conditions were used: compound concentration 10 μM, voltage 450 V (a) and 500 V (b, c), excitation wavelengths 309 nm (a), 318 nm (b) and 310 nm (c).

This is not common behaviour, even though it is observed, for instance, for multiple-side-chained perylene derivatives.[17] On the contrary, because of the large aromatic core, the perylene derivatives have usually a strong tendency to self-aggregate in water as a result of hydrophobic interactions, leading to π–π stacking of several molecules one upon the other.[18] It is worth noting that very recently self-association of perylene-based G-quadruplex ligands has been reported to favour binding to duplex DNA, compromising the specific recognition of G-quadruplex structures.[19] This could explain the good selectivity shown by AZATRUX for the G-quadruplex with respect to duplex DNA.[8]

The synthesized triazatruxene derivatives also showed very interesting fluorescence properties. In fact, unsubstituted triazatruxene core **3** showed a very high fluorescence intensity in DMSO even at a low voltage (Figure 6a), as also reported for several other lipophilic N-substituted derivatives in organic solvents.[14,20] In contrast, no fluorescence was detected in water under the same conditions, whereas a high voltage (800 V) was necessary to detect a weak fluorescence (Supporting Information, Figure S4). This is probably due to a very scarce solubility of this compound in water and/or a strong self-aggregation, as suggested by the strong hypochromic effect observed in the UV spectrum (Supporting Information, Figure S4). On the contrary, the hydrophilic triazatruxene derivatives showed a good fluorescence both in DMSO and in water at a voltage of 500 V (Figure 6b,c; Supporting Information, Figure S5). This means that the reported compounds represent the first example of triazatruxene derivatives with a significant fluorescence in water (Supporting Information, Figure S6).

Conclusions

In this paper we have analyzed and compared different synthetic routes for the preparation of hydrophilic triazatruxene derivatives. The best synthetic approach involved cyclocondensation of unsubstituted 2-oxindole, followed by a two-step insertion of the desired side chains. The synthesized compounds do not show appreciable tendency to self-aggregate in water, despite the wide aromatic surface, and are fluorescent both in organic solvents and in water. Their promising characteristics, in terms of solubility and quantum yield, make them particularly suitable to develop also hydrophilic fluorescent dyes.

It is worth noting that the proposed pathway represents a versatile synthetic approach for the preparation of many compounds of this series with different side chains in terms of length and basicity, which are two critical parameters for modulating G-quadruplex ligands properties.[7] Currently we are studying the biological activity of the new compounds by several biophysical and biochemical techniques to compare the results with those reported for AZATRUX.[8]

Experimental Section

General: All the commercial reagents and solvents were purchased from Fluka and Sigma–Aldrich and used without further purifica-

tion. TLC glass plates (silica gel 60 F_{254}) and silica gel 60 (0.040–0.063 mm) were purchased from Merck. ^1H and ^{13}C NMR spectra were acquired with Varian Gemini 200 and Varian Mercury 300 instruments, with a digital resolution of 0.5 Hz. DOSY experiment on AZATRUX was performed with a Bruker Avance 400 spectrometer, and as described in the Supporting Information MS (ESI) spectra were recorded with a Micromass Q-TOF MICRO spectrometer. Elemental analyses (C, H, N) were carried out with an EA1110 CHNS-O (CE instruments). UV/Vis absorption spectra and fluorescence emission spectra were registered by using a JASCO V-530 spectrophotometer and a Varian Cary Eclipse Fluorescence spectrophotometer, respectively. MES-KCl buffer was composed of 10 mM MES at pH 6.5 and 50 mM KCl.

***CAUTION*:** $POCl_3$ (phosphoryl chloride) is corrosive and very toxic by inhalation. It reacts violently with water, liberating toxic gases, and can cause severe burns.

2,3,7,8,12,13-Hexabromo-10,15-dihydro-5*H*-diindolo[3,2-*a*:3′,2′-*c*]-carbazole (2): A solution of Br_2 (4.10 g, 25.5 mmol) in CH_3CN (5 mL) was added to a mixture of indole **1** (1 g, 8.5 mmol) and CH_3CN (15 mL) with stirring at room temperature over 5 min. The mixture was left to stir overnight, and then the resulting dark-green solid was filtered and washed with acetonitrile (150 mL). Obtained product **2** was recrystallized by addition of acetone (80 mL) to a DMSO (3 mL) solution of the crude product (181 mg, 10%). ^1H NMR (200 MHz, [D_6]DMSO): δ = 12.30 (br., 3 H, N_{arom}-H), 8.59 (s, 3 H, aromatic H), 7.89 (s, 3 H, aromatic H) ppm. HRMS (ESI): calcd. for $C_{24}H_8N_3Br_6$ $[M - H]^-$ 811.5818; found 811.5857.

