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4-Amino-1,8-naphthalimide-Based Tröger's Bases As High Affinity DNA Targeting Fluorescent Supramolecular Scaffolds

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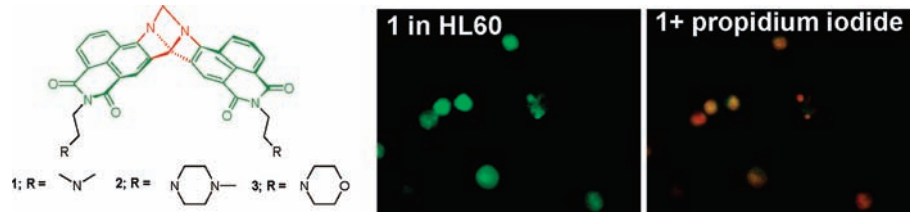
ABSTRACT



The synthesis and photophysical and biological investigation of fluorescent 1,8-naphthalimide conjugated Tröger's bases 1−3 are described. These structures bind strongly to DNA in competitive media at pH 7.4, with concomitant modulation in their fluorescence emission. These structures also undergo rapid cellular uptake, being localized within the nucleus within a few hours, and are cytotoxic against HL60 and (chronic myeloid leukemia) K562 cell lines.

The ability to specifically target nucleic acid structures using small or medium sized organic molecules and conjugates has become a highly topical area of research within chemistry.[1−4] With the aim of developing novel luminescent supramolecular structures as probes for nucleic acids, and as novel imaging agents, we have recently developed both Ru(II)[5] and dual visibly and NIR emitting Ru−Yb/Nd(III)[6] conjugates and shown that these can bind strongly to DNA with concomitant changes in their photophysical properties. Herein, we present the synthesis of all organic-based DNA targeting systems, **1**−**3**, based on 4-amino-1,8-naphthalimide[7] derived Tröger's bases (Scheme 1).[8,9] Compounds **1**−**3**, which possess good water solubility, were designed so that at physiological pH their two cationic amino terminus would give rise to strong electrostatic interaction/binding at the phosphate

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(1) Dervan, P. B.; Poulin-Kerstien, A. T.; Fechter, E. J.; Edelson, B. S. *Top. Curr. Chem.* **2005**, *253*, 31.
(2) Lalor, R.; Baillie-Johnson, H.; Redshaw, C.; Matthews, S. E.; Mueller, A. *J. Am. Chem. Soc.* **2008**, *130*, 2892.
(3) (a) McDonnell, S. O.; Hall, M. J.; Allen, L. T.; Byrne, A.; Gallagher, W. M.; O'Shea, D. F. *J. Am. Chem. Soc.* **2005**, *127*, 16360. (b) Gallagher, W. M.; Allen, L. T.; O'Shea, C.; Kenna, T.; Hall, M.; Gorman, A.; Killoran, J.; O'Shea, D. F. *Br. J. Cancer* **2005**, *92*, 1702.
(4) (a) Andersson, J.; Li, S. M.; Lincoln, P.; Andreasson, J. *J. Am. Chem. Soc.* **2008**, *130*, 11836. (b) Fujimoto, J.; Bando, T.; Minoshima, M.; Kashiwazaki, G.; Nishijima, S.; Shinohara, K.; Sugiyama, H. *Bioorg. Med. Chem.* **2008**, *16*, 9741. (c) Ott, I.; Xu, Y. F.; Liu, J. W.; Kokoschka, M.; Harlos, M.; Sheldrick, W. S.; Qian, X. H. *Bioorg. Med. Chem.* **2008**, *16*, 7107. (d) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *10*, 2215.
(5) Ryan, G. J.; Quinn, S.; Gunnlaugsson, T. *Inorg. Chem.* **2008**, *47*, 401.
(6) Nonat, A. M.; Quinn, S. J.; Gunnlaugsson, T. *Inorg. Chem.* **2009**, *48*, 4646.
(7) (a) Veale, E. B.; Tocci, G. M.; Pfeffer, F. M.; Kruger, P. E.; Gunnlaugsson, T. *Org. Biomol. Chem* **2009**, *7*, in press. (b) Veale, E. B.; Gunnlaugsson, T. *J. Org. Chem.* **2008**, *73*, 8073.
(8) Deprez, N. R.; McNitt, K. A.; Petersen, M. E.; Brown, R. G.; Lewis, D. E. *Tetrahedron Lett.* **2005**, *46*, 2149.

