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Synthesis, spectroscopic and DNA alkylating properties of malondialdehyde (MDA) *bis*-imine fluorescent adducts†

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The synthesis of a series of malondialdehyde (MDA) fluorescent adducts that mimic the well-known pentamethine cyanine dyes is reported. This new subclass of *bis*-imino dyes shares some common spectroscopic properties with their polymethine analogues although absorbing at significantly shorter wavelengths. A small library of trimethine and pentamethine cyanine dye *bis*-imino analogues have been synthesised and characterised that cover a spectral range from blue to orange. Of particular interest is their capacity to act as mono- and *bis*-alkylating agents of nucleosides in general and of cytidine (and 2′-deoxycytidine) in particular.

Introduction

DNA alkylating agents, which include nitrogen mustards, have been used extensively in cancer chemotherapy.[1] These agents can be either mono- or bi-functional. The former have a single reactive group and therefore react with a single nucleophilic centre of a unique nucleobase (most commonly guanine or adenine).[2] The latter have two reactive groups and are therefore capable of reacting with two different nucleobases belonging to either a unique or two different DNA strands, thus resulting in intrastrand and interstrand cross-links, respectively.[3] Interstrand cross-links prevent strand separation and hence constitute complete blocks to DNA replication and transcription.[4] For this reason, a number of *bis*-alkylating agents have been designed that show promising anticancer activity. These include aziridines,[5] nitrosoureas,[6] alkane sulfonates[7] or platinum derivatives.[8] A major drawback of this class of compounds is their high cytotoxicity as a consequence of their high chemical reactivity. Reaction of the drug with intracellular nucleophiles (*e.g.* glutathione, proteins,...) significantly lower their potential potency by preventing them from reaching their DNA target.[9]

There also exist a number of endogeneous metabolites, products of enzymatic reactions, that are responsible for DNA damage and mutation. For instance, numerous studies have shown that formaldehyde (FA) and acetaldehyde (AA) were genotoxic and mutagenic to mammalian cells, mainly *via* formation of DNA–protein cross-links in target tissues.[10,11] Malondialdehyde (MDA) is an endogenous product of lipid peroxidation and prostaglandin biosynthesis with mutagenic properties in bacteria and human cells.[12] Although MDA can also form reversible Schiff adducts with deoxyadenosine and deoxycytosine, the most abundant MDA-DNA adduct is the so-called M_1dG that is formed by reaction of MDA with the exocyclic amine N(2) of deoxyguanosines followed by cyclization onto the N(1) position (Fig. 1).[12] This adduct, previously found in healthy human tissues recently proved mutagenic in human cells with MDA being suspected to be involved in the formation of DNA interstrand cross-links. MDA is also a widely used marker of oxidative lipid injury whose concentration varies in response to biotic and abiotic plant stress.[13]

Herein, we report on the synthesis and characterisation of an original family of fluorescent and reversible MDA adducts as *bis*-imino analogues of symmetrical pentamethine cyanine dyes. Analogues of trimethine cyanine dyes were also synthesized following a similar strategy. Although absorbing and emitting at slightly shorter wavelengths than their parent all-methine cyanine dyes, those reversible dyes have potential as MDA fluorescent sensors. More important are their potential applications as alkylating and/or cross-linking agents. The synthesis, stability and spectroscopic and DNA binding properties of this novel subclass of dyes are described.

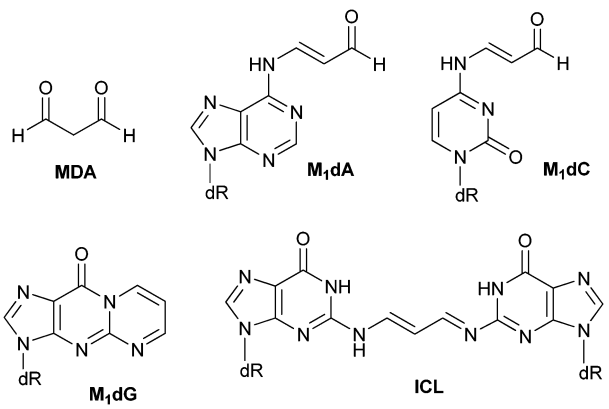

Fig. 1 Structures of MDA and of its mono- and *bis*-adducts with deoxyadenosine (M_1dA), deoxycytosine (M_1dC), deoxyguanosine (M_1dG). An example of interstrand cross-link (ICL) is also represented.

