Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: C. De Schutter, V. Roy, P. Favetta , C. Pavageau, S. Maisonneuve, N. Bogliotti, J. Xie and L. A. Agrofoglio, *Org. Biomol. Chem.*, 2018, DOI: 10.1039/C8OB01606B.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C8OB01606B

Journal Name

ARTICLE

Synthesis and Characterization of various 5'-Dye-labeled Ribonucleosides

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Coralie De Schutter,[a] Vincent Roy,[a] Patrick Favetta,[a] Corentin Pavageau,[b] Stéphane Maisonneuve,[b] Nicolas Bogliotti,[b] Juan Xie[b] and Luigi A. Agrofoglio*[a]

Hitherto *unknown* chromophoric nucleosides are reported. This novel set of visibly coloured dye-labeled 5'-nucleosides including 1,2,4,5-tetrazine, dicyanomethylene-4H-pyran, benzophenoxazinone, 9,10-anthraquinone and azobenzene chromophores, were prepared mainly under Cu-catalyzed azide-alkyne cycloaddition (CuAAC). Design criteria are outlined. Several derivatives possess in supplement a fluorescence property The absorption and fluorescence spectra of all coloured nucleosides were recorded to study their potential as visible-range probes. Such nucleodyes are of great interest for future competitive lateral flow test MIP-based strips.

Introduction

Nucleosides are structural subunits of nucleic acids and the biochemical precursors of nucleotides, which play an important role in cell metabolism. Their analogues are largely used to combat cancer and viral infections and some nucleoside metabolites of RNA, are well known potential biomarkers of cancer.[1] Labeled nucleos(t)ides using fluorescence polarization are critical elements for various applications,[2] including study of nucleic acid functions and structure, sequence detection, nucleoside and nucleotide cellular interaction, nucleotide-binding proteins or for competitive nucleotide binding assay. The first fluorescent nucleotide, reported by Hiratsuka and Uchida,[3] was the 2',3'-*O*-(2,4,6-trinitrocyclohexadienylidene) adenosine 5'-triphosphate. The site of incorporation of the dye on the nucleoside is chosen in order to minimize the effect on the structures of the corresponding oligonucleotides. Thus, the dye is generally introduced by conjugation through linkers to the sugar hydroxyl groups[4] or more generally to the nucleobase[5] at the C5 position of pyrimidines or C8 position of purines.[4]

Recently we have reported the quantitative detection of urinary modified nucleoside biomarkers by new sensors based on molecularly imprinted polymer (MIP).[6-8] In order to explore competitive lateral flow test MIP-based strips[9] (Figure S1), a rapid growing strategies for qualitative and quantitative analysis, the development of 5'-dye-labeled nucleosides is now mandatory.

If fluorescent nucleosides have been largely developed, chromophoric nucleoside analogues have been less explored[10] (and only for azo dye) but offer often advantage of a high degree of stability under most application conditions. In the current study, we have prepared several *hitherto unknown* uridine and adenosine dye analogues and we turn our attention to visibly coloured nucleoside probes with intense dyes such as 1,2,4,5-tetrazine (or *s*-tetrazine), dicyanomethylene-4H-pyran (DCM), benzophenoxazinone, 9,10-anthraquinone and azobenzene. The 5'-position of nucleoside analogues was chosen for the introduction of the dye. Their photophysical features were characterized.

Results and Discussion

Synthesis of 5'-dye labelled nucleoside

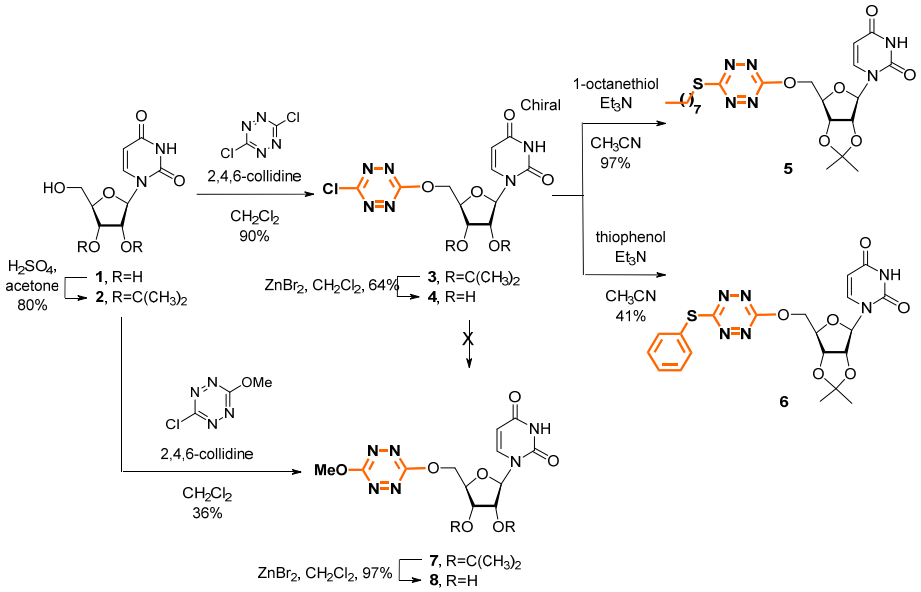
First of all, we turned our attention to the introduction of various chromophoric 1,2,4,5-tetrazines at C5' of uridine in order to obtain coloured uridines ranging from purple to orange to red. According to the nature of the *s*-tetrazine substituent, fluorescence properties can also be displayed.[11] All *s*-tetrazine derivatives were prepared starting from commercially available uridine (**1**), which, after protection of the 2'-and 3'-hydroxyls by *isopropylidene* group (to **2**), was reacted with 3,6-dichloro-*s*-tetrazine[12] in presence of 2,4,6-collidine as a non-nucleophilic base (ratio **2**/*s*-tetrazine/2,4,6-collidine = 1/1/1) . The monosubstituted derivative **3** was obtained in high yield (90%). Removal of the *iso*propylidene moiety by a dilute solution of 0.5 M hydrochloric acid in methanol at 55°C for 3h[13] unfortunately led also to the cleavage of the 6-chloro-*s*-tetrazine moiety. The C-O bond between the uridine- and tetrazine-moiety was similarly cleaved when the reaction was carried out in HCl 0.5 M in dioxane at room temperature, and with 10% acetic acid at 90°C for 10 min, degradation products were observed.[14] We thus explored some Lewis acid reagents which have been fairly successful in selective removing *iso*propylidene ketal in the presence of other acid-sensitive moieties.

[a] Univ. Orléans, CNRS, ICOA, UMR 7311, F-45067 Orléans, France
E-mail : luigi.agrofoglio@univ-orleans.fr
[b] PPSM, ENS Paris-Saclay, CNRS, Univ. Paris-Saclay, F-94253 Cachan, France
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8OB01606B

Journal Name

ARTICLE



Scheme 1. Synthesis of nucleosides bearing 1,2,4,5-tetrazine moiety

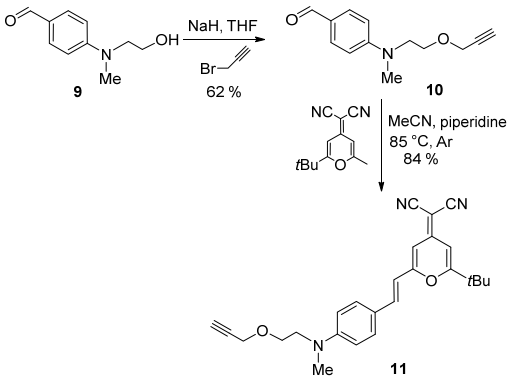
The obtained *s*-tetrazine-uridine **3** was treated, under mild aprotic condition, with a large excess of anhydrous $ZnBr_2$ (8 equiv.) in dichloromethane at room temperature. [15] After 21h stirring, the deprotected *s*-tetrazine-uridine **4** was obtained in 64% yield. Substitution of the remaining chlorine atom of the *s*-tetrazine-uridine **3** by alkyl- or aromatic-thiol proceeded readily in presence of triethylamine in acetonitrile to give thioethers **5** and **6**, respectively.[16] Unfortunately, all our attempts to selectively remove the *iso*propylidene group, using either the previous Lewis acid conditions, a Dowex-H^+ resin (50Wx8-100) in methanol at 40°C for 16h.[17] or deionized water under microwave irradiation (10 min, 120°C),[18] failed. We observed either no reaction either the released of the substituted *s*-tetrazine-moiety.

A second nucleophilic substitution of chorine atom of **3** by sodium methoxide didn't lead to compound **7** but the starting material was recovered. As reported by Clavier and Audebert,[12] such unsymmetrical disubstitution of *s*-tetrazines is often achieved with poor to moderate yields (25% average) and required the use of an alcoolate. Thus, using the previous conditions, 3,6-dichloro-*s*-tetrazine was treated with sodium methoxide to afford 3-chloro-6-methoxy-s-tetrazine in a 71% yield.[19] Under initial conditions (ratio **2**/*s*-tetrazine/2,4,6-collidine = 1/1/1), the desired *s*-tetrazine **7** was isolated after 32h stirring in 3% yields. After an optimization (ratio **2**/*s*-tetrazine/2,4,6-collidine = 1/2/4), the *s*-tetrazine-uridine **7** was obtained in 36% yield. Finally, deprotection in presence of $ZnBr_2$ led to 5'-*O*-(6-methoxy-1,2,4,5-tetrazin-3-yl)-uridine (**8**) with a good yield.

We faced similar difficulties to obtain other asymmetric disubstituted *s*-tetrazines and we failed to obtain the 3-morpholino-6-uridine-*s*-tetrazine. Thus, in order to circumvent these difficulties and to obtain desired 5'-dye-labeled ribonucleosides, we focused our attention on the Huisgen 1,3-dipolar cycloaddition between 5'-azido-uridine derivative **17**[20] and various alkynes **11**, **15** and **16,** bearing a various chromophoric group.

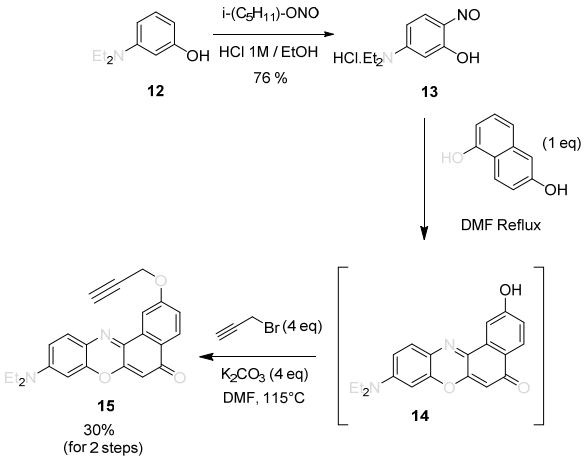
(i) The DCM fluorophore **11**, an analogue of 4-dicyanomethylene-2-*t*-butyl-6-methyl-4H-pyran, can be readily prepared from the commercially available aldehyde **9**, by *O*-propargylation to **10**, followed by Knoevenagel condensation with the *tert*-butyl-DCM (Scheme 2).