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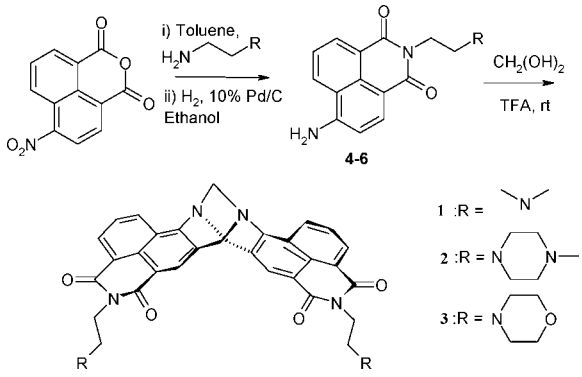
Scheme 1. Synthesis of **4**−**6** and Tröger's Bases **1**−**3**[a]



[a] See text for the nature of R.

backbone of DNA, while the Tröger's base part, which has been employed in various supramolecular structures,[10,11] due to its unique V-shaped geometry would allow these structures to adopt an almost orthogonal geometry,[12] to facilitate binding via the grooves and/or through intercalation. In this letter, we describe the synthesis and the photophysical evaluation of **1**−**3** and the binding of these structures to DNA. Furthermore, we also demonstrate that **1**−**3** undergo rapid cellular uptake, localizing within 24 h within the nucleus, and that they possess low micromolar toxicity in drug resistant chronic myeloid leukemia K562 cell lines.

The synthesis of **1**−**3** (see Supporting Information), which gives racemic mixtures, involved the initial formation of the 4-amino-1,8-naphthalimide derivatives **4**−**6**, achieved in good yields in two steps from the commercially available 4-nitro-1,8-naphthalic anhydride, by first reacting it with *N*,*N*-dimethylethylenediamine, 1-(2-aminoethyl)-4-methyl-piperazine, and 4-(2-aminoethyl)-morpholine, respectively (**7**−**9** see in Supporting Information), followed by hydrogenlysis in MeOH at 3 atm H_2 in the presence of 10% Pd/C catalyst. Compounds **4**−**6** were then converted into the Tröger's bases **1**−**3**, by using 1.5 equiv of formaldehyde in neat TFA over 12 h at room temperature. This was followed by basification of the resulting mixture to pH 12 and extraction with CH_2Cl_2, which gave compounds **1**−**3** in 49−60% yield, after crystallization from ethanol. The ^{1}H NMR of **1**−**3** (400 MHz, $CDCl_3$) (see Figures S1−S3 in Supporting Information for ^{1}H and ^{13}C NMR of **1**−**3** and S5−S7 for ^{1}H−^{1}H-COSY) confirmed their identity by the presence of a well-separated doublet of doublets between 5.14 and 4.34 ppm, pertaining to the methylene protons of the diazocine ring, while also clearly reflecting the C_2 plane of symmetry of the molecule.

4-Amino-1,8-naphthalimides are known to be strongly colored and fluorescent molecules, due to their internal charge transfer (ICT) nature, which is caused by their "push−pull" character.[5,7] Hence, they have been used as fluorescent and colorimetric PET sensors as well as drug candidates.[7,13,14] Similarly, **1**−**3** all displayed broad ICT bands in their absorption spectra when recorded in a range of solvents (see inset in Figure 1) similar to those observed for **4**−**6** and a high energy band centered around 240 nm. These were, however, blue-shifted in comparison to **4**−**6**, due to the lack of the 4-amino diazocine ring moiety to participate as strongly in the ICT. In the case of **2**, excitation at λ_{max} = 385 nm gave rise to broad emission with λ_{Fmax} = 517 nm and Φ_F = 0.0114, with relatively long excited state lifetimes (τ_F) of 10 ns, at pH 7.4. Similar results were observed for **2**, with Φ_F = 0.0114, with τ_F = 9.6 ns. The pH dependence of both their ground and the excited states was also investigated. While **4**−**6** displayed only minor changes in their absorption, significant changes were observed in the ground state of **1**−**3** (Figure 1 for **1**), from which pK_a values of 8.7, 8.2, and 6.0 were determined, respectively. These were assigned to the protonation of their terminal amines. In contrast, only minor changes were observed in the fluorescence of **1**−**3** as a function of pH, which is due to directional PET quenching, which is not "allowed" in such structures, having the quencher at the imide terminus.[15,16]