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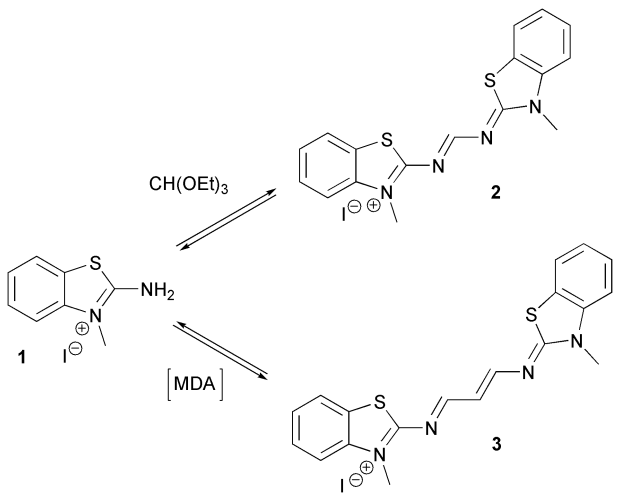
Results and discussion

Synthesis and spectroscopic properties of *bis*-imino analogues of symmetrical trimethine and pentamethine cyanine dyes

As a proof-of-concept experiment, firstly, analogues of trimethine and pentamethine cyanine dyes were synthesised by reaction of 1-methyl-2-aminobenzothiazolium iodide with triethyl orthoformate and 1,1,3,3-tetramethoxypropane (*i.e.* MDA precursor), respectively, using pyridine or acetic acid as a solvent (Scheme 1). Under such conditions, dyes **2** and **3** were isolated as pure solids by precipitation from the reaction mixture. They differ from commonly used symmetrical cyanine dyes by two C→N substitutions within the polymethine chain.

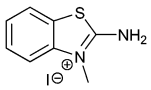
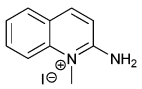
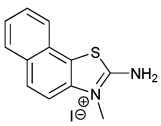
In order to demonstrate the versatility of our synthesis, a small number of *bis*-imino adducts was synthesized that cover a spectral range from blue to orange. They were obtained from reactions of triethylorthoformate or 1,1,3,3-tetramethoxypropane with either *N*-methyl-2-amino-quinolinium iodide[14] or *N*-methyl-2-amino-naphthathiazolium iodide.[15] The spectroscopic properties of the isolated dyes taken in DMSO are summarized in Table 1.

We recently reported the synthesis of unsymmetrical imino dyes from reaction between the aminobenzothiazolium derivative **1** and a Fisher's base aldehyde.[16] From this example, it appeared that the introduction of one nitrogen atom within the polymethine bridge of a cyanine dye was responsible for a loss of around 60–70 nm of the maximum absorption and emission wavelengths of the dye. Interestingly, the substitution of a second carbon atom by a nitrogen atom accounts for an additional 70 nm drop of the maximum absorption wavelength. While *bis*-imino dyes **2** and **3** absorb maximally at 412 and 525 nm, respectively, (Table 1) their parent trimethine and pentamethine symmetrical cyanine dyes have their maximum absorption wavelengths at 562 and 660 nm respectively. It is also noteworthy that both imino dyes **2** and **3** are characterised by moderately large Stoke's shifts of 47 and 34 nm, respectively. Comparable effects are observed when replacing the benzothiazole heterocycles by either quinolines or naphthothiazoles. Although *bis*-quinoline and *bis*-benzothiazole dyes absorb and emit at nearly identical wavelengths, dyes from the naphthobenzothiazole family offer the advantage of absorbing (and emitting) at significantly longer wavelengths (maximum absorption wavelengths of λ_{max} = 412 and 465 nm for dyes **2** and **6**, respectively).



Scheme 1 Synthetic route to fluorescent *bis*-imino adducts **2** and **3** as analogues of trimethine and pentamethine cyanine dye.