View Article Online
DOI: 10.1039/C8OB01606B



Scheme 2. Synthesis of alkyne-functionalized DCM **11**

(ii) The benzophenoxazinone **15**[21a,b] was obtained from the 2-(diethylamino)-phenol (**12**) through an optimized one-pot procedure. Compound **12** was first converted into its nitroso derivative **13** with amyl nitrite, the latter being reacted with 1,6-dihydroxynaphtalene at refluxed DMF[21c] and submitted to a Williamson ether synthesis to its propargyl ether analogue (Scheme 3).

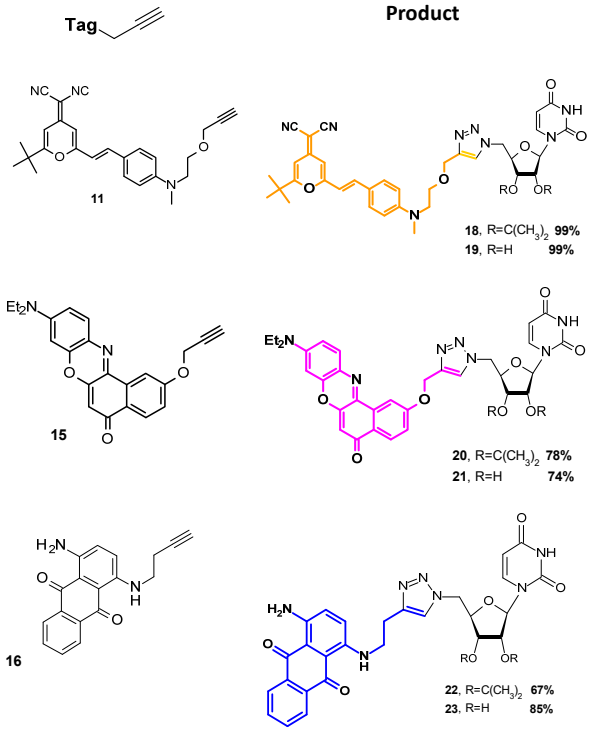


Scheme 3. Synthesis of alkyne-functionalized benzophenoxazinone **15**

(iii) The 1-amino-4-(but-3-ynylamino)anthracene-9,10-dione (Tag **16**) was easily obtained from a solution of commercially available 1,4-diaminoanthraquinone and K_2CO_3 in ACN with 4-bromobutyne.

Copper-catalyzed azide-alkyne Huisgen cycloaddition (CuAAC) between those coloured tags **11**, **15** and **16** and 5'-azido-5'-deoxy-2',3'-*O*-*iso*propylidene-uridine was then explored, (Scheme 4). No reaction was observed in a *t*BuOH/H_2O (8:2) mixture at 28°C for 16h.[22] An increase of CuSO4 (from 5 to 10 mol%), of sodium ascorbate (from 10 to 20 mol%), and in DMSO at 60°C led to the desired compounds **18** (98% yield) and **20** (74% yield), respectively. Similar protected uridines **18**, **20** and **22,** respectively, were better obtained in high yields after 3 minutes under microwave irradiation in presence of copper(I) sulfate catalyst and sodium ascorbate.

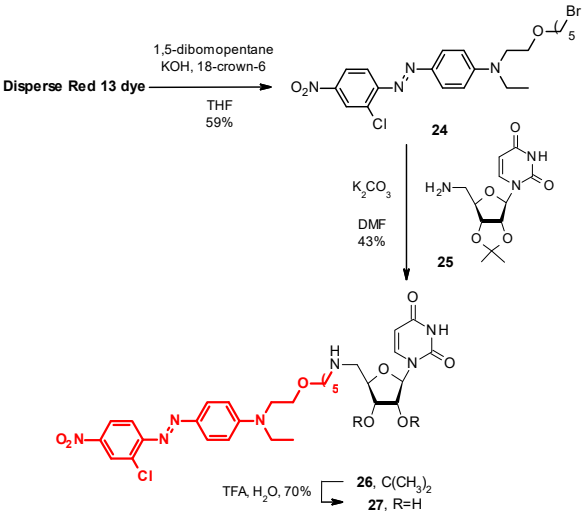
Compounds **18**, **20** and **22** were then dissolved in a 90% TFA in water mixture and stirred at room temperature for 10 min to afford the corresponding deprotected tagged-nucleosides dicyanomethylene-4*H*-pyran **19**, naphthooxazine **21** and 9,10-anthracenedione **23**, respectively, in high yields. This method can be applied to a large range of coloured tag with other 5'-azido-5'-deoxy nucleosides (Scheme 4).



Scheme 4. Synthesis of various coloured nucleosides through click chemistry

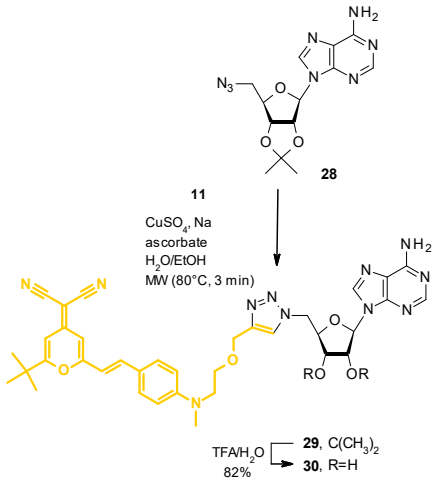
The synthesis of azo dye Disperse Red 13 uridine derivative **27** started from commercially available Disperse Red 13 which has a deep dark red color. We first tried the direct substitution of 2',3'-*O*-*iso*propylidene-5'-*O*-toluenesulfonyluridine by Disperse red 13 in presence of a large excess of sodium hydride in THF. Unfortunately, the degradation of the reagents was observed; likewise, the nucleophilic substitution, under these conditions, of 4-bromobut-1-yne (for CuAAC) with the OH of this tag failed. The difficulties met to introduce the alkyne group on the red-tag led us to investigate another pathway than the [3+2] cycloaddition reaction. The introduction of the azo dye at 5'-OH of uridine was thought to be easily done through a nucleophilic substitution of a halo azo dye. Thus, the hydroxyl group of Disperse Red 13 dye was reacted with 1,5-dibromopentane in presence of potassium hydroxide and 18-crown-6 to afford the bromo derivative **24**[23] in 59% yields, (Scheme 5). The nucleophilic displacement of bromine of **24** by the 5'-hydroxy group of protected uridine **2** with sodium hydride or potassium hydroxide failed. Based on the work reported by Yao,[24] compound **26** was obtained by reacting the 5'-amino-5'-deoxy-2',3'-*O*-*iso*propylideneuridine (**25**) with azo dye **24** using potassium carbonate in DMF with a moderate yield. *Iso*propylidene deprotection was performed in presence of TFA in water to afford the red azo-uridine derivative **27** in 70% yields.

View Article Online
DOI: 10.1039/C8OB01606B



Scheme 5. Synthesis of red azo dye uridine derivative **27**

Finally, yellow-coloured adenosine nucleoside **30** was prepared by CuAAC of 5'-azido-5'-deoxy-2',3'-*O*-*iso*propylideneadenosine **28**,[25] with the alkyne yellow-**11**, under microwave irradiation to **29** in 81% yields, and subsequent deprotection of the sugar moiety by TFA, (Scheme 6).



Scheme 6. Synthesis of yellow adenine derivative

Photophysical Properties

The absorption and fluorescence spectra of all coloured nucleosides were recorded in acetonitrile unless otherwise stated. The absorption and fluorescence (when relevant) spectra of the *s*-tetrazine derivatives **3** to **8** are displayed in Figure 1 and the data are collected in Table 1. All absorption spectra of compounds **3** to **8** showed an important signal around 260 nm (λ_3). The absence of this band in the 3,6-dichloro-*s*-tetrazine (green line) spectrum clearly indicates that this signal belong to the uridine moiety (black line) of the compounds. The *s*-tetrazine moiety presents two smaller bands (λ_1 and λ_2), which are approximately at 350 and 515 nm. The introduction of the uridine group on the 3,6-dichloro-*s*-tetrazine increased only slightly λ_1 (501 to 509 nm, entries 1 and 2) but the change was more significant on λ_2 (303 to 323 nm, entries 1 and 2). The same pattern is observed when the chlorine of **3** is replaced by a methoxy group (entry 6; Figure 1, purple line). This bathochromic effect was more pronounced when chlorine is replaced by a thioether moiety (entries 4 and 5; Figure 1, blue lines). This shifting allowed the *s*-tetrazine nucleosides to range from pink to orange as illustrated in Figure 2 (a) and (b). This range of colour could be very useful in the case where the coloured derivatives are immobilized on a urinary strip used in the semi-quantitative analysis of nucleoside biomarkers by displacement method. No modification of the three wavelengths has been observed after deprotection of the sugar ring even when ethanol was used for *s*-tetrazine **4** to solve solubility problems. This bathochromic shift was still present for the emission maxima of **3** and **7** (or **4** and **8**) but weaker ($\Delta\lambda^{em} \approx 10$ nm). Thus, both compounds appeared yellow under UV-light (Figure 2, c). The major difference between these two *s*-tetrazines was the significant drop of fluorescence, to its complete disappearance for the thioethers *s*-tetrazines **5** and **6**.

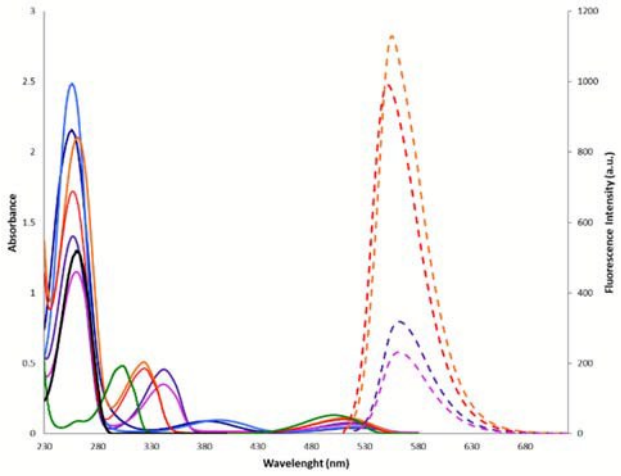


Figure 1. Absorbance (solid line) and Fluorescence (dotted line) spectra for *s*-tetrazine compounds in CH_3CN, except compound 4 in EtOH, at 0.1 mM: uridine **1** (black), 3,6-dichloro-*s*-tetrazine (green), **3** (red), **4** (orange), **5** (blue), **6** (dark blue), **7** (purple) and **8** (pink).