10,15-Dihydro-5*H*-diindolo[3,2-*a*:3′,2′-*c*]carbazole (3)

Method A (Scheme 1): A mixture of **2** (175 mg, 0.21 mmol), Et_3N (0.24 mL, 1.71 mmol), HCOOH (0.06 mL, 1.71 mmol), and 10% Pd/C (68 mg, 0.06 mmol) in MeOH (15 mL) was heated for 30 min under reflux. The mixture was filtered through Celite, and the filtrate was diluted with CH_2Cl_2, washed with aqueous HCl (10%) and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give **3** as a pale-yellow solid (44 mg, 60%). ^1H NMR (200 MHz, [D_6]acetone): δ = 11.08 (s, 3 H, N_{arom}-H), 8.52 (d, J = 7 Hz, 3 H, aromatic H), 7.68 (d, J = 7.4 Hz, 3 H, aromatic H), 7.4–7.2 (m, 6 H, aromatic H) ppm. ^{13}C NMR (50.2 MHz, [D_6]acetone): δ = 141.0, 136.4, 124.8, 124.5, 121.5, 121.4, 112.9, 103.3 (8 C, aromatic) ppm. UV (DMSO): λ ($\varepsilon \times 10^{-4}$, $M^{-1}cm^{-1}$) = 274 (3.3), 309 (6.8), 325 (3.6), 342 (1.5) nm. MS (ESI): m/z = 344.01 $[M - H]^-$.

Method B (Scheme 1): Following the initial procedure described above, a mixture of indole **1** (1 g, 8.5 mmol) and CH_3CN (15 mL) was stirred at room temperature and a solution of Br_2 (4.10 g, 25.5 mmol) in CH_3CN (5 mL) was added over 5 min. The mixture was stirred overnight, and the resulting dark-green solid was filtered and washed with acetonitrile (150 mL). At this point, without further purification, the crude product (2 g) was mixed with Et_3N (4.20 mL, 30.23 mmol), HCOOH (1.14 mL, 30.23 mmol) and 10% Pd/C (200 mg, 0.18 mmol) in MeOH (30 mL), and the resulting mixture was heated for 30 min under reflux. After a work up similar to that described above, the crude product was dissolved in methanol, adsorbed onto silica gel and purified by flash chromatography (ethyl acetate/*n*-hexane, 15:85) to give pure **3** as a pale-yellow solid (128 mg, 13%) with the same characterization data as that reported above.

Method C (Scheme 1): A mixture of 2-indolinone **4** (2.0 g, 15 mmol) and $POCl_3$ (10 mL) was heated at 100 °C for 8 h. Then, the reaction mixture was poured into ice and neutralized carefully with KOH until pH 7–8. After neutralization, the precipitate was filtered to give the crude product as a brown solid. The latter was dissolved in methanol, adsorbed onto silica gel and purified by flash chromatography (ethyl acetate/*n*-hexane, 15:85) to give pure **3** as a pale-yellow solid (830 mg, 48%) with the same characterization data as that reported above.

General Procedure for the Synthesis of Intermediate Compounds 6a–d: A mixture of triazatruxene core **3** and KOH (10 equiv.) in THF was heated under reflux for 10 min. An excess amount of the appropriate dihalide was then added, and the mixture was heated under reflux for 6 h. The mixture was diluted with AcOEt, washed with 10% aqueous HCl and then with brine solution. The organic layer was dried with Na_2SO_4 and the solvents were evaporated under vacuum. The crude product was dissolved in chloroform, adsorbed onto silica gel and purified by flash chromatography under the appropriate conditions.