Table 1 Maximum absorption and emission wavelengths (taken in DMSO) of $CH(OEt)_3$ and MDA fluorescent adducts

Amine	Electrophile	*Bis*-imine dye	λ_{abs}/nm	λ_{em}/nm
	$CH(OEt)_3$	**2**	412	459
	MDA	**3**	525	559
	$CH(OEt)_3$	**4**	437	462
	MDA	**5**	530	560
	$CH(OEt)_3$	**6**	465	506
	MDA	**7**	557	602

Due to the high molar absorptivity and tunable optical properties of these original MDA adducts, we believe that this dynamic and versatile system could represent the basis for the highly sensitive detection of MDA in biological media. MDA is commonly used as a biomarker of oxidative stress with respect to lipid peroxidation in body fluids or cells and is often quantified *via* detection of the fluorescent adduct it forms with thiobarbituric acid.[17] Our strategy therefore represents a possible alternative to thiobarbituric acid that also offers the advantage of a much higher versatility with respect to the absorption and emission wavelengths of the adducts formed.

Stability of *bis*-imino cyanine dyes 2 and 3

Due to their reversible nature, the reactions of imine formation from amines and carbonyl derivatives have been widely used in dynamic combinatorial chemistry.[18] In order to assess the stability of these *bis*-imino dyes, the half-life values for compounds **2** and **3** in DMSO were determined by ^{1}H NMR and mass spectrometry. Because of their bright colour in solution, the degradation of both dyes could also be easily monitored by naked eye, looking at the slow disappearance of the bright yellow and bright red colours of solutions of **2** and **3**, respectively.

Bis-imine **2** proved significantly more stable than *bis*-imine **3** with a half-life of 36 h (*versus* 10 h for compound **3**) in DMSO. Interestingly, both dyes **2** and **3** were shown to undergo comparable mono-hydrolysis reactions in solution, leading to the formation of *N*-methyl-2-amino-benzothiazolium **1** and of imino-formamide **2a** and imino-aldehyde **3a**, respectively (Fig. 2 and 3). While appearance of formamide **2a** is characterised by a singlet at 8.96 ppm, formation of aldehyde **3a** is characterised by a doublet centred at 9.52 ppm. However, no traces of MDA[19] (resulting from a complete hydrolysis of compound **3**) were ever detectable even after 72 h, thus

demonstrating the relative stability of mono-imine **3a**. It is also noteworthy that, in the case of compound **3**, a third degradation product (in addition to compounds **1** and **3a**) was also detectable by ^{1}H NMR although we were not able to unambiguously determine its structure.

Malondialdehyde (MDA) *bis*-imine fluorescent adducts as DNA alkylating prodrugs

Exposure of double-stranded DNA to MDA results in the formation of covalent adducts which are responsible for high levels of mutations when the DNA is replicated. Interestingly, more than 90% of the MDA-induced mutations occur at GC base pairs, consistent with the preferential reaction of MDA with deoxyguanosines to form M_1dG adducts (Fig. 1). There is also evidence that MDA can form DNA–DNA interstrand cross-links (ICL), a critical DNA damage that interferes with essential aspects of cellular metabolism (*e.g.* DNA replication and transcription). While endogenous alkylating molecules like MDA contribute to carcinogenesis through mutagenesis, an increasing number of antitumor agents have been designed that form covalent adducts with DNA and that can also form, in some cases, ICLs. A significant drawback of this class of drugs is a consequence of their extremely high reactivity, hence responsible for a serious lack of specificity. For instance, non-specific reactions with thiol and/or amine nucleophiles in the cytoplasm are common examples of drug detoxification.[9]