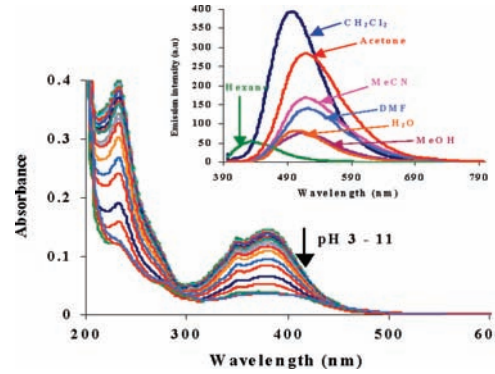


Figure 1. Changes in the absorption spectra of **1** as a function of pH. Inset: The emission of **1** in various solvents.

The binding of **1**−**3** to DNA was investigated at pH 7.4 (10 mM phosphate buffer), by observing the changes in their absorption and emission spectra, in the presence of 0 mM and,

(9) (a) Valík, M.; Malina, J.; Palivec, L.; Foltýnová, J.; Tkadlecová, M.; Urbanová, M.; Brabec, V.; Král, V. *Tetrahedron* **2006**, *62*, 8591. (b) Baldeyrou, B.; Tardy, C.; Bailly, C.; Colson, P.; Houssier, C.; Charmantray, F.; Demeunynck, M. *Eur. J. Med. Chem.* **2002**, *37*, 315. (c) Tatibouet, A.; Demeunynck, M.; Andraud, C.; Collet, A.; Lhomme, J. *Chem. Commun.* **1999**, 161.

(10) (a) Sergeyev, S. *Helv. Chem. Acta* **2009**, *92*, 415. (b) Dolensky, B.; Elguero, J.; Kral, V.; Pardo, C.; Valik, M. *Adv. Heterocycl. Chem.* **2007**, *93*, 1. (c) Valik, M.; Strongin, R. M.; Kral, V. *Supramol. Chem.* **2005**, *17*, 347. (d) Valik, M.; Kral, V.; Dolensky, B. *Org. Lett.* **2006**, *8*, 4867.

(11) (a) Havlik, M.; Kral, V.; Kaplanek, R.; Dolensky, B. *Org. Lett.* **2008**, *10*, 4767. (b) Kiehne, U.; Weilandt, T.; Lutzen, A. *Eur. J. Org. Chem.* **2008**, 2056. (d) Goswami, S.; Ghosh, K.; Dasgupta, S. *J. Org. Chem.* **2000**, *65*, 1907. (e) Goswami, S.; Ghosh, K. *Tetrahedron Lett.* **1997**, *38*, 4503. (f) Wilcox, C. S.; Cowart, M. D. *Tetrahedron Lett.* **1986**, *27*, 5563.

(12) (a) Weilandt, T.; Kiehne, U.; Schnakenburg, G.; Lutzen, A. *Chem. Commun.* **2009**, 2320. (b) Brotherhood, P. R.; Luck, I. J.; Blake, I. M.; Jensen, P.; Turner, P.; Crossley, M. J. *Chem.*−*Eur. J.* **2008**, *14*, 10967.

(13) (a) Hariprakasha, H. K.; Kosakowska-Cholody, T.; Meyer, C.; Cholody, W. M.; Stinson, S. F.; Tarasova, N. I.; Michejda, C. J. *J. Med. Chem.* **2007**, *23*, 5557. (b) Cholody, W. M.; Kosakowska-Cholody, T.; Hollingshead, M. G.; Hariprakasha, H. K.; Michejda, C. J. *J. Med. Chem.* **2005**, *48*, 4474. (c) Brãna, M. F.; Ramos, A. *Curr. Med. Chem. Anti-Cancer Agents* **2001**, *1*, 237.

in the case of **1** and **2**, also in 50 and 160 mM NaCl solutions (Table S1−S6 in Supporting Information). On all occasions, the absorption spectra of **1**−**3** were substantially affected by the successive addition of calf-thymus (*ct*-) DNA. As an example, the changes observed for the titration of **1** in 160 mM NaCl solution are shown Figure 2, resulting in a 26% hypochromism, with an accompanying bathochromic shift and the appearance of an isosbestic point at 410 nm.