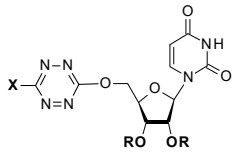
These observations were correlated with the results obtained by Audebert, Clavier and co-workers with non-nucleosides-s-tetrazines.[13] Modifications of the para positions of the tetrazine from Cl-Cl (3,6-dichloro-s-tetrazine) to Ur-Cl (**3**, **4**), or Ur-OR (**7**, **8**), or Ur-SR (**5**, **6**) results in a bathochromic shift for the λ¬2 absorption wavelength (303 to 393 nm) thus changing the colour from orange to pink. In addition of colours from *s*-tetrazine derivatives, the visible colours panel available was increased with tags **11**, **13**, **15** and **16**, from blue to red, passing by yellow-orange and pink. For example, the colours given by the dicyanomethylene-4H-pyran derivative **18** and naphthooxazine derivative **20**, were showed in Figure 3 (a and c). These both tagged-uridines possessed also a high intensity emission fluorescence (Figure 3, b and c). This property could help to decrease the detection limit of the urinary nucleosides by strip type sensors coupled to spectrofluorometric reading.

View Article Online
DOI: 10.1039/C8OB01606B

Journal Name

ARTICLE

Table 1: Absorption and fluorescence maxima for *s*-tetrazine derivatives recorded in CH_3CN



	Substituent		Compd	UV/Vis absorption				Fluorescence	
	X	R		λ_1^{abs} (nm)	λ_2^{abs} (nm)	λ_3^{abs} (nm)	ε ($L.mol^{-1}.cm^{-1}$)[a]	λ^{em} [b] (nm)	ϕ_f
1	3,6-dichloro-*s*-tetrazine			501	303	_	_	551; 567[c]	0.14[c]
2	Cl	$C(CH_3)_2$	**3**	509	323	256	569	551	0.15
3	Cl	H	**4** [d]	512	323	261	320	555	0.17
4	$S(CH_2)_7CH_3$	$C(CH_3)_2$	**5**	521	393	256	486	_	_
5	SPh	$C(CH_3)_2$	**6**	522	382	256	430	_	_
6	OCH_3	$C(CH_3)_2$	**7**	519	342	256	492	562	0.10
7	OCH_3	H	**8**	518	342	260	219	562	0.08

[a] molar absorption coefficient determined at λ_1^{abs}. [b] λ^{ex} = 509 nm for **3** and **4**; 519 nm for **7** and **8**. [c] From ref.[13a] [d] UV/Vis absorption and fluorescence spectra were recorded in EtOH.

The photophysical properties evaluation of the other chromophore substituents is listed in Table 2. For a clearer reading, the absorbance intensities of tagged-nucleosides and the emission intensities of fluorescent tagged-nucleosides were plotted according wavelength in **Error! Reference source not found.**2, and in **Error! Reference source not found.**, respectively. The absorbance capacities of the *s*-tetrazines chromophore tags were lower than the other ones.

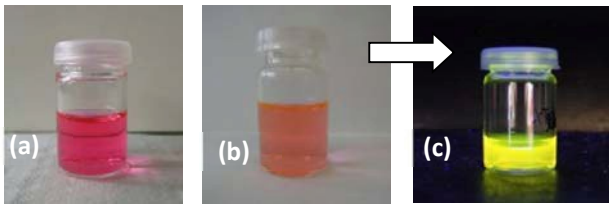


Figure 2. Pictures of two *s*-tetrazine compounds at 0.2 mM in CH_3CN. (a) **7**, (b) **3**, (c) **3** under 365 nm UV-light

Table 2: Absorption and fluorescence maxima recorded in CH_3CN

	Product	UV/Vis absorption					Fluorescence[a]	
		λ_1^{abs} (nm)	λ_2^{abs} (nm)	λ_3^{abs} (nm)	λ_4^{abs} (nm)	ε ($L.mol^{-1}.cm^{-1}$)[b]	λ^{em} (nm)	ϕ_f
1	**11**	465	372	352	290[c]	$3.95.10^4$	597	0.13
2	UrPrBV **18**	466	373	352	262	$3.03.10^4$	599	0.14
3	UrBVOH **19**	466	373	352	265	$3.31.10^4$	599	0.14
4	**15**	535	266	_	_	$3.04.10^4$	607	0.24
5	UrPrCP **20**	535	264	_	_	$3.35.10^4$	607	0.24
6	UrCPOH **21**	535	265	_	_	$2.46.10^4$	607	0.24
7	**16**	608	566	250	_	$1.19.10^4$	_	_
8	UrPrAQ **22** [d]	611	568	251	_	$1.28.10^4$	_	_
9	UrAQOH **23** [d, e]	614	570	253	_	$0.82.10^4$	_	_
10	**24**	507	288	_	_	$3.54.10^4$	_	_
11	UrPrRed **26**	507	288[f]	261	_	$2.85.10^4$	_	_
12	UrRedOH **27**	507	288[f]	263	_	$2.09.10^4$	_	_
13	AdPrBV **29**	467	373	352	262	$3.80.10^4$	599	0.13
14	AdBVOH **30**	466	373	352	262	$3.12.10^4$	599	0.14

View Article Online
DOI: 10.1039/C8OB01606B

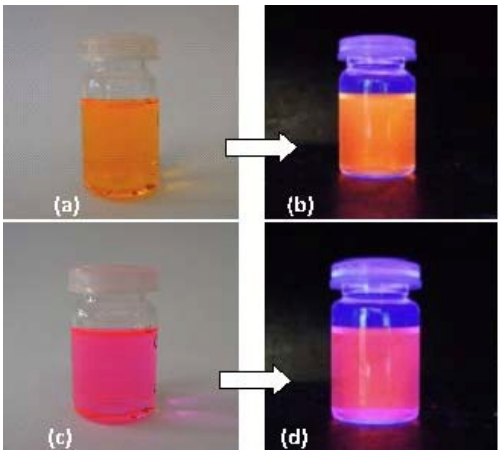


Figure 3. Picture of compounds: (a) **18** and (c) **20** at 0.05 mM in ACN; (b) **18** and (d) **20** at 0.05 mM in ACN under 365 nm UV-light.

Conclusion

We present herein hitherto unknown visibly coloured chromophoric nucleoside analogues in which the dye has been introduced at the 5' position of the sugar moiety. Absorption and fluorescence spectra (when relevant) of all coloured nucleosides are presented to study their potential as visible-range probes. The complementarity of these coloured derivatives of nucleoside, possibly being a urinary modified nucleoside biomarker of human cancer, and a selective recognition strip would open the road to user-friendly bedside sensors.

Experimental

Materials and methods

Commercially available chemicals were of reagent grade and used as received. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60F254, E. Merck). Compounds were visualized by UV irradiation. Column chromatography was performed on Silica Gel 60 M (40-63 µm, E. Merck). ^{1}H and ^{13}C NMR spectra were recorded at 250 nm (^{13}C, 62.9 MHz) or at 400 nm (^{13}C, 100.62 MHz). Chemical shifts are given in parts per million using tetramethylsilane (TMS) as internal standard. Coupling constants (J) are reported in Hertz (Hz) and multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad signal) and m (multiplet). High Resolution Mass spectra were performed on a Bruker maxis mass spectrometer by the "Fédération de Recherche" ICOA/CBM (FR2708) platform. Melting point was performed only after recrystallization in adequate solvent. All reactions under microwave irradiation were performed using the Microwave Biotage Initiator in 2–5 mL sealed tubes

Jasco FP-8200 Fluorescence Spectrometer (Jasco Corporation, Lisses, France) equipped with a 150 W xenon lamp was utilized for all fluorescence measurements. The bandwidths for excitation and emission spectra were adjusted at 5.0 nm. Scan speed, data interval and response were 200 nm/min, 0.5 nm and 0.5 sec, respectively. SpectraManager software was used for changing the format of the recorded spectra to ASCII. For all spectroscopic measurements a 1 cm four-sided Hellma quartz cuvette was used.

Absorption spectra were measured on a Shimadzu UV-1800 UV-Vis spectrophotometer (Shimadzu France, Noisiel) with 1 nm resolution and corrected for the blank with the second cell containing only solution solvent. Hellma® absorption cuvettes, pathlength 10 mm, were used for all absorption spectra.

Here, the quantum yield was measured by relative method, where the relative quantum yield of synthesized molecules was calculated according to a standard fluorophore with a well-known quantum yield. So, standards fluorophores were fluorescein in ethanol (ϕ = 0.79) and rhodamine B in ethanol (f = 0.5) using dilute sample solutions using the following equation:

$$\phi_x = \left(\frac{I_x}{I_{ref}}\right) \cdot \left(\frac{A_{ref}}{A_x}\right) \cdot \left(\frac{n_x}{n_{ref}}\right) \cdot \left(\frac{D_x}{D_{ref}}\right) \cdot \phi_{ref}$$

Where f, I, A, n and D stand for quantum yield, integrated emission intensity, absorbance at l_{exc}, refractive index of solvent, and dilution ratio, respectively. Sample and reference are denoted by x and *ref*, respectively. The λ_{exc} is in a very close proximity for the sample and reference solutions to circumvent correction of the difference in excitation energy at different wavelengths.

Spectroscopic grade solvents were obtained from Sigma Aldrich, Saint Quentin Fallavier, France. Aqueous samples were prepared with ultra-high pure water from Elga apparatus. The spectroscopic samples were prepared from concentrated acetonitrile or Ethanol stock solutions, hence, all samples contain 200 µM.

5'-*O*-(6-Chloro-1,2,4,5-tetrazin-3-yl)-2',3'-*O*-*iso*propylideneuridine (3). To a mixture of 2',3'-*O*-*iso*propylideneuridine (100 mg, 0.35 mmol) and 3,6-dichloro-*s*-tetrazine (58 mg, 0.39 mmol) in CH_2Cl_2 (10 mL) was introduced dropwise over 30 min 2,4,6-collidine (50 µL, 0.35 mmol). After 16 h stirring at RT, the mixture was washed with water, brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexanes/AcOEt 2:8) to obtain **3** (119 mg, 90%) as an orange-pink oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H, NH), 7.28 (d, *J* = 8.0 Hz, 1H, H-6), 5.72 (d, *J* = 8.0 Hz, 1H, H-5), 5.62 (s, 1H, H-1'), 5.12 (d, *J* = 6.2 Hz, 1H, H-2'), 5.04 (dd, *J* = 6.2, 4.2 Hz, 1H, H-3'), 4.92 (dd, *J* = 11.5, 6.5 Hz, 1H, H-5'a), 4.87 (dd, *J* = 11.5, 4.0 Hz, 1H, H-5'b), 4.62 – 4.58 (m, 1H, H-4'), 1.57 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (C-Cl), 164.7 (C-O), 163.6 (C-4), 150.4 (C-2), 143.2 (C-6), 114.8 ($C(CH_3)_2$), 103.0 (C-5), 96.2 (C-1'), 85.7 (C-4'), 84.5 (C-2'), 81.4 (C-3'), 70.1 (C-5'), 27.2 ($C(CH_3)_2$), 25.3 ($C(CH_3)_2$). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{14}H_{16}ClN_6O_6$ 399.0814, found 399.0812.