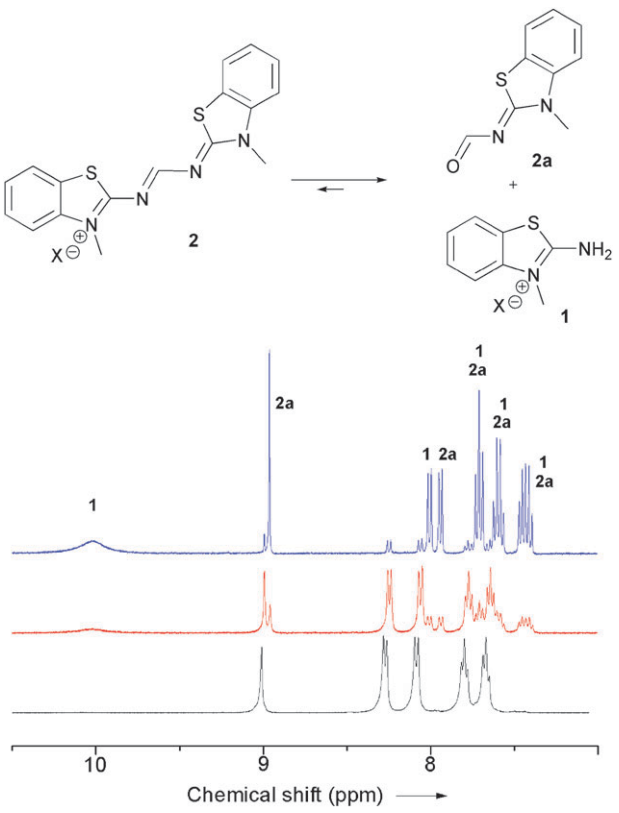


Fig. 2 ^{1}H NMR spectra of compound **2** (15 mM in d6-DMSO) recorded at t = 0 (black), 16 h (red) and 96 h (blue). After 4 days at rt, dye **2** is almost fully converted into amine **1** and aldehyde **2a**. Characteristic signals corresponding to imino aldehyde **2a** and amine **1** are highlighted. Half-life ($t_{1/2}$) of dye **2** was found to be 36 h.

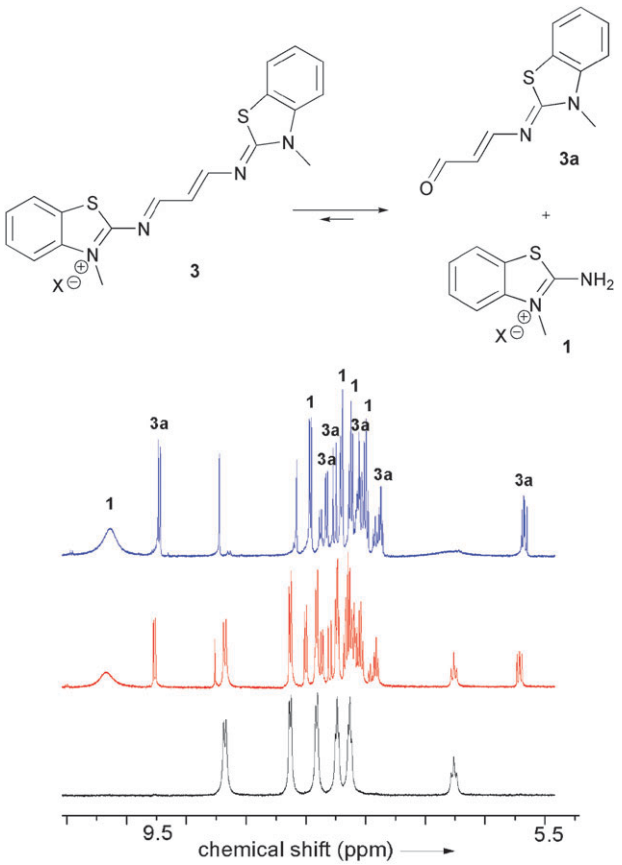


Fig. 3 ^{1}H NMR spectra of compound **3** (15 mM in d6-DMSO) recorded at rt at t = 0 (black), 12 h (red) and 36 h (blue). Characteristic signals corresponding to imino aldehyde **3a** and amine **1** are highlighted. Half-life ($t_{1/2}$) of dye **3** was found to be 10 h.

Successful strategies to render such molecules more selective for tumor cells include their conjugation with sequence-specific minor groove binders in order to direct the alkylating drug to the intended target site.[20] The use of prodrugs also proved to be an efficient alternative to increase either the lifetime or cell penetration of the DNA alkylator.[21]

Herein, we investigated whether our reversible cyanine dye analogues could act as DNA-alkylating prodrugs. The ability of pentamethine cyanine dyes to bind into the minor groove of double-stranded DNA is well documented in the literature.[22] Therefore we reasoned that our *bis*-imino dyes could serve as vectors to deliver MDA within the minor groove of B-DNA. DNA alkylation could then proceed either (i) *via* hydrolysis of the dye once bound to DNA, thus liberating MDA at the viscinity of its target, or (ii) *via* direct *trans*-imination reaction between the nucleophilic nucleobases (G, A or C) and the imino dye. In order to demonstrate the potential of our *bis*-imino dyes as DNA alkylating agents, a reaction between compound **3** and each of the four natural nucleosides (adenosine, guanosine, cytidine and thymidine) was monitored by ^{1}H NMR, UV spectroscopy and mass spectrometry in DMSO. Briefly, to a solution of dye **3** (10 mM) in DMSO was added 5 equivalents of one nucleoside (A, T, C or G) and the four reactions were monitored individually by LC-MS over 72 h at room temperature. A nucleoside-free solution of dye was also used