5,10,15-Tris(4-iodobutyl)diindolo[3,2-*a*:3′,2′-*c*]carbazole (6a): Following the procedure described above, a mixture of **3** (355 mg, 1.03 mmol) and KOH (576 mg, 10.3 mmol) in THF (20 mL) was treated with 1,4-diiodobutane **5a** (2.0 mL, 15 mmol). After the work up as above, the crude product was purified by flash chromatography (ethyl acetate/*n*-hexane, 5:95) to give **6a** (373 mg, 40%) as a dark-yellow viscous oil. ^1H NMR (200 MHz, [D_6]acetone): δ = 8.30 (d, J = 7.8 Hz, 3 H, aromatic H), 7.79 (d, J = 7.8 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 5.03 (t, J = 7.4 Hz, 6 H, N_{arom}-CH_2), 3.07 (t, J = 7.0 Hz, 6 H, I-CH_2), 1.89 (m, 6 H, $N_{arom}CH_2$-CH_2), 1.61 (m, 6 H, ICH_2-CH_2) ppm. ^{13}C NMR (50.2 MHz, [D_6]acetone): δ = 141.4, 138.8, 123.5, 123.3, 121.8, 120.3, 111.3, 103.6 (8 C, aromatic), 45.7, 30.6, 30.4, 6.1 ppm. MS (ESI): m/z = 892.43 $[M + H]^+$.

5,10,15-Tris(5-iodopentyl)diindolo[3,2-*a*:3′,2′-*c*]carbazole (6b): Following the procedure described above, a mixture of 3 (100 mg, 0.29 mmol) and KOH (81 mg, 1.40 mmol) in THF (2 mL) was treated with 1,5-diiodopentane 5b (0.64 mL, 4.35 mmol). After the work-up as above, the crude product was purified by flash chromatography (ethyl acetate/*n*-hexane, 10:90) to give the compound of formula 6b (97 mg, yield 36%), as a dark yellow viscous oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.23 (d, J = 7.5 Hz, 3 H, aromatic H), 7.61 (d, J = 8.1 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 4.89 (t, J = 7.8 Hz, 6 H, N_{arom}-CH_2), 3.03 (t, J = 6.6 Hz, 6 H, I-CH_2), 1.95 (m, 6 H, $N_{arom}CH_2$-CH_2), 1.74 (m, 6 H, ICH_2-CH_2), 1.35 (m, 6 H, ICH_2CH_2-CH_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 140.9, 138.6, 123.4, 122.9, 121.4, 119.9, 110.5, 103.3 (8 C, aromatic), 46.5, 32.8, 28.5, 27.4, 6.3 ppm. MS (ESI): m/z = 934.34 $[M + H]^+$.

5,10,15-Tris(5-bromo-3-oxapentyl)diindolo[3,2-*a*:3′,2′-*c*]carbazole (6c): Following the procedure described above, a mixture of **3** (130 mg, 0.38 mmol) and KOH (0.71 mL, 5.7 mmol) in THF (2 mL) was treated with **5c** (1.0 mL, 15 mmol). After the work up as above, the crude product was purified by flash chromatography (ethyl acetate/*n*-hexane, 15:85) to give **6c** (120 mg, 40%) as a yellow viscous oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.32 (d, J = 8.4 Hz, 3 H, aromatic H), 7.74 (d, J = 8.2 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 5.19 (t, J = 6.6 Hz, 6 H, N_{arom}-CH_2), 3.99 (t, J = 6.6 Hz, 6 H, $N_{arom}CH_2$-CH_2-O), 3.61 (t, J = 5.8 Hz, 6 H, O-CH_2-CH_2Br), 3.25 (t, J = 5.8 Hz, 6 H, OCH_2-CH_2-Br) ppm. ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = 141.2, 138.7, 123.2, 123.1, 121.6, 120.4, 110.6, 103.5 (8 C, aromatic), 71.0, 69.3, 46.1, 30.0 ppm. MS (ESI): m/z = 800.10 $[M + H]^+$.

5,10,15-Tris(6-iodohexyl)diindolo[3,2-*a*:3′,2′-*c*]carbazole (6d): Following the procedure described above, a mixture of **3** (130 mg, 0.38 mmol) and KOH (211 mg, 3.80 mmol) in THF (5 mL) was treated with **5d** (0.94 mL, 5.7 mmol). After the work up as above, the crude product was purified by flash chromatography (ethyl ace-

tate/*n*-hexane, 10:90) to give **6d** (150 mg, 40%), as a dark-yellow viscous oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.26 (d, J = 7.8 Hz, 3 H, aromatic H), 7.63 (d, J = 8.2 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 4.92 (t, J = 7.4 Hz, 6 H, N_{arom}-CH_2), 3.02 (t, J = 7.4 Hz, 6 H, I-CH_2), 1.95 (m, 6 H, $N_{arom}CH_2$-CH_2), 1.66 (m, 6 H, ICH_2-CH_2), 1.27 (m, 12 H, ICH_2CH_2-CH_2) ppm.