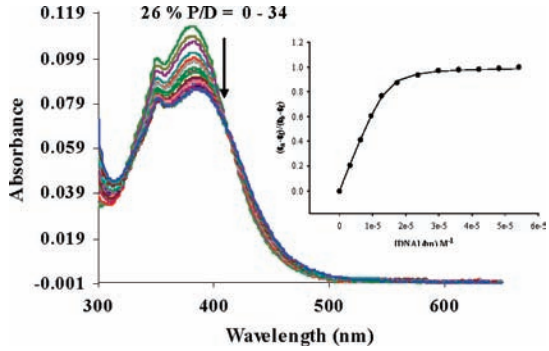


Figure 2. Changes in the absorption spectrum of **1** (8.7 μM) in 10 mM phosphate buffer (pH 7.4) and 160 mM NaCl with increasing concentration of *ct*-DNA (0−245 μM). Inset: Plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ vs DNA (M, bp) (•) and the best fit of the data (−) using the Bard equation[12] for **1**.

Using the method of Bard et al.,[17] the binding constants K_b were determined from these changes, which in the case of **1** confirmed a very strong binding affinity for DNA with $K_b = 7.40\ (\pm 0.2) \times 10^6\ M^{-1}$ in the absence of NaCl and $K_b = 0.61\ (\pm 0.3) \times 10^6\ M^{-1}$ in the presence of 160 mM NaCl. Similarly, strong binding was observed for **2** and **3** (Table 1). From these results, the order of binding affinity correlates well with the pK_a values determined above, where **3** binds the least efficiently to DNA within the physiological pH range. This was clear when **3** was titrated at pH 6.5, as significantly stronger binding was observed than that seen at pH 7.4, with $K_b = 0.80\ (\pm 0.08) \times 10^6\ M^{-1}$, reflecting the relationship between DNA binding and the effect of the pK_a of the side chains of **1**−**3**.

Table 1. Intrinsic Binding Constant K_b Determined from the Absorption (Abs) and the Fluorescence Emission (Flu) Spectra of **1**−**3** at pH 7.4 (10 mM Phosphate Buffer) at NaCl = 0, 50, and 160 mM[a,b]

K_b (10^6 M^{-1})	**1**	**2**	**3**
	0 mM		
Abs[a]	7.40 (±0.2)	4.24 (±0.3)	0.79 (±0.2)
Flu[b]	1.75 (±0.04)	2.56 (±0.09)	0.18 (±0.012)
	50 mM		
Abs[a]	3.00 (±0.3)	2.70 (±0.07)	−
Flu[b]	1.03 (±0.05)	0.96 (±0.03)	−
	160 mM		
Abs[a]	0.61 (±0.31)	0.50 (±0.1)	−
Flu[b]	0.72 (±0.03)	0.10 (±0.02)	−

[a] K_b obtained by the methods of Bard et al. [b] K_b obtained by the methods of McGhee and von Hippel.

The changes in fluorescence emission of **1**−**3** were also monitored at pH 7.4 at various NaCl concentrations. Figure 3 shows the changes in the emission spectra of **1** upon titration with *ct*-DNA at pH 7.4 in 160 mM NaCl solution and the normalized changes at 510 nm, in different ionic strengths. These results demonstrate that the singlet excited state was significantly affected upon binding to DNA, even in such competitive media, causing 86% quenching with a concomitant bathochromic shift of 28 nm in the presence of 160 mM NaCl. Analyzing these changes using the model of McGhee and von Hippel[18] gave $K_b = 1.75\ (\pm 0.04) \times 10^6\ M^{-1}$ with a binding size of $n = 1.70\ (\pm 0.03)$ at pH 7.4 and 0 mM NaCl, while at 150 mM NaCl $K_b = 0.72\ (\pm 0.03) \times 10^6\ M^{-1}$ was determined. See Table 1 for **2** and **3**. In contrast to these results, the changes in the naphthalimide precursors **4**−**6** were much less, being ca. 42% quenched for **4**, with a $K_b = 0.98\ (\pm 0.03) \times 10^6\ M^{-1}$, while a $K_b = 0.23\ (\pm 0.04) \times 10^6\ M^{-1}$ was determined for **5** at 0 mM NaCl. This clearly confirms that the incorporation of the Tröger's base moiety has a major influence on both the binding affinity and the photophysical properties of these naphthalimide systems in the presence of DNA. The strong affinity of **1**−**3** for DNA was further confirmed by carrying out Ethidium Bromide (EtBr) displacement assays[19] which showed full displacement of the dye from *ct*-DNA by **1**−**3**.