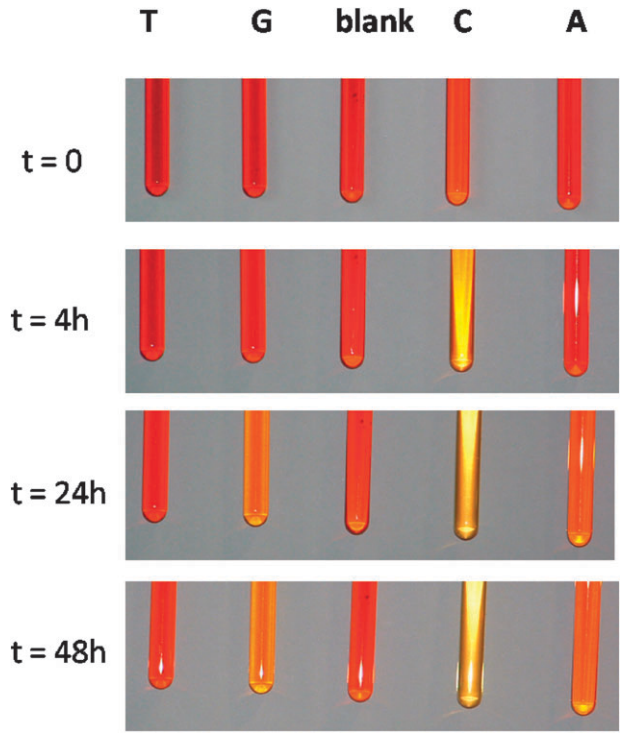


Fig. 4 Reaction between compound **3** (10 mM) and 5 equivalents of each of the four nucleosides (50 mM A, T, C or G) in DMSO. From left to right: thymidine, guanosine, no nucleoside, cytidine, adenosine. Reactions were monitored over 72 h at room temperature.

as a negative control. As anticipated, no reaction was ever observed with thymidine, the only nucleobase lacking an exocyclic primary amine. However, compound **3** proved capable of reacting covalently with the other three nucleosides, but with significantly different reactivities. Conveniently, the differences in reactivity of the four nucleobases could be easily monitored by naked eye, by looking at the solution's color change (Fig. 4).

Cytidine proved to be by far the most reactive nucleoside, inducing a red-to-yellow colour change within less than 4 h whilst a red-to-orange colour change appeared after 20–24 h only with either purine (A or G).

In order to further investigate the nature of the interaction between *bis*-imine **3** and nucleosides C, A and G, these three reactions were monitored by both NMR and LC-MS. This combined analysis allowed us to characterize the major covalent adducts formed upon reaction of dye **3** with each individual nucleoside.

Reaction of dye **3** with adenosine resulted in the slow and simultaneous appearance of the half-dye **3a** (resulting from the slow hydrolysis of *bis*-imine **3**) and of the adenosine-MDA covalent adduct (**M_1A**, Fig. 1). Both products were characterised by ^{1}H NMR, with the appearance of two new aldehyde signals, centred at 9.52 and 9.44 ppm, and corresponding to **3a** and **M_1A**, respectively. Formation of **M_1A** could potentially proceed *via* three different mechanisms: (i) complete hydrolysis of dye **3** into MDA and amine **1** followed by reaction of adenosine with the MDA generated *in situ*; (ii) *trans*-imination between dye **3** and adenosine followed by a mono-hydrolysis of either imine, leading either to the formation of **M_1A** or **3a**; (iii) partial hydrolysis of dye **3** into mono-imine **3a** and amine **1** followed by reaction between adenosine and **3a**. In a similar way, reaction of **3** with guanosine led to the slow formation of **M_1G** (Fig. 1).