5'-*O*-(6-Chloro-1,2,4,5-tetrazin-3-yl)-uridine (4). Anhydrous $ZnBr_2$ (250 mg, 1 mmol) was added to a solution of compound **3** (100 mg, 0.25 mmol) in CH_2Cl_2 (4 mL). Another batch of anhydrous $ZnBr_2$ (250 mg) was introduced again after 5 h stirring at room temperature and then the mixture was stirred overnight. The reaction was quenched with a saturated solution of EDTA.Na_2. The residual starting material was removed by extraction with CH_2Cl_2 and then the unprotected uridine derivative was extracted from the aqueous layer with AcOEt

View Article Online
DOI: 10.1039/C8OB01606B

(5 x 10 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. After a quick purification by flash column chromatography (AcOEt), *s*-tetrazine **4** (58 mg, 64%) was obtained as an orange-pink oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (d, *J* = 2.2 Hz, 1H, NH), 7.72 (d, *J* = 8.1 Hz, 1H, H-6), 5.83 (d, *J* = 5.1 Hz, 1H, H-1'), 5.62 (dd, *J* = 8.1, 2.2 Hz, 1H, H-5), 4.82 (dd, *J* = 11.7, 3.3 Hz, 1H, H-5'a), 4.75 (dd, *J* = 11.7, 6.1 Hz, 1H, H-5'b), 4.30 – 4.25 (m, 1H, H-4'), 4.21 (dd, *J* = 5.2 Hz, 1H, H-2'), 4.15 (dd, *J* = 5.2 Hz, 1H, H-3'). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3 (C-Cl), 163.2 (C-O), 163.0 (C-4), 150.7 (C-2), 141.0 (C-6), 102.1 (C-5), 88.9 (C-1'), 80.9 (C-4'), 72.6 (C-2'), 69.9 (C-3'), 69.9 (C-5'). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{11}H_{12}ClN_6O_6$ 359.0501, found 359.0501.

5'-*O*-(6-Octanthio-1,2,4,5-tetrazin-3-yl)-2',3'-*O*-*iso*propylideneuridine (5). A solution of 1-octanethiol (0.04 mL, 0.25 mmol) and triethylamine (0.03 mL, 0.25 mmol) in acetonitrile (20 mL) was added dropwise through a dropping funnel to a solution of *s*-tetrazine **3** (50 mg, 0.13 mmol) in acetonitrile (20 mL). The mixture was stirred at room temperature for 1 hour and the solvent was evaporated. Purification by flash column chromatography (CH_2Cl_2/MeOH 96:4) gives compound **5** (60 mg, 94%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 1H, NH), 7.34 (d, *J* = 8.1 Hz, 1H, H-6), 5.74 (d, *J* = 1.9 Hz, 1H, H-1'), 5.73 (d, *J* = 8.1, 1.9 Hz, 1H, H-5), 5.08 (dd, *J* = 6.4, 1.9 Hz, 1H, H-2'), 5.03 (dd, *J* = 6.4, 3.9 Hz, 1H, H-3'), 4.88 – 4.78 (m, 2H, H-5'), 4.64 – 4.58 (m, 1H, H-4'), 3.26 (t, *J* = 7.4 Hz, 2H, CH_2-S), 1.77 (quint, *J* = 7.0 Hz, 2H), 1.58 (s, 3H, CH_3), 1.46 (quint, *J* = 7.0 Hz, 2H, CH_2), 1.37 (s, 3H, CH_3), 1.35 – 1.21 (m, 8H, 4 x CH_2), 0.87 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6 (C-S), 165.9 (C-O), 163.3 (C-4), 150.2 (C-2), 142.4 (C-6), 114.9 ($C(CH_3)_2$), 103.1 (C-5), 95.0 (C-1'), 85.3 (C-4'), 84.6 (C-2'), 81.2 (C-3'), 69.0 (C-5'), 31.9 (CH_2-S), 31.1 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 27.2 (CH_2), 25.4 ($C(CH_3)_2$), 22.7 ($C(CH_3)_2$), 14.2 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{22}H_{33}N_6O_6S$ 509.2177, found 509.2175.

5'-*O*-(6-Phenylthio-1,2,4,5-tetrazin-3-yl)-2',3'-*O*-*iso*propylideneuridine (6). A solution of thiophenol (0.03 mL, 0.25 mmol) and triethylamine (0.03 mL, 0.25 mmol) in acetonitrile (20 mL) was added dropwise through a dropping funnel to a solution of *s*-tetrazine **3** (50 mg, 0.13 mmol) in acetonitrile (20 mL). The mixture was stirred at room temperature for 2 h and the solvent was evaporated under reduced pressure. Purification by flash column chromatography (CH_2Cl_2/MeOH 96:4) gives compound **6** (24 mg, 41%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H, NH), 7.66 – 7.64 (m, 2H, CH(Ar)), 7.49 – 7.45 (m, 3H, CH(Ar)), 7.29 (d, *J* = 8.0 Hz, 1H, H-6), 5.72(d, *J* = 1.9 Hz, 1H, H-1'), 5.70 (d, *J* = 8.0, 1.9 Hz, 1H, H-5), 5.05 (dd, *J* = 6.4, 1.9 Hz, 1H, H-2'), 5.00 (dd, *J* = 6.4, 4.0 Hz, 1H, H-3'), 4.79 (d, *J* = 4.6 Hz, 2H, H-5'), 4.58 (dd, *J* = 4.6 Hz, 1H, H-4'), 1.58 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1 (C-S), 166.0 (C-O), 163.1 (C-4), 150.1 (C-2), 142.4 (C-6), 135.6 (CH_{Ar}), 130.5 (CH_{Ar}), 130.0 (CH_{Ar}), 126.5 (C_{Ar}-S), 114.9 ($C(CH_3)_2$), 103.0 (C-5), 95.0 (C-1'), 85.2 (C-4'), 84.5 (C-2'), 81.2 (C-3'), 69.1 (C-5'), 27.3 ($C(CH_3)_2$), 25.4 ($C(CH_3)_2$). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{20}H_{21}N_6O_6S$ 473.1238, found 473.1236.

5'-*O*-(6-Methoxy-1,2,4,5-tetrazin-3-yl)-2',3'-*O*-*iso*propylideneuridine (7). To a mixture of 2',3'-*O*-*iso*propylideneuridine (189 mg, 0.67 mmol) and 3-chloro-6-methoxy-*s*-tetrazine (195 mg, 1.33 mmol) in anhydrous CH_2Cl_2 (3 mL) was introduced 2,4,6-collidine (0.36 mL, 2.66 mmol). After 72 h stirring at RT, the mixture was diluted with AcOEt, washed with water (3 x 5 mL), brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexanes/AcOEt 2:8) to obtain compound **7** (93.2 mg, 36%) as an orange-pink oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H, NH), 7.36 (d, *J* = 8.0 Hz, 1H, H-6), 5.77 (d, *J* = 2.1 Hz, 1H, H-1'), 5.62 (dd, *J* = 8.0, 2.1 Hz, 1H, H-5), 5.08 (dd, *J* = 6.5, 2.2 Hz, 1H, H-2'), 5.03 (dd, *J* = 6.2, 3.7 Hz, 1H, H-3'), 4.84 (dd, *J* = 11.4, 5.5 Hz, 1H, H-5'), 4.79 (dd, *J* = 11.4, 3.5 Hz, 1H, H-5'), 4.64 – 4.60 (m, 1H, H-4'), 4.24 (s, 3H, OCH_3), 1.60 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (*C*-OCH_3), 166.1 (C-O), 162.9 (C-4), 150.1 (C-2), 142.3 (C-6), 114.9 ($C(CH_3)_2$), 103.1 (C-5), 94.8 (C-1'), 85.2 (C-4'), 84.6 (C-2'), 81.1 (C-3'), 69.2 (C-5'), 57.0 (OCH_3), 27.3 ($C(CH_3)_2$), 25.4 ($C(CH_3)_2$). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{15}H_{19}N_6O_7$ 395.1310, found 395.1309.

5'-*O*-(6-Methoxy-1,2,4,5-tetrazin-3-yl)-uridine (8). General procedure used for tetrazine derivative **4** was followed with compound **7** (93.2 mg, 0.24 mmol) to give deprotected compound **8** (80.3 mg, 97%) as a pink oil. ^{1}H NMR (250 MHz, acetone-d_6) δ 10.30 (s, 1H, NH), 7.89 (d, *J* = 8.1 Hz, 1H, H-6), 5.99 (d, *J* = 3.4 Hz, 1H, H-1'), 5.75 (d, *J* = 8.1 Hz, 1H, H-5), 4.92 – 4.80 (m, 3H, H-5', OH), 4.54 – 4.38 (m, 4H, H-2', H-3', H-4', OH), 4.21 (s, 3H, OCH_3). ^{13}C NMR (63 MHz, acetone-d_6) δ 167.5 (C-OCH_3), 166.9 (C-O), 164.5 (C-4), 151.3 (C-2), 142.0 (C-6), 102.6 (C-5), 90.7 (C-1'), 82.6 (C-4'), 74.6 (C-2'), 71.2 (C-3'), 69.5 (C-5'), 56.9 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{12}H_{15}N_6O_7$ 355.0997, found 355.0999.

4-[*N*-Methyl-*N*-(2-*O*-propargylethyl)]aminobenzaldehyde (10). To a stirred suspension of NaH (60%, 3.91 g, 0.098 mol) in distilled THF (50 mL) cooled in an ice bath and under argon, were dripped over 20 min a solution of commercially available 4-[*N*-(2-hydroxyethyl)-*N*-methyl]aminobenzaldehyde (10.29 g, 0.057 mol) in distilled THF (90 mL) followed by a solution of propargyl bromide (80% in toluene, 9.2 mL, 0.083 mol) over 5 minutes. After 15 h, the excess of sodium hydride was destroyed by addition of crushed ice in the mild and organic solvent was evaporated under reduced pressure. The residue was then partitioned in a mixture CH_2Cl_2/H_2O (150/150 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL). Organic layers were combined, washed with brine, dried over $MgSO_4$, evaporated under reduced pressure and purified by column chromatography (hexanes/EtOAc from 9:1 to 6:4) to give 62% of the desired compound **10** (7.72 g, 0.036 mol) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H, H_{CHO}), 7.73 (d, *J* = 9.2 Hz, 2H, 2 x CH_{Ar}), 6.73 (d, *J* = 9.2 Hz, 2H, 2 x CH_{Ar}), 4.15 (d, *J* = 2.3 Hz, 2H, O-CH_2-C≡), 3.73 (t, *J* = 5.3 Hz, 2H, O-CH_2), 3.66 (t, *J* = 5.3 Hz, 2H, N-CH_2), 3.10 (s, 3H, N-CH_3), 2.43 (t, *J* = 2.3 Hz, 1H, *H*-C≡). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.4 (CHO), 153.6, 132.2 (CH_{Ar}), 125.5, 111.1 (CH_{Ar}), 79.4 ($C_{q\ C\equiv C}$), 74.9 ($CH_{C\equiv C}$), 67.3 (O-CH_2), 58.6 (O-CH_2), 52.0 (N-CH_2), 39.4 (N-CH_3). HRMS-ESI m/z $[M+H]^+$ calcd for $C_{13}H_{16}NO_2$ 218.1177, obsd 218.1165.