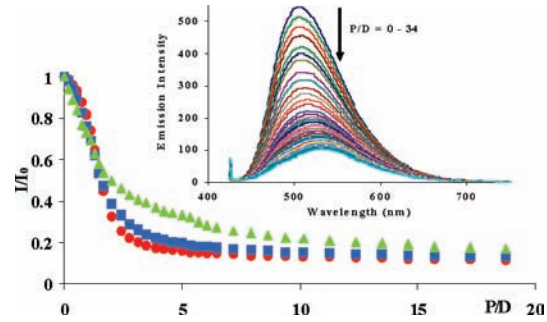


Figure 3. Plot of the changes in the emission spectra (λ_{ex} 415 nm) of **1** in the presence of *ct*-DNA in: (●) 0 mM (■) 50 mM and (▲) 160 mM NaCl. Inset: The changes in the fluorescence of **1** (8.7 μM) in 10 mM phosphate buffer, pH 7.4, and 160 mM NaCl solution with *ct*-DNA.

(14) (a) Ali, H. D. P.; Kruger, P. E.; Gunnlaugsson, T. *New. J. Chem.* **2008**, *32*, 1153. (b) Parkesh, R.; Lee, T. C.; Gunnlaugsson, T. *Org. Biomol. Chem.* **2007**, *5*, 310. (c) Duke, R. M.; Gunnlaugsson, T. *Tetrahedron Lett.* **2007**, *48*, 8043. (d) Gunnlaugsson, T.; Kruger, P. E.; Jensen, P.; Tierney, J.; Ali, H. D. P.; Hussey, G. M. *J. Org. Chem.* **2005**, *70*, 10875. (e) Gunnlaugsson, T.; Kruger, P. E.; Jensen, P.; Pfeffer, F. M.; Hussey, G. M. *Tetrahedron Lett.* **2003**, *35*, 8909. (f) Gunnlaugsson, T.; Kruger, P. E.; Lee, T. C.; Parkesh, R.; Pfeffer, F. M.; Hussey, G. M. *Tetrahedron Lett.* **2003**, *35*, 6575.

(15) de Silva, A. P.; Rice, T. E. *Chem. Commun.* **1996**, 163.

(16) Gao, Y. Q.; Marcus, R. A. *J. Phys. Chem. A* **2002**, *106*, 1956.

(17) Carter, M. T.; Rodriguez, M.; Bard, A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8901.

(18) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.

(19) Tse, W. C.; Boger, D. L. *Acc. Chem. Res.* **2004**, *37*, 61.

The striking difference between the displacement of **2** and **3**, vs that observed for the precursors **4** and **5**, is shown in Figure 4. From these, C_{50} values of 0.38 and 0.625 μM and apparent

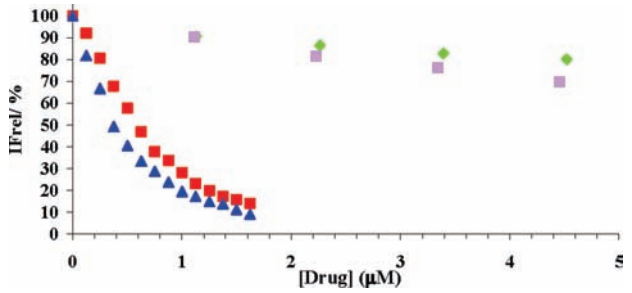


Figure 4. Rhanges in the fluorescence emission of EtBr upon competitive binding of the Tröger's bases 1 (▲) and 2 (red ■) and the naphthalimide precursors **4** (purple ■) and **5** (♦) to *ct*-DNA.

binding constant K_{app} of 3.4 and 2.4 $\times$ 10^7 M^{-1} were determined for **1** and **2**, respectively. In contrast, for **4** and **5**, significantly higher concentrations were needed to displace EtBr, with C_{50} values of 10 and 25 μM and K_{app} of 0.15 and 0.054 $\times$ 10^7 M^{-1} being determined for these precursors, respectively. Moreover, this strong binding was also observed using thermal denaturation studies, where **1** and **2** significantly stabilized the *ct*-DNA structure, with $\Delta T_m > 15$ °C, in contrast to that seen for **4** and **5**, respectively (Figure S8, Supporting Information).[20]