General Procedure for the Synthesis of Final Compounds 7a–d: A mixture of intermediate compound **6** and piperidine was heated in THF under reflux for 2 h. The mixture was evaporated under reduced pressure and purified by flash chromatography (ethyl acetate saturated with 30% aqueous ammonia solution) to give the corresponding compound of formula **7** (Scheme 2).

5,10,15-Tris[4-(1-piperidino)butyl]diindolo[3,2-*a*:3′,2′-*c*]carbazole (7a, AZATRUX): Following the general procedure, **6a** (209 mg, 0.23 mmol) was treated with piperidine (0.69 mL, 7.0 mmol) in THF (5 mL). Purification of the crude product gave **7a** (161 mg, 91%) as a dark-yellow viscous oil. Product **7a** crystallized by slowly cooling a saturated methanol solution to obtain white needles (m.p. 122–123 °C). A part of compound **7a** (110 mg) was precipitated in the form of its hydrochloride salt by dissolving it in a mixture of methanol/HCl (methanol/37% aqueous HCl, 95:5) and adding diethyl ether: 81 mg of a white solid was obtained with a yield of 58%. ^{1}H NMR (300 MHz, D_2O): δ = 7.61 (d, J = 7.8 Hz, 3 H, aromatic H), 7.40 (m, 6 H, aromatic H), 7.25 (m, 3 H, aromatic H), 3.98 (m, 6 H, N_{arom}-CH_2), 2.89 (br., 6 H, $N_{piperidine}$-CH_2), 2.53 (m, 6 H, $N_{piperidine}$-CH_2), 2.29 (br., 6 H, $N_{piperidine}$-CH_2), 1.49 (m, 24 H, $N_{piperidine}CH_2$-CH_2, $N_{arom}CH_2$-CH_2), 1.06 (m, 6 H, $CH_{2piperidine}$) ppm. UV (aq. MES-KCl): λ ($\varepsilon \times 10^{-4}$, $M^{-1}cm^{-1}$) = 255 (2.6), 313 (4.8) nm. HRMS (ESI): calcd. for $C_{51}H_{67}N_6$ $[M + H]^+$ 763.5427; found 763.5393. $C_{51}H_{66}N_6 \cdot 3HCl \cdot 5H_2O$ (962.61): calcd. C 63.6, H 8.3, N 8.7; found C 63.5, H 8.3, N 8.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.26 (d, J = 8.4 Hz, 3 H, aromatic H), 7.68 (d, J = 8.2 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 4.95 (t, J = 7.4 Hz, 6 H, N_{arom}-CH_2), 2.25 (m, 18 H, $N_{piperidine}$-CH_2), 1.99 (m, 6 H, $N_{arom}CH_2$-CH_2), 1.51 (m, 18 H, $N_{piperidine}CH_2$-CH_2), 1.39 (m, 6 H, γ-$CH_{2piperidine}$) ppm. ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = 140.8, 138.6, 123.2, 122.7, 121.3, 119.7, 110.6, 103.0 (8 C, aromatic), 58.2, 54.2, 46.5, 27.5, 25.5, 24.1, 23.4 ppm. UV (DMSO): λ ($\varepsilon \times 10^{-4}$, $M^{-1}cm^{-1}$) = 267 (1.9), 318 (6.1), 354 (1.0) nm.