(*E*)-2-(2-[4-(*N*-Methyl-*N*-(2-O-propargylethyl)amino)styryl]-6-(*tert*-butyl)-4H-pyran-4-ylidene)malononitrile (11). To a stirred solution of aldehyde **10** (5.95 g, 0.027 mol) and 4-(dicyanomethylene)-2-(*tert*-butyl)-6-methyl-4*H*-pyran (5.40 g, 0.025 mol) in distilled CH_3CN (60 mL) under argon, was added piperidine (2.9 mL, 0.029 mol). The mixture was heated at 85 °C overnight. After completion of the reaction, the mild was cooled to room temperature and the precipitate was filtered and washed with cold CH_3CN (10 mL)

View Article Online
DOI: 10.1039/C8OB01606B

followed by cyclohexane (200 mL). The filtrate was evaporated under reduced pressure and the residue was then triturated (sonication) with a minimum of cold CH_3CN then filtered. Combination of the two solids afforded 84% of the title compound **11** (10.6 g, 0.026 mol) as an orange/red solid. Mp 156°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 9.2 Hz, 2H, 2 x CH_{Ar}), 7.34 (d, *J* = 16.0 Hz, 1H, CH=), 6.74 (d, *J* = 8.7 Hz, 2H, 2 x CH_{Ar}), 6.59 (d, *J* = 2.3 Hz, 1H, CH_{pyran}), 6.51 (d, *J* = 2.3 Hz, 1H, CH_{pyran}), 6.50 (d, *J* = 16.9 Hz, 1H, CH=), 4.16 (d, *J* = 2.7 Hz, 2H, O-CH_2), 3.74 (t, *J* = 5.7 Hz, 2H, O-CH_2), 3.64 (t, *J* = 5.7 Hz, 2H, N-CH_2), 3.09 (s, 3H, N-CH_3), 2.43 (t, *J* = 2.5 Hz, 1H, H-C≡), 1.37 (s, 9H, 3 x CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 160.3, 156.9, 150.7, 138.4 (CH=), 129.9 (CH_{Ar}), 122.8, 115.9, 115.8, 113.2 (CH=), 112.3 (CH_{Ar}), 105.7 (CH_{pyran}), 102.5 (CH_{pyran}), 79.5, 77.4, 74.9 (HC≡), 67.4 (O-CH_2), 58.7, 57.9, 52.3 (N-CH_2), 39.4 (N-CH_3), 36.8 ($C_{q,\ tBu}$), 28.3 ($CH_{3\ tBu}$). HRMS-ESI m/z $[M+Na]^+$ calcd for $C_{26}H_{27}N_3O_2Na$ 436.2001, obsd 436.1992.

5-(Diethylamino)-2-nitrosophenol chlorhydrate (13). 1M HCl solution in ethanol was prepared by adding dropwise AcCl (5.7 mL, 0.10 mol) in 100 mL of EtOH at 0 °C and the solution stirred for 15 minutes. Diethylaminophenol **12** (5.00 g, 30.3 mmol) was added and then amyl nitrite (4.0 mL, 30 mmol, 1 eq) was added dropwise at 0 °C and the solution stirred for 2.5 h. Et_2O was added (ca. 500 mL) and the precipitate was filtered, washed with Et_2O and vacuum dried. Nitrosophenol was obtained as a brown powder (5.32 g, 23.1 mmol, 76%). ^{1}H NMR (400 MHz, CD_3OD) *δ* 7.70 (d, *J* = 10.4 Hz, 1H, H-3), 7.20 (dd, *J* = 10.4, 2.4 Hz, 1H, H-4), 6.40 (d, *J* = 2.4 Hz, 1H, H-6), 3.95 (q, *J* = 7.2 Hz, 2H, N-CH_2), 3.87 (q, *J* = 7.2 Hz, 2H, N-CH_2), 1.39 (t, *J* = 7.2 Hz, 6H, 2 x CH_3). ^{13}C NMR (100 MHz, CD_3OD): *δ* 164.0, 145.8, 124.8 (C-3), 121.0 (C-4), 98.9 (C-6), 49.8 (CH_2), 15.5 (CH_3), 14.0 (CH_3).

9-(Diethylamino)-2-(prop-2-yn-1-yloxy)-5*H*-benzo[*a*]phenoxazin-5-one (15). To a solution of phenol **13** (1.52 g, 6.59 mmol) in DMF (50 mL) was added 1,6-dihydroxynaphtalene (1.06 g, 6.62 mmol). The mixture was refluxed for 4 h. Compound **14** can be isolated by preparative chromatography. To the resulting solution was added K_2CO_3 (3.51 g, 25.4 mmol, 4 eq), then 3-bromoprop-1-yne (80%, 2.8 mL, 25 mmol, 4 eq) was added dropwise and stirred 1 h at 115 °C. After concentration under reduced pressure, the crude was purified by column chromatography (hexanes/EtOAc 2:1) to obtain alkyne **15** (708 mg, 30% for two steps) as a dark green powder. Mp 184°C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.24 (d, *J* = 8.7 Hz, 1H, H-4), 8.13 (d, *J* = 2.6 Hz, 1H, H-1), 7.59 (d, *J* = 9.1 Hz, 1H, H-11), 7.24 (dd, *J* = 8.7, 2.7 Hz, 1H, H-3), 6.65 (dd, *J* = 9.1, 2.7 Hz, 1H, H-10), 6.44 (d, *J* = 2.7 Hz, 1H, H-8), 6.30 (s, 1H, H-6), 4.89 (d, *J* = 2.4 Hz, 2H, *CH*$_2$-C≡), 3.46 (q, *J* = 7.1 Hz, 4H, 2 x N-*CH*$_2$), 2.59 (t, *J* = 2.4 Hz, 1H, H-C≡), 1.26 (t, *J* = 7.1 Hz, 6H, 2 x CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 183.3 (C=O), 160.2, 152.3, 151.0, 147.0, 139.8, 134.1, 131.3 (C-11), 128.0 (C-4), 126.4, 124.9, 118.5 (C-3), 109.7 (C-10), 107.2 (C-1), 105.4 (C-6), 96.3 (C-8), 78.1 (-*C*≡), 76.1 (H-*C*≡), 56.2 (*CH*$_2$-C≡), 45.2 (CH_2), 12.7 (CH_3). HRMS-ESI m/z $[M+H]^+$ calcd for $C_{23}H_{21}N_2O_3$ 373.1547, found 373.1545.

1-Amino-4-(but-3-ynylamino)anthracene-9,10-dione (Tag 16). To a solution of 1,4-diaminoanthraquinone (500 mg, 2.10 mmol) and K_2CO_3 (1.2 g, 8.40 mmol) in ACN (8 mL) was added 4-bromobutyne (0.85 mL, 9.06 mmol) in ACN (2 mL). The solution was stirred at 65 °C for 6 days. After concentration under reduced pressure, the residue was purified by flash column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 99:1) to give compound **16** as a blue solid (62 mg, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.74 (s, 1H, NH), 8.43 – 8.31 (m, 2H, CH-(C-C=O)), 7.77 – 7.69 (m, 2H, CH-(CH-C-C=O)), 7.18 (d, *J* = 9.5 Hz, 1H, CH-(C(NH_2))), 7.15 – 7.05 (m, 2H, NH_2), 7.03 (d, *J* = 9.5 Hz, 1H, CH-(C(NH))), 3.61 (q, *J* = 7.0 Hz, 2H, CH_2N), 2.63 (td, *J* = 7.0, 2.6 Hz, 2H, CH_2), 2.12 (t, *J* = 2.6 Hz, 1H, H-C≡). ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.0 (C=O-(C-C(NH_2))), 183.2 (C=O-(C-C(NH))), 146.5, 144.5, 134.8, 134.2, 132.7 (CH-(CH-C-C=O)), 132.4 (CH-(CH-C-C=O)), 128.7 (CH-(C(NH_2))), 126.4 (CH-(C-C=O)), 122.4 (CH-(C(NH))), 111.1 (C-N), 110.2 (C-N), 81.2 (C≡), 70.7(C≡), 41.9 (CH_2N), 20.0 (CH_2). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{18}H_{15}N_2O_2$ 291.1128, found 291.1131.

2-[2-*tert*-Butyl-6-[(*E*)-2-[4-[2-[[1-[[4-(2,4-dioxopyrimidin-1-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]methyl]triazol-4-yl]methoxy]ethyl-methyl-amino]phenyl]vinyl]pyran-4-ylidene]propanedinitrile (18). 5'-azido-5'-deoxy-2',3'-*O-iso*propylideneuridine (150 mg, 0.49 mmol), Tag **11** (201 mg, 0.49 mmol), sodium ascorbate (73 mg, 0.29 mmol) and copper sulfate pentahydrate (30 mg, 0.15 mmol) were suspended in a 1:1 mixture of water and ethanol (2.5 mL each) in a closed microwave reaction vessel. After microwave-irradiation for 3 min at 80 °C, the mixture was concentrated under reduced pressure. The crude residue was extracted with CH_2Cl_2, washed with brine, dried with $MgSO_4$, filtrated and concentrated under reduced pressure. Purification by flash column chromatography (hexanes/AcOEt 3:7 followed by CH_2Cl_2/MeOH 96:4) afforded compound **18** (154 mg, 99%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 9.36 (s, 1H, NH), 7.49 (s, 1H, H-triazole), 7.41 (d, *J* = 8.9 Hz, 2H), 7.32 (d, *J* = 15.9 Hz, 1H, H-alkene), 7.03 (d, *J* = 8.0 Hz, 1H, H-6), 6.68 (d, *J* = 8.9 Hz, 2H), 6.59 (d, *J* = 2.1 Hz, 1H, H-pyran), 6.51 (d, *J* = 2.1 Hz, 1H, H-pyran), 6.49 (d, *J* = 15.9 Hz, 1H, H-alkene), 5.72 (dd, *J* = 8.0, 1.7 Hz, 1H, H-5), 5.51 (d, *J* = 1.7 Hz, 1H, H-1'), 5.06 (dd, *J* = 6.5, 1.7 Hz, 1H, H-2'), 4.95 (dd, *J* = 6.5, 4.3 Hz, 1H, H-3'), 4.71 (dd, *J* = 14.0, 4.1 Hz, 1H, H-5'), 4.65 (dd, *J* = 14.0, 7.1 Hz, 1H, H-5'), 4.63 (s, 2H, C-CH_2-O), 4.49 (ddd, *J* = 7.1, 4.3, 4.1 Hz, 1H, H-4'), 3.73 (t, *J* = 5.9 Hz, 2H, CH_2-O), 3.60 (t, *J* = 5.9 Hz, 2H, CH_2-N), 3.04 (s, 3H, CH_3-N), 1.53 (s, 3H, C(CH_3)$_2$), 1.37 (s, 9H, CH_3(*t*Bu)), 1.34 (s, 3H, C(CH_3)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 172.0 (C-pyran), 163.2 (C-4), 160.4 (C-pyran), 156.9, 150.9, 150.1 (C-2), 145.0 (C-triazole), 143.4 (C-6), 138.5 (CH-alkene), 129.9 (CH_{Ar}), 123.9 (CH-triazole), 122.5, 115.9, 115.0 (s, *C*(CH_3)$_2$), 113.1 (CH-alkene), 112.1 (2C, CH_{Ar}), 105.6 (CH-pyran), 103.0 (C-5), 102.5 (CH-pyran), 96.4 (C-1'), 86.4 (C-4'), 84.4 (C-2'), 81.9 (C-3'), 67.9 (CH_2-O), 64.7 (C-*C*H_2-O), 57.8 (CN), 52.1 (CH_2-N), 51.9 (C-5'), 39.2 (*C*H_3-N), 36.7 (*C*(*t*Bu)), 28.3 (CH_3), 27.21 (CH_3), 25.38 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{38}H_{43}N_8O_7$ 723.3249, found 723.3246.