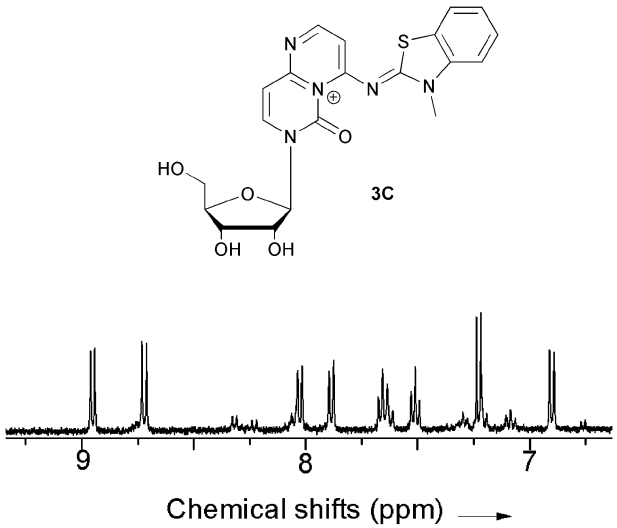


Fig. 5 Proposed structure of cytidine adduct **3C** (m/z = 442) and its ^{1}H NMR spectrum.

Interestingly, reaction between **3** and cytidine led to the formation of multiple covalent adducts which proved significantly different to those obtained from adenosine and guanosine. Among them, one drew our attention because of its surprisingly high maximum absorption wavelength (λ_{max} = 392 nm) and because of its fluorescent properties (λ_{em} = 472 nm). This adduct **3C** was isolated by HPLC and a proposed structure based on tandem NMR and mass spectrometry studies is proposed in Fig. 5.

It is noteworthy that a similar chemical behaviour was observed when reacting dye **3** with 2′-deoxycytidine (dC). Again, the 2′-deoxy analogue of **3C** formed (**3dC**, m/z = 426) which showed identical UV absorption and fluorescence emission spectra as **3C**. The formation of such adducts would demonstrate the *bis*-alkylating reactivity of *bis*-imino dye **3** towards cytosines. A similar adduct was recently reported by Richter *et al.* which resulted from the reaction of clerocidin with unpaired cytosines.[23] Although the possible formation of such fluorescent adducts under biological conditions and in the context of an RNA (*e.g.* **3C**) or a DNA (*e.g.* **3dC**) strand remains to be demonstrated, imino dyes of general structure **3** could potentially serve as cytosine-specific alkylating agents, the formation of DNA adducts being detectable by fluorescence spectroscopy. Unfortunately, and due to the very poor water-solubility of the *bis*-imino dyes **3**, **5** and **7**, it was not yet possible to assess the stability and reactivity of this class of compounds under near-physiological aqueous conditions.

Conclusions

We report herein the first family of symmetrical cyanine dye *bis*-imino analogues that differ from the well-known trimethine and pentamethine cyanine dyes by two C → N substitutions within the polymethine chain. These changes account for

(i) a 120–140 nm decrease in the maximum absorption and emission wavelengths of the dyes and (ii) a relative instability of the dyes in solution due to the intrinsic reversible nature of imines. Of particular interest are the *bis*-imino dyes, analogues of pentamethine cyanine dyes, which are formed from reaction between MDA and two molecules of amino-substituted nitrogen-containing heterocycles. This fluorogenic reaction could serve as a basis for a versatile (*e.g.* tunable absorption and emission wavelengths by varying the nature of the heterocycle) alternative to thiobarbituric acid for sensing MDA *in vitro* and maybe also *in vivo*. More interesting is the reactivity of *bis*-imino dyes of general structure **3** towards natural nucleobases in general and cytosines in particular. We have demonstrated that dye **3** reacted selectively with cytidine and 2′-deoxycytidine to form a stable fluorescent adduct. The possible application of these dyes for alkylating cytosines with the context of a DNA or an RNA strand is currently underway in our group.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400 and 100.6 MHz, respectively. Chemical shifts are reported as δ values (ppm) with reference to the residual solvent peaks. All reagents and solvents were obtained from commercial sources and used without further purification. Fluorescence emission spectra were recorded in quartz cells at 20 °C on a Jobin Yvon Fluorolog 3.22 instrument. The excitation and emission bandwidths were fixed to 3 and 3 nm, respectively. UV spectra were recorded on a V-670 UV-Visible spectrophotometer from JASCO in a 10 mm pathlength cuvette.