5,10,15-Tris[5-(1-piperidino)pentyl]diindolo[3,2-*a*:3′,2′-*c*]carbazole (7b, AZATRUX-2): Following the general procedure, **6b** (100 mg, 0.29 mmol) was treated with piperidine (0.31 mL, 3.1 mmol) in THF (2 mL). Purification of the crude product gave **7b** (73 mg, 91%) as a dark-yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, J = 8.1 Hz, 3 H, aromatic H), 7.60 (d, J = 8.4 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 4.92 (t, J = 6.6 Hz, 6 H, N_{arom}-CH_2), 2.5–2.3 (m, 18 H, $N_{piperidine}$-CH_2), 1.9–1.7 (m, 18 H, $N_{arom}CH_2$-CH_2-CH_2-CH_2-$CH_2N_{piperidine}$), 1.47 (m, 12 H, $N_{piperidine}CH_2$-CH_2), 1.02 (m, 6 H, γ-$CH_{2piperidine}$) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 140.8, 138.5, 123.1, 121.4, 120.2, 110.9, 103.3 (7 C, aromatic; two peaks are superimposed at 123.1 ppm in a broad and intense signal), 57.5, 53.2, 46.3, 28.7, 24.0, 23.8, 23.7, 22.5 ppm UV (DMSO): λ ($\varepsilon \times 10^{-4}$, $M^{-1}cm^{-1}$) = 264 (2.2), 317 (6.1), 354 (0.9) nm. HRMS (ESI): m/z = 805.5864 $[M + H]^+$.

5,10,15-Tris[5-(1-piperidino)-3-oxapentyl]diindolo[3,2-*a*:3′,2′-*c*]carbazole (7c, AZATRUX-3): Following the general procedure, **6c** (120 mg, 0.15 mmol) was treated with piperidine (0.38 mL, 3.8 mmol) in THF (2 mL). Purification of the crude product gave **7c** (94 mg, 89%) as a dark-yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.31 (d, J = 7.8 Hz, 3 H, aromatic H), 7.72 (d, J = 9 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 5.16 (t, J = 6.6 Hz, 6 H, N_{arom}-CH_2), 3.93 (t, J = 6.0 Hz, 6 H, $N_{arom}CH_2$-CH_2-O), 3.47 (t, J = 6.6 Hz, 6 H, O-CH_2-$CH_2N_{piperidine}$), 2.40 (t, J = 6.3 Hz, 6 H, OCH_2-CH_2-$N_{piperidine}$) 2.27 (m, 12 H, $N_{piperidine}$-CH_2), 1.49 (m, 12 H, $N_{piperidine}CH_2$-CH_2), 1.33 (m, 6 H, γ-$CH_{2piperidine}$) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$). δ = 141.1, 138.8, 123.1, 123.0, 121.6, 120.1, 110.6, 103.4 (8 C, aromatic), 69.3, 69.1, 58.3, 54.7, 46.2, 25.6, 24.0 ppm. UV (DMSO): λ ($\varepsilon \times 10^{-4}$, $M^{-1}cm^{-1}$) = 263 (2.3), 317 (6.1), 352 (1.0) nm. HRMS (ESI): m/z = 811.5270 $[M + H]^+$.

5,10,15-Tris[6-(1-piperidino)hexyl]diindolo[3,2-*a*:3′,2′-*c*]carbazole (7d, AZATRUX-4): Following the general procedure, **6d** (150 mg, 0.15 mmol) was treated with piperidine (0.46 mL, 4.6 mmol) in THF (2 mL). The purification of the crude product gave **7d** (114 mg, 90%) as a dark-yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.25 (d, J = 7.8 Hz, 3 H, aromatic H), 7.61 (d, J = 8.7 Hz, 3 H, aromatic H), 7.5–7.3 (m, 6 H, aromatic H), 4.89 (m, 6 H, N_{arom}-CH_2), 2.5–2.2 (m, 18 H, $N_{piperidine}$-CH_2), 1.7–1.2 (m, 42 H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 140.9, 138.8, 123.4, 122.7, 121.4, 119.6, 110.5, 103.1 (8 C, aromatic), 59.1, 54.4, 46.8, 29.6, 27.2, 26.4, 25.7, 24.3 ppm. HRMS (ESI): m/z = 847.6355 $[M + H]^+$.

1-(4-Iodobutyl)indolin-2-one (8): 2-Indolinone **4** (200 mg, 1.5 mmol) was added to a suspension of sodamide (58 mg, 1.5 mmol) in dry dioxane (4 mL); the mixture was stirred under reflux until evolution of ammonia ceased. 1,4-Diiodobutane **5a** (1 mL, 7.5 mmol) was then added, and the mixture was heated under reflux for 5 h. The mixture was diluted with AcOEt, washed with water and then with brine solution. The organic layer was dried with Na_2SO_4 and the solvents were evaporated under vacuum. The crude product was dissolved in chloroform, adsorbed onto silica gel and purified by flash chromatography (chloroform/*n*-hexane, 70:30) to give compound **8** (70 mg, 15%) as a colourless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27 (m, 2 H, aromatic H), 7.03 (t, J = 7.8 Hz, 1 H, aromatic H), 6.84 (d, J = 7.8 Hz, 1 H, aromatic H), 3.73 (t, J = 6.6 Hz, 2 H, N-CH_2), 3.52 (s, 2 H, CH_2-CO), 3.22 (t, J = 6.6 Hz, 2 H, I-CH_2), 1.86 (m, 4 H, NCH_2-CH_2-CH_2-I) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 175.0 (carbonyl C), 144.4, 127.9, 124.6, 124.5, 122.3, 108.3, (6 C, aromatic), 38.7, 35.7, 30.5, 28.3, 5.8 ppm.