2-[2-tert-Butyl-6-[(*E*)-2-[4-[2-[[1-[[5-(2,4-dioxopyrimidin-1-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl]triazol-4-yl]methoxy]ethyl-methyl-amino]phenyl]vinyl]pyran-4-ylidene]propanedinitrile (19). To a solution of compound **18** (154 mg, 0.21 mmol) in water (0.12 mL) was added TFA (1.08 mL). The mixture was stirred at room temperature until completion (10 min). After evaporation under reduced pressure, the crude product was extracted with AcOEt, washed with $NaHCO_3$, brine, dried with $MgSO_4$, filtrated and concentrated. The product was purified by flash column chromatography (CH_2Cl_2/MeOH 96:4 to 90:10) followed by CH_2Cl_2/MeOH/ammoniac (90:9:1) to give compound **19** (146 mg, 99%) as a red solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) *δ* 11.37 (s, 1H, NH), 8.00 (s, 1H, H-triazole), 7.56 – 7.51 (m, 3H, H-6, H-Ar), 7.45 (d, *J* = 15.9 Hz, 1H, H-alkene), 7.03 (d, *J* = 15.9 Hz, 1H, H-alkene), 6.76 – 6.73 (m,

View Article Online
DOI: 10.1039/C8OB01606B

3H, H-Ar, H-pyran), 6.41 (d, *J* = 2.0 Hz, 1H, H-pyran), 5.74 (d, *J* = 5.6 Hz, 1H, OH), 5.63 (dd, *J* = 8.1, 1.4 Hz, 1H, H-5), 5.51 (d, *J* = 5.6 Hz, 1H, H-1'), 5.39 (d, *J* = 5.3 Hz, 1H, OH), 4.71 (dd, *J* = 14.4, 4.4 Hz, 1H, H-5'), 4.62 (dd, *J* = 14.4, 7.7 Hz, 1H, H-5'), 4.53 (s, 2H, C-CH_2-O), 4.16 – 4.10 (m, 1H, H-4'), 4.08 (dd, *J* = 10.5, 5.6 Hz, 1H, H-2'), 3.97 (dd, *J* = 10.5, 5.1 Hz, 1H, H-3'), 3.65 – 3.57 (m, 4H, CH_2-N, CH_2-O), 2.98 (s, 3H, CH_3-N), 1.35 (s, 9H, CH_3 (*t*Bu)). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) *δ* 172.3 (C-pyran), 162.9 (C-4), 160.9 (C-pyran), 156.5, 150.6 (C-2, (C-Ar)-N), 143.9 (s, C-triazole), 141.1 (s, C-6), 138.6 (s, CH-alkene), 130.1 (C_{Ar}), 124.6 (CH-triazole), 122.1, 115.8, 112.9 (CH-alkene), 111.7 (C_{Ar}), 104.9 (CH-pyran), 102.1 (C-1'), 101.5 (CH-pyran), 88.7 (C-5), 81.7 (C-4'), 72.0 (C-2'), 70.5 (C-3'), 67.1 (CH_2-O), 63.5 (C-CH_2-O), 54.9 (CN), 51.2 (C-5'), 51.0 (CH_2-N), 39.0 (CH_3-N), 36.3 (*C*(*t*Bu)), 27.5 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{34}H_{39}N_8O_7$ 683.2936, found 683.2932.

1-[6-[[4-[[9-(Diethylamino)-5-oxo-benzo[a]phenoxazin-2-yl]oxymethyl]triazol-1-yl]methyl]-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl]pyrimidine-2,4-dione (20). Following the procedure used for **18** from 5'-azido-5'-deoxy-2',3'-*O*-*iso*propylideneuridine (83 mg, 0.27 mmol), **Tag 15** (100 mg, 0.27 mmol), sodium ascorbate (40 mg, 0.16 mmol) and copper sulfate pentahydrate (15 mg, 0.08 mmol) in H_2O/EtOH (1:1) (4 mL), compound **20** was obtained as a purple solid (143 mg, 78%) after purification by flash column chromatography (hexanes/AcOEt 3:7 then CH_2Cl_2/MeOH 95:5). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.49 (s, 1H, NH), 8.33 (s, 1H, H-triazole), 8.07 – 8.00 (m, 2H, H-Ar), 7.69 (d, *J* = 7.6 Hz, 1H, H-6), 7.56 (d, *J* = 8.8 Hz, 1H, H-Ar), 7.32 (d, *J* = 8.4 Hz, 1H, H-Ar), 6.78 (d, *J* = 8.5 Hz, 1H, H-Ar), 6.59 (s, 1H, H-Ar), 6.15 (s, 1H, CH-C=O), 5.80 (s, 1H, H-1'), 5.65 (d, *J* = 7.6 Hz, 1H, H-5), 5.34 (s, 2H, CH_2-O), 5.18 – 5.11 (m, 1H, H-2'), 4.95 – 4.88 (m, 1H, H-3'), 4.80 (dd, *J* = 14.1, 5.0 Hz, 1H, H-5'), 4.68 (dd, *J* = 14.1, 7.6 Hz, 1H, H-5'), 4.50 – 4.36 (m, 1H, H-4'), 3.55 – 3.40 (m, 4H, CH_2-N), 1.48 (s, 3H, C(CH_3)$_2$), 1.29 (s, 3H, C(CH_3)$_2$), 1.16 (t, *J* = 6.3 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 181.8 (C=O), 163.8 (C-4), 161.0 (C-Ar-(OCH_2)), 152.2 (O-C-(CHC=O)), 151.3 (C-Ar), 150.8 (C-2), 146.9 (O-C-(CH-Ar)), 144.0 (C-6), 142.9 (C-triazole), 138.6 (C=N), 133.9 (C-Ar), 131.4 (CH-Ar), 127.7 (CH_{Ar}), 125.7 (C_{Ar}), 125.6 (s, CH-triazole), 124.4 (C_{Ar}), 118.3 (CH_{Ar}), 113.9 (*C*(CH_3)$_2$), 110.5 (CH_{Ar}), 107.3 (CH_{Ar}), 104.5 (*CH*-C=O), 102.4 (C-5), 96.4 (CH_{Ar}), 93.8 (C-1'), 85.8 (C-4'), 84.1 (C-2'), 81.9 (C-3'), 61.9 (CH_2-O), 51.8 (C-5'), 44.9 (CH_2-N), 27.3 (CH_3), 25.5 (CH_3), 12.9 (2 x CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{35}H_{36}N_7O_8$ 682.2620, found 682.2618.

1-[5-[[4-[[9-(Diethylamino)-5-oxo-benzo[a]phenoxazin-2-yl]oxymethyl]triazol-1-yl]methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione (21). The same **p**rocedure used for **19** was followed with **20** (25 mg, 0.04 mmol) and TFA (0.19 mL) in H_2O (0.02 mL), to afford compound **21** (17 mg, 74%) as a purple solid after purification by flash column chromatography (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.37 (s, 1H, NH), 8.29 (s, 1H, H-triazole), 8.05 (d, *J* = 2.5 Hz, 1H, H-Ar), 8.04 (d, *J* = 8.6 Hz, 1H, H-Ar), 7.59 (d, *J* = 9.1 Hz, 1H, H-Ar), 7.55 (d, *J* = 8.1 Hz, 1H, H-6), 7.33 (dd, *J* = 8.6, 2.5 Hz, 1H, H-Ar), 6.79 (dd, *J* = 9.1, 2.5 Hz, 1H, H-Ar), 6.61 (d, *J* = 2.5 Hz, 1H, H-Ar), 6.17 (s, 1H, CH-C=O), 5.77 (d, *J* = 5.5 Hz, 1H, H-1'), 5.64 (d, *J* = 8.1 Hz, 1H, H-5), 5.53 (d, *J* = 5.5 Hz, 1H, OH-2'), 5.41 (d, *J* = 5.5 Hz, 1H, OH-3'), 5.35 (s, 2H, CH_2-O), 4.78 (dd, *J* = 14.4, 4.2 Hz, 1H, H-5'), 4.70 (dd, *J* = 14.4, 7.6 Hz, 1H, H-5'), 4.24 – 4.15 (m, 1H, H-4'), 4.10 (ddd, *J* = 5.5 Hz, 1H, H-2'), 4.01 (ddd, *J* = 5.5 Hz, 1H, H-3'), 3.49 (q, *J* = 6.8 Hz, 4H, CH_2-N), 1.16 (t, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 181.3 (C=O), 162.9 (C-4), 160.6 (C-Ar-(OCH_2)), 151.73 (O-C-(CHC=O)), 150.8 (C-2), 150.6 (C-Ar), 146.4 (C-Ar), 142.33 (C-triazole), 141.1 (C-6), 138.1 (C=N), 133.5 (C_{Ar}), 130.9 (CH_{Ar}), 127.2 (CH_{Ar}), 125.5 (CH-triazole), 125.2 (C_{Ar}), 123.9 (C_{Ar}), 117.9 (CH_{Ar}), 110.1 (CH_{Ar}), 106.8 (CH_{Ar}), 104.1 (CH-C=O), 102.1 (C-5), 95.9 (CHAr), 88.8 (C-1'), 81.7 (C-4'), 72.1 (C-2'), 70.6 (C-3'), 61.5 (CH_2-O), 51.3 (C-5'), 44.4 (CH_2-N), 125 (2 x CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{32}H_{32}N_7O_8$ 642.2307, found 642.2303.