Having demonstrated strong DNA binding affinities for **1**−**3**, we next investigated the cellular uptake and the toxicity of **1**−**3** in a promyelocytic leukemia HL-60 cell line by using flow cytometry. Both **1** and **2** exhibit rapid cellular uptake, with 85% and 80% accumulation within 3 h, while **3**, showed the slowest uptake, reaching 85% accumulation after 6 h in HL-60 cells (Figures S10−11, Supporting Information). These results are in good agreement with that seen above, where at pH 7.4, both **1** and **2** are cationic, which decreases their lipophilicity and facilitates the transport across the cell membrane, as has been demonstrated by Qian et al.[21]

Bright field images of HL-60 cells, treated with **1**−**3**, also suggested that **1** and **2** were potentially more cytotoxic than **3** as cells treated with **1** and **2** were condensed and formed blebs at the surface of the cell membrane, while cells treated with **3** exhibited intact membrane integrity and a larger cytoplasm (Figure 5A). Furthermore, localization studies of **1**−**3** using confocal fluorescent microscopy confirmed that **1**−**3** crossed the cell membrane of HL-60 cells within 24 h incubation (Figure 5B). From Figure 5B, it is also clear that despite the fact that the naphthalimide emission was quenched upon binding to DNA these novel supramolecular scaffolds still give rise to significant green emission within the cellular environment. Furthermore, these images also indicated that **1**−**3** are mainly localized within the

(20) The DNA binding was also investigated for **1** and **2** using the homopolymer poly(dA-dT). See Figure S9, Supporting Information.

(21) Li, Z.; Yang, Q.; Qian, X. H. *Biorg. Med. Chem. Lett.* **2005**, *15*, 3143.

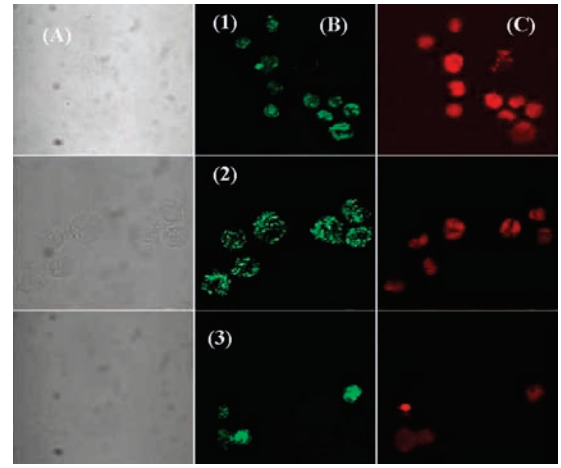


Figure 5. Cellular imaging pictures, taken after 24 h of **1**−**3** showing localization in the nucleus: (A) phase, (B) fluorescence arising from **1**−**3** (10 μM), (C) costained with propium iodide (see Figure S13, Supporting Information).

nucleus and to a lesser extent within the cytoplasm. This was further confirmed using the nuclear stain propidium iodide (Figure 5C).

The cytotoxic potential of **1**−**3**, after 24 h incubation using HL-60 cells, was determined using the MTT proliferation assay. From these results, the cytotoxic parameters, IC_{50}, TGI, and LD_{50}, were calculated (see Figure S12 and Tables S7 and S8, Supporting Information). Here, **1** and **2** induced a sharp decrease in cell viability after 24 h with LD_{50} values of 5.21 μM and 5.50 μM, respectively, which was considerably lower than seen for **4** and **5** (LD_{50} values of 27.7 μM and 80.9 μM, respectively). This further demonstrates the significance of the Tröger's base unit in these structures. Similar studies were also carried out using the cell line K562, which showed the same trend, where **1** was shown to be the most cytotoxic, with IC_{50} = 5.53 μM after 48 h incubation, while **2** gave IC_{50} = 17.0 μM after the same time. As predicted from the above DNA binding studies, **3** and **6** showed an apparent lack of toxicity.

In summary, we have developed novel supramolecular DNA-targeting motives using 4-amino-1,8-naphthalimide derived Tröger's bases. We showed that **1**−**3** bind DNA with high affinity and, by using confocal fluorescence imaging studies, demonstrated rapid cellular uptake of **1**−**3** and that these compounds localize within the nucleus. We also showed that various biological results correlate well with these DNA binding studies. We are in the process of further evaluating the photophysical and biological properties of **1**−**3** and related structures.

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Supporting Information Available: Synthesis and characterization of **1**−**7**, Figures S1−S12, and Tables S1−S8. This material is available free of charge via the Internet at http://pubs.acs.org.

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