1-[4-(1-Piperidino)butyl]indolin-2-one (9): A mixture of **8** (150 mg, 4.76 mmol) and piperidine (0.69 mL, 7.0 mmol) was heated in THF (3 mL) under reflux for 2 h. The mixture was evaporated under reduced pressure and purified by flash chromatography (chloroform/methanol/30% aqueous ammonia solution, 95:5:0.5) to give **9** (116 mg, 90%) as a colourless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.23 (m, 2 H, aromatic H), 6.99 (t, J = 7.8 Hz, 1 H, aromatic H), 6.83 (d, J = 7.8 Hz, 1 H, aromatic H), 3.70 (t, J = 6.6 Hz, 2 H, N-CH_2), 3.52 (s, 2 H, CH_2-CO), 3.15 (m, 2 H, $N_{piperidine}$-CH_2), 2.01 (m, 4 H) 1.77 (m, 4 H), 1.65 (m, 6 H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 175.0 (carbonyl C), 144.2, 127.8, 124.5, 124.4, 122.2, 108.3, (6 C, aromatic), 57.9, 54.0, 39.3, 35.7, 25.1, 24.8, 23.5, 23.0 ppm. HRMS (ESI): m/z = 295.1800 $[M + Na]^+$.

5,10,15-Tris[4-(1-piperidino)butyl]diindolo[3,2-a:3′,2′-c]carbazole (7a, AZATRUX): Following a similar procedure to that described above, a mixture of **9** (100 mg, 0.37 mmol) and $POCl_3$ (2 mL) was heated at 100 °C for 24 h. Then, the reaction mixture was poured into ice and basified carefully with KOH, and the resulting precipitate was washed with H_2O and then taken up in $CHCl_3$. This solution was washed with H_2O, dried with Na_2SO_4 and the solvent was evaporated under reduced pressure to give the crude product as a brown solid. The latter was purified by flash chromatography (chloroform/methanol/30% aqueous ammonia solution, 90:10:1) to

give **7a** (Scheme 3), as a dark-yellow viscous oil (14 mg, 15%) with the same characterization data as that reported above.

UV/Vis Absorption and Fluorescence Spectroscopy: UV/Vis absorption spectra and fluorescence emission spectra were performed at 298 K by using the instruments indicated above. Absorbance of AZATRUX in MES/KCl buffer was registered at an initial concentration of 17 μM, following further dilutions up to 1.7 μM. As for fluorescence spectroscopy, for all the spectra compounds concentration was 10 μM, the excitation and emission slits width was set to 5 nm, whereas the excitation wavelength and the emission range where suitably changed. The emission PMT (PhotoMultiplier Tubes) detector voltage was modulated from 450 to 800 V. In particular, for the spectra of **7a**–**c** in DMSO the excitation wavelength was set to 318 nm, the emission range was set from 325 to 550 nm and the emission PMT voltage was fixed to 500 V. For the spectra of **7a**–**c** in MES-KCl buffer the excitation wavelength was set to 310 nm, the emission range was set from 320 to 550 nm and the emission PMT voltage was fixed to 500 V. For the spectra of **3** in DMSO the excitation wavelength was set to 309 nm, the emission range was set from 320 to 550 nm and the emission PMT voltage was fixed to 450 V. For the spectra of **3** in MES-KCl buffer the excitation wavelength was set to 310 nm, the emission range was set from 320 to 550 nm and the emission PMT voltage was fixed to 800 V.

Supporting Information (see footnote on the first page of this article): UV/Vis absorption and fluorescence spectra, ^{1}H and ^{13}C NMR spectra, DOSY experiment on AZATRUX.

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