1-[6-[[4-[2-[(4-Amino-9,10-dioxo-1-anthryl)amino]ethyl]triazol-1-yl]methyl]-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl]pyrimidine-2,4-dione (22). Following the general procedure used for **18**, 5'-azido-5'-deoxy-2',3'-*O*-*iso*propylideneuridine (97 mg, 0.31 mmol), **Tag 16** (91 mg, 0.31 mmol), sodium ascorbate (47 mg, 0.19 mmol) and copper sulfate pentahydrate (19 mg, 0.09 mmol) were reacted in H_2O/EtOH (1:1) (4 mL) to yield compound **22** as a blue solid (125 mg, 67%) after purification by flash column chromatography (hexanes/AcOEt 1:9 then CH_2Cl_2/MeOH 96:4). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.46 (d, *J* = 1.9 Hz, 1H, NH_{uracil}), 10.83 (t, *J* = 5.7 Hz, 1H, NH_{AQ}), 8.53 – 8.25 (m, 2H, NH_2), 8.25 – 8.18 (m, 2H, CH-(C-C=O)), 8.00 (s, 1H, H-triazole), 7.81 – 7.74 (m, 2H, CH-(CH-C-C=O)), 7.64 (d, *J* = 8.0 Hz, 1H, H-6), 7.42 (d, *J* = 9.6 Hz, 1H, CH-(C(NH))), 7.30 (d, *J* = 9.6 Hz, 1H, CH-(C(NH_2))), 5.77 (d, *J* = 1.8 Hz, 1H, H-1'), 5.62 (dd, *J* = 8.0, 1.9 Hz, 1H, H-5), 5.10 (dd, *J* = 6.5, 1.8 Hz, 1H, H-2'), 4.87 (dd, *J* = 6.5, 4.5 Hz, 1H, H-3'), 4.73 (dd, *J* = 14.2, 4.5 Hz, 1H, H-5'), 4.62 (dd, *J* = 14.2, 7.7 Hz, 1H, H-5'), 4.36 (dt, *J* = 7.7, 4.5 Hz, 1H, H-4'), 3.72 (q, *J* = 6.9 Hz, 2H, CH_2N), 3.01 (t, *J* = 6.9 Hz, 2H, CH_2), 1.45 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ^{13}C NMR (63 MHz, DMSO-*d*$_6$) δ 181.3 (C=O-(C-C(NH_2))), 180.5 (C=O-(C-C(NH))), 163.3 (C-4), 150.3 (C-2), 146.4, 146.1, 144.12 (s, C-triazole), 143.4 (C-6), 134.1, 133.7, 132.3 (CH-(CH-C-C=O)), 132.2 (CH-(CH-C-C=O)), 129.6 (CH-(C(NH_2))), 125.7 (2 x C, CH-(C-C=O)), 123.7 (CH-(C(NH))), 123.2 (CH-triazole), 113.4 (*C*(CH_3)$_2$), 108.1 (C-N), 107.9 (C-N), 101.8 (C-5), 93.2 (C-1'), 85.3 (C-4'), 83.6 (C-2'), 81.3 (C-3'), 51.2 (C-5'), 41.8 (CH_2N), 26.8 (CH_3), 25.7 (CH_2), 25.1 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{30}H_{30}N_7O_7$ 600.2201, found 600.2200.

1-[5-[[4-[2-[(4-Amino-9,10-dioxo-1-anthryl)amino]ethyl]triazol-1-yl]methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione (23). General procedure used for **19** was followed with **22** (250 mg, 0.42 mmol) and TFA (2.2 mL) in H_2O (0.2 mL), to afford compound **23** (199 mg, 85%) as a blue solid after purification by flash column chromatography (CH_2Cl_2/MeOH from 95:5 to 90:10). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.84 (t, *J* = 5.7 Hz, 1H, NH_{AQ}), 8.68 – 8.25 (brs, 2H, NH_2), 8.22 (dd, *J* = 5.8, 3.2 Hz, 2H, CH-(C-C=O)), 7.97 (s, 1H, H-triazole), 7.84 – 7.71 (m, 2H, CH-(CH-C-C=O)), 7.45 (d, *J* = 8.0 Hz, 1H, H-6), 7.42 (d, *J* = 9.5 Hz, 1H, CH-(C(NH))), 7.30 (d, *J* = 9.5 Hz, 1H, CH-(C(NH_2))), 5.75 (d, *J* = 5.2 Hz, 1H, H-1'), 5.55 (d, *J* = 8.0 Hz, 1H, H-5), 4.70 (dd, *J* = 4.8, 14.0 Hz, 1H, H-5'), 4.61 (dd, *J* = 7.4, 14.0 Hz, 1H, H-5'), 4.20 – 4.09 (m, 1H, H-4'), 4.05 (t, *J* = 5.2 Hz, 1H, H-2'), 3.96 (t, *J* = 5.2 Hz, 1H, H-3'), 3.73 (q, *J* = 6.7 Hz, 2H, CH_2N), 3.02 (t, *J* = 7.1 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 181.3 (C=O-(C-C(NH_2))), 180.5 (C=O-(C-C(NH))), 164.4 (C-4), 151.5 (C-2), 146.3 (C_{quat}), 146.1 (C_{quat}), 144.0 (C-triazole), 140.7 (C-6), 134.1, 133.7, 132.3 (CH-(CH-C-C=O)), 132.2 (CH-(CH-C-C=O)), 129.7 (CH-(C(NH_2))), 125.8 (CH-(C-C=O)), 125.7 (CH-(C-C=O)), 123.7 (CH-(C(NH))), 123.4 (CH-triazole), 108.1 (C-

View Article Online
DOI: 10.1039/C8OB01606B

N), 107.9 (C-N), 102.1 (C-5), 89.1 (C-1'), 81.6 (C-4'), 72.3 (C-2'), 70.6 (C-3'), 51.2(C-5'), 41.9 (CH_2N), 25.8 (CH_2). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{27}H_{26}N_7O_7$ 560.1888, found 560.1884.

5-Bromopentyl 2-(*N*-ethyl-*N*-{4'-[(2''-chloro-4''-nitrophenyl)-phazo]phenyl}amino)ethyl ether (Tag 24) was prepared following the procedure reported by Isaad *et al.*[23] from commercially available 2-[4-(2-chloro-4-nitrophenylazo)-*N*-ethylphenylamino]ethanol (Disperse Red 13) in 59% yield.

1-[6-[[5-[2-[4-[(*E*)-(2-Chloro-4-nitro-phenyl)azo]-*N*-ethyl-anilino]ethoxy]pentylamino]methyl]-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl]pyrimidine-2,4-dione (26). To a solution of 5'-amino-5'-deoxy-2',3'-*O-iso*propylideneuridine (240 mg, 0.85 mmol) and K_2CO_3 (88 mg, 0.64 mmol) in DMF (50 mL) was added red **Tag 24** (205 mg, 0.42 mmol). The resulting mixture was stirred at room temperature for 16 h. The mixture was extracted with AcOEt, washed with water (5 x 150 mL), brine, dried with $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH from 99:1 to 97:3) to give compound **26** as a red oil (127 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 2.4 Hz, 1H, H-Ar), 8.13 (dd, *J* = 8.9, 2.4 Hz, 1H, H-Ar), 7.92 (d, *J* = 8.7 Hz, 2H, H-Ar), 7.76 (d, *J* = 8.9 Hz, 1H, H-Ar), 7.32 (d, *J* = 8.0 Hz, 1H, H-6), 6.76 (d, *J* = 8.7 Hz, 2H, H_{-Ar}), 5.74 (d, *J* = 8.0 Hz, 1H, H-5), 5.69 (d, *J* = 2.5 Hz, 1H, H-1'), 4.91 (dd, *J* = 6.6, 2.5 Hz, 1H, H-2'), 4.75 (dd, *J* = 6.6, 4.5 Hz, 1H, H-3'), 4.09 (d, *J* = 4.5 Hz, 1H, H-4'), 3.92 – 3.83 (m, 2H, H-5'), 3.62 (s, 4H, N-CH_2-CH_2-O), 3.55 (q, *J* = 7.0 Hz, 2H, CH_2N), 3.44 (t, *J* = 6.4 Hz, 2H, CH_2O), 1.67 – 1.58 (m, 4H, CH_2N, CH_2), 1.56 (s, 3H, $C(CH_3)_2$), 1.44 – 1.36 (m, 2H, CH_2), 1.34 (s, 3H, $C(CH_3)_2$), 1.27 – 1.20 (m, 2H, CH_2), 1.24 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (C-4), 153.7 (C-N=N), 152.5 (C-N), 151.0 (C-2), 147.4 (C-NO_2), 144.6 (C-N=N), 140.1 (C-6), 134.2 (C-Cl), 127.5 (CH_{Ar}), 126.4 (CH_{Ar}), 123.0 (CH_{Ar}), 118.4 (CH_{Ar}), 115.1 (*C*$(CH_3)_2$), 111.9 (2 x C, CH_{Ar}), 102.6 (C-5), 94.7 (C-1'), 87.6 (C-4'), 84.9 (C-2'), 81.7 (C-3'), 71.7 (CH_2O), 68.6 (OCH_2-(CH_2N)), 50.8 (NCH_2-(CH_2O)), 46.5 (CH_2N), 41.5 (C-5'), 30.1, 29.7 (CH_2NH), 27.6, 27.6 (CH_3), 25.8 (CH_3), 23.9, 12.6 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{33}H_{43}ClN_7O_8$ 700.2856, found 700.2856.

1-[5-[[5-[2-[4-[(*E*)-(2-Chloro-4-nitro-phenyl)azo]-N-ethyl-anilino]ethoxy]pentylamino]methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione (27). The procedure used for **19** was followed with **26** (160 mg, 0.23 mmol) and TFA (1.2 mL) in H_2O (0.1 mL), to afford compound **27** (105 mg, 70%) as a black solid after purification by flash column chromatography (CH_2Cl_2/MeOH from 95:5 to 80:20). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H, H-Ar), 8.24 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.93 (d, *J* = 7.9 Hz, 1H, H-6), 7.85 (d, *J* = 8.8 Hz, 2H, H-Ar), 7.78 (d, *J* = 8.5 Hz, 1H, H-Ar), 6.91 (d, *J* = 8.8 Hz, 2H, H-Ar), 5.86 – 5.67 (m, 2H, H-1', H-5), 4.11 – 4.05 (m, 1H, H-2'), 3.97 – 3.90 (m, 1H, H-3'), 3.85 – 3.70 (m, 3H, H-4', CH_2NH), 3.70 – 3.50 (m, 6H, N-CH_2-CH_2-O, CH_2N), 3.41 (s, 2H, CH_2O), 2.86 – 2.73 (m, 2H, H-5'), 1.50 (s, 4H, CH_2), 1.34 – 1.23 (m, 2H, CH_2), 1.17 (t, *J* = 6.7 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.8 (C-4), 152.4 (C-N=N), 152.2 (C-N), 150.7 (C-2), 146.6 (C-NO_2), 143.2 (C-N=N), 139.7 (C-6), 132.2 (C-Cl), 126.7 (2 x C, C_{Ar}), 125.7 (CH_{Ar}), 123.4 (CH_{Ar}), 118.0 (CH_{Ar}), 111.8 (CH_{Ar}), 101.0 (C-5), 89.1 (C-1'), 84.8 (C-4'), 73.1 (C-2'), 70.2 (C-3', CH_2O), 67.8 (OCH_2-(CH_2N)), 49.7 (CH_2N), 45.4 (NCH_2-(CH_2O)), 42.9 (C-5'), 40.6 (CH_2NH), 28.7, 26.8, 23.0, 12.0 (CH_3). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{30}H_{39}ClN_7O_8$ 660.2543, found 660.2542.