General procedure for the synthesis of *bis*-imino analogues of trimethine cyanine dyes

To a solution of *N*-methyl-2-amino-benzothiazolium, *N*-methyl-2-amino-quinolinium iodide or *N*-methyl-2-amino-naphthathiazolium iodide (3.5 mmol) in acetic acid (20 mL) was added triethylorthoacetate (2 mL) and the reaction mixture was stirred at 85 °C for 5 h. The solvent was then removed under reduced pressure and the residue was triturated with diethyl ether. The solid was collected by filtration under reduced pressure and washed with methanol (3 × 30 mL) and diethyl ether (3 × 30 mL). The desired *bis*-imino dyes were thus obtained pure as amorphous brown/yellow solids.

Compound 2 (yield 80%): ^{1}H NMR (400 MHz, DMSO): δ = 8.94 (s, 1H), 8.20 (d, J = 7.7 Hz, 2H), 8.00 (d, J = 8.2 Hz, 2H), 7.73 (t, J = 7.5 Hz, 2H), 7.60 (t, J = 7.5 Hz, 2H), 4.14 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 163.3, 138.0, 128.4 (× 3), 126.4 (× 3), 125.8, 123.8 (× 3), 115.0 (× 3), 34.0 (× 2) ppm. HR-MS ESI positive mode: m/z: calcd for $C_{17}H_{15}N_4S_2^+$ 339.073; Found 339.071. **Compound 4** (yield 20%): ^{1}H NMR (400 MHz, DMSO): δ = 9.26 (s, 1H), 8.62 (d, J = 9.4 Hz, 2H), 8.16–8.12 (m, 4H), 8.00–7.96 (m, 4H), 7.69 (t, J = 7.2 Hz, 2H), 4.24 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 164.7, 161.1 (× 2), 142.6 (× 2), 140.0 (× 2), 133.8 (× 2), 130.0 (× 2), 126.5 (× 2), 124.5 (× 2), 118.1 (× 2), 116.9 (× 2), 34.8 (× 2) ppm. HR-MS ESI positive mode: m/z: calcd for $C_{21}H_{19}N_4^+$ 327.160; found 327.158. **Compound 6** (yield 45%): ^{1}H NMR (400 MHz, DMSO): δ = 9.04 (s, 1H), 8.32 (d, J = 8.0 Hz, 2H), 8.27 (d, J = 8.0 Hz, 2H), 8.24–8.18 (m, 4H), 7.83 (t, J = 7.3 Hz, 2H), 7.74 (t, J = 7.5 Hz, 2H), 4.31 (s, 6H) ppm. HR-MS ESI positive mode: m/z: calcd for $C_{25}H_{19}N_4S_2^+$ 439.105; found 439.101.

General procedure for the synthesis of *bis*-imino analogues of pentamethine cyanine dyes

To a solution of *N*-methyl-2-amino-benzothiazolium, *N*-methyl-2-amino-quinolinium iodide or *N*-methyl-2-amino-naphthathiazolium iodide (3.5 mmol) in acetic acid (20 mL) was added triethylorthoacetate (2 mL) and the reaction mixture was stirred at 85 °C for 5 h. The solvent was then removed under reduced pressure and the residue was triturated with diethyl ether. The solid was collected by filtration under reduced pressure and washed with methanol (3 × 30 mL) and diethyl ether (3 × 30 mL). The desired *bis*-imino dyes were thus obtained pure as amorphous dark red solids.

Compound 3 (yield 50%): ^{1}H NMR (400 MHz, DMSO): δ = 8.81 (d, J = 10.8 Hz, 2H), 8.15 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.0 Hz, 2H), 7.68 (t, J = 7.8 Hz, 2H), 7.55 (t, J = 7.7 Hz, 2H), 6.51 (t, J = 10.9 Hz, 1H), 3.96 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 172.0, 171.9 (×2), 139.3 (× 2), 128.3 (× 2), 126.1 (× 2), 123.9 (× 2), 123.7 (× 3), 118.4, 114.5 (× 3), 33.2 (× 2) ppm. HR-MS ESI positive mode: m/z: calcd for $C_{19}H_{17}N_4S_2^+$ 365.089; found 365.085. **Compound 5** (yield 5%): ^{1}H NMR (400 MHz, DMSO): δ = 8.86 (d, J = 11.1 Hz, 2H), 8.58 (d, J = 7.9 Hz, 2H), 8.11–8.07 (m, 4H), 7.96 (t, J = 7.7 Hz, 2H), 7.72 (d, J = 9.2 Hz, 2H), 7.66 (t, J = 7.5 Hz, 2H), 6.43 (t, J = 10.8 Hz, 1H), 4.23 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 164.7, 159.2, 142.6 (× 2), 139.2 (× 2), 133.8 (× 3), 133.0 (× 3), 126.3 (× 2), 124.4 (× 2), 118.0 (× 3), 114.8 (× 2), 34.3 (× 2) ppm. HR-MS ESI positive mode: m/z: calcd for $C_{23}H_{21}N_4^+$ 353.176; found 353.173. **Compound 7** (yield 40%): ^{1}H NMR (400 MHz, DMSO): δ = 8.85 (d, J = 11.1 Hz, 2H), 8.24 (d, J = 8.9 Hz, 2H), 8.15 (d, J = 8.0 Hz, 2H), 8.08–8.03 (m, 4H), 7.78 (t, J = 7.5 Hz, 2H), 7.66 (t, J = 7.5 Hz, 2H), 6.56 (t, J = 11.1 Hz, 1H), 4.07 (s, 6H) ppm. HR-MS ESI positive mode: m/z: calcd for $C_{27}H_{21}N_4S_2^+$ 465.120; found 465.121.

General procedure for the synthesis of and isolation of the covalent adduct 3C (or 3dC)

To a solution of *bis*-imino dye **3** (5 mg, 0.01 mmol) in DMSO (1 mL) was added cytidine or 2′-deoxycytidine (5 equivalents, 0.05 mmol from Sigma) and the reaction mixture was shaken at room temperature for 24 h. The crude mixture was finally purified by HPLC to afford the fluorescent adduct **3C** (or **3dC**) pure as a yellow/orange oil. **Compound 3C**: HR-MS ESI positive mode: m/z: calcd for $C_{20}H_{20}N_5O_5S^+$ 442.118; found 442.119. **Compound 3dC**: HR-MS ESI positive mode: m/z: calcd for $C_{20}H_{20}N_5O_4S^+$ 426.123; found 426.125.

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15 2-Naphthylamine (1 g, 6.98 mmol) and sodium thiocyanate (1.13 g, 13.96 mmol) were dissolved in methanol (60 mL) at rt and the pale yellow/brown solution was cooled to −25 °C. Bromine (0.3 mL, 5.82 mmol) was addeddropwise and the reaction mixture was stirred below −10 °C for 2 h. The pink suspension was then warmed up to rt and 400 mL of water was added. The solid was then collected by filtration under vacuum, washed with water and dried under vacuum. To this pale yellow solid were added ethanol (40 mL) and 1.5 M HCl (50 mL) and the suspension was refluxed for 2 h. The hot suspension was then filtered under vacuum and the filtrate was neutralized with K_2CO_3 at 0 °C until reaching pH 7–8. The aqueous solution was then extracted with CH_2Cl_2 (3 × 30 mL), the organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to giving the desired naphtha-[2,1-*d*]thiazol-2-amine as a brown powder (700 mg, 60%). To a solution of naphtha[2,1-*d*]thiazol-2-amine (700 mg, 3.49 mmol) in a mixture of acetonitrile (15 mL) and methanol (8 mL) was added iodomethane (2 mL) and the reaction mixture was stirred for 1 h at 45 °C. After evaporation under vacuum, the residue was triturated in acetonitrile. The solid was then filtered off solution, and was washed 3 times with hot acetonitrile and then with diethyl ether. The desired *N*-methyl-2-amino-naphthathiazolium iodide was thus obtained pure as a yellow solid (901 mg, 75%). NMR ^{1}H (DMSO) δ = 10.14 (s broad, 2H); 8.21 (d, J = 8.1 Hz, 1H); 8.15 (d, J = 8.1 Hz, 1H); 8.07 (d, J = 8.1 Hz, 1H); 7.93 (d, J = 8.9 Hz, 1H); 7.75 (t, J = 8.1 Hz, 1H); 7.66 (t, J = 7.9 Hz, 1H); 3.86 (s,3H). NMR ^{13}C (DMSO) δ = 167.4, 136.6, 130.2, 129.1, 128.9, 128.4, 126.4, 123.5, 123.0, 117.8, 113.4, 32.7. HR-MS ESI positive mode: m/z: calcd for $C_{12}H_{11}N_2S^+$ 215.066; found 215.066.

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