2-[2-[(*E*)-2-[4-[2-[[1-[[4-(6-Aminopurin-9-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]methyl]triazol-4-yl]methoxy]ethyl-methyl-amino]phenyl]vinyl]-6-tert-butyl-pyran-4-ylidene]propanedinitrile (29) Following general procedure used for **18**, 5'-azido-5'-deoxy-2',3'-*O-iso*propylideneadenosine (140 mg, 0.42 mmol) was reacted with **Tag 11** (174 mg, 0.0.42 mmol), sodium ascorbate (63 mg, 0.25 mmol) and copper sulfate pentahydrate (25 mg, 0.13 mmol) in H_2O/EtOH (1:1) (4 mL) to afford compound **29** as an orange oil (256 mg, 81%) after purification by flash column chromatography (AcOEt/CH_2Cl_2/MeOH 3:1:0.1 then CH_2Cl_2/MeOH 96:4). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.33 (s, 1H, H-2), 7.78 (s, 1H, H-8), 7.37 (d, *J* = 8.8 Hz, 2H, CH_{Ar}), 7.31 (d, *J* = 15.8 Hz, 1H, H-alkene), 7.23 (s, 1H, H-triazole), 6.65 (d, *J* = 8.8 Hz, 2H, CH_{Ar}), 6.58 (d, *J* = 1.7 Hz, 1H, H-pyran), 6.50 (d, *J* = 1.7 Hz, 1H, H-pyran)), 6.47 (d, *J* = 15.8 Hz, 1H, H-alkene), 6.05 (d, *J* = 1.8 Hz, 1H, H-1'), 5.89 (s, 2H, NH_2), 5.40 (dd, *J* = 6.3, 1.8 Hz, 1H, H-2'), 5.19 (dd, *J* = 6.3, 3.6 Hz, 1H, H-3'), 4.77 (dd, *J* = 14.3, 4.2 Hz, 1H, H-5'), 4.70 (dd, *J* = 14.3, 7.4 Hz, 1H, H-5'), 4.63 – 4.56 (m, 1H, H-4'), 4.55 (s, 2H, C-CH_2O), 3.67 (t, *J* = 5.6 Hz, 2H, CH_2O), 3.56 (t, *J* = 5.6 Hz, 2H, CH_2N), 2.99 (s, 3H, CH_3N), 1.59 (s, 3H, $C(CH_3)_2$), 1.36 (s, 12H, $C(CH_3)_2$, CH_3(*t*Bu)). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 171.9 (C-pyran), 160.3 (C-pyran), 156.9, 155.8, 153.3 (C-2), 150.9, 149.1, 144.9 (C-triazole), 140.2 (C-8), 138.4 (CH-alkene), 129.8 (2 x C, CH_{Ar}), 123.7, 122.4, 120.5, 115.9, 115.8, 115.08, 112.9 (CH-alkene), 112.0 (2C, CH_{Ar}), 105.5 (CH-pyran), 102.4 (CH-pyran), 90.7 (C-1'), 85.5 (C-4'), 84.0 (C-2'), 82.0 (C-3'), 67.8 (CH_2O), 64.6 (C-CH_2O), 57.8 (2C, CN), 52.0 (CH_2N), 51.7 (C-5'), 39.1 (CH_3N), 36.7, 28.2 (CH_3 (*t*Bu)), 27.2 ($C(CH_3)_2$), 25.4 ($C(CH_3)_2$). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{39}H_{44}N_{11}O_5$, 746.3521 found 746.3519.

2-[2-[(*E*)-2-[4-[2-[[1-[[5-(6-Aminopurin-9-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl]triazol-4-yl]methoxy]ethyl-methyl-amino]phenyl]vinyl]-6-tert-butyl-pyran-4-ylidene]propanedinitrile (30). Following general procedure used for **19**, compound **29** (240 mg, 0.32 mmol) was reacted with TFA (1.6 mL) in H_2O (0.2 mL) to afford compound **30** as a dark red solid (186 mg, 82%) after purification by flash column chromatography (CH_2Cl_2/MeOH 95:5 to 80:20) as eluent. ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 8.26 (s, 1H, H-2), 8.16 (s, 1H, H-8), 7.84 (s, 1H, H-triazole), 7.53 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 7.43 (d, *J* = 15.9 Hz, 1H, H-alkene), 7.32 (s, 2H, NH_2), 7.00 (d, *J* = 15.9 Hz, 1H, H-alkene), 6.74 (d, *J* = 2.1 Hz, 1H, H-pyran), 6.69 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 6.40 (d, *J* = 2.1 Hz, 1H, H-pyran), 5.91 (d, *J* = 5.4 Hz, 1H, H-1'), 5.62 (d, *J* = 5.5 Hz, 1H, OH-2'), 5.50 (d, *J* = 4.4 Hz, 1H, OH-3'), 4.81 – 4.72 (m, 2H, H-5'), 4.69 (d, *J* = 5.5 Hz, 1H, H-2'), 4.46 (s, 2H, C-CH_2O), 4.28 – 4.24 (m, 2H, H-3', H-4'), 3.56 (t, *J* = 3.6 Hz, 4H, O-CH_2CH_2-N), 2.91 (s, 3H, CH_3N), 1.34 (s, 9H, CH_3, (*t*Bu)).^{13}C NMR (101 MHz, DMSO-d_6) *δ* 172.2 (C-pyran), 160.9 (C-pyran), 156.4, 156.1, 152.6 (C-2), 150.6, 149.2, 143.8, 139.9 (C-8), 138.5 (CH-alkene), 130.0 (2 x C, CH_{Ar}), 124.4, 122.0, 119.2, 115.8, 115.7, 112.8 (CH-alkene), 111.7 (2 x C, CH_{Ar}), 104.9 (CH-pyran), 101.5 (CH-pyran), 87.8 (C-1'), 82.4 (C-4'), 72.5 (C-2'), 70.9 (C-3'), 67.0 (CH_2O), 63.5 (C-CH_2O), 51.3 (C-5'), 50.9 (CH_2N), 36.3 (CH_3N), 27.52 (CH_3(*t*Bu)). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{36}H_{40}N_{11}O_5$, 706.3208 found 706.3201.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

View Article Online
DOI: 10.1039/C8OB01606B

This work was supported by institutional support from the University of Orleans and from ANR-10-TECS-0004.

Notes and references

1. a) L.P. Jordheim, D. Durantel, F. Zoulim, C. Dumontet, *Nat. Rev. Drug Discov*., 2013, **12**, 447–464. b) L. A. Agrofoglio, S. R. Challand in "Acyclic, Carbocyclic and L-Nucleosides"; Kluwer Academic Publishers: Dordrecht, 1998. c) E. De Clercq, A. Holý, *Nat. Rev. Drug Discov*., 2005, **4**, 928–940.
2. a) R. W. Sinkeldam, N. J. Greco, Y. Tor, Chem Rev., 2010, **110**, 2579–2619; b) M. S. Noe, Y. Xie, Y. Tor, in "Methods for Studying Nucleic Acid/Drug Interactions"; CRC Press, 2011, pp. 159–182; c) U. Asseline, *Curr. Org. Chem.*, 2006, **10**, 491–518; d) D. M. Jameson, J. F. Eccleston, Methods Enzymol., 1997, **178**, 363-390; e) A. A. Tanpure, M. G. Pawar, S. G. Srivatsan, *Isr. J. Chem.*, 2013, **53**, 366–378.
3. T. Hiratsuka, K. Uchida, *Biochim. Biophys. Acta*, 1973, 320, 365-647.
4. a) T. Hiratsuka, *Eur. J. Biochem*., 2003, **270**, 3479-3485; b) C. M. McKeen, L. J. Brown, J. T. Nicol, J. M. Mellor, T. Brown, *Org. Biomol. Chem*., 2003, **1**, 2267–2275. c) H. Asanuma, T. Yoshida, T. Ito, M. Komiyama, *Tetrahedron Lett.* 1999, **40**, 7995–7998.
5. a) A. R. Katritzky, L. Khelashvili, J. Kovacs, K. Shanab, *Chem. Biol. Drug. Des*.,2009, **73**, 396–402; b) M. Adamczyk, S. R. Akireddy, P. G. Mattingly, R. E. Reddy, *Tetrahedron*, 2003, **59**, 5749–5761.
6. A. Krstulja, S. Lettieri, A.J. Hall, V. Roy, P. Favetta, L. A. Agrofoglio, *Macromol. Biosci.* 2017, **17**, 1700250.
7. C. Dejous, H. Hallil, V. Raimbault, J.L. Lachaud, B. Plano, R. Delépée, P. Favetta, L. A. Agrofoglio, D. Rebière, *Sensors*, 2016, **16**, 915; b) L. A. Agrofoglio, A. Krstulja, C. De Schutter, P. Favetta, R. Delépée, V. Roy, C. Dejous, H. Hallil, J.-L. Lachaud, N. Lebal, N. Omar Aouled, V. Raimbault, D. Rebière, S. Dulong, F. Levi, C. Junot, F. Théodoro, A. Pruvost, R. Vidal, *IRBM* 2014, *35*, 66–71; c) N. Lebal, D. Rebière, R. Delepée, L. A. Agrofoglio, B. Plano, H. Hallil, A. Krstulja, C. Dejous, *Micro Amp Nano Lett*. 2013, **8**, 563–566.
8. A. Krstulja, S. Lettieri, A. J. Hall, R. Delépée, P. Favetta, L. A. Agrofoglio, *Anal. Bioanal. Chem.,* 2014, **406**, 6275-6284.
9. a) K. M. Koczula, A. Gallotta, *Essays Biochem*., 2016, **60**, 111-120; b) G. Kim, J. Lim, C. Mo, J. Biosys. Eng., 2015, 40, 277-283.
10. N. S. Freeman, C. E. Moore, L. M. Wilhelmsson, Y. Tor, *J. Org. Chem*., 2016, **81**, 4530-4539.
11. G. Clavier, P. Audebert, *Chem. Rev.,* 2010, **110**, 3299-3314.
12. a) Y.-H. Gong, F. Miomandre, R. Méallet-Renaud, S. Badré, L. Galmiche, J. Tang, P. Audebert, G. Clavier, *Eur. J. Org. Chem.,* 2009, 6121-6128; b) J. Malinge, C. Allain, L. Galmiche, F. Miomandre, P. Audebert, *Chem. Mater.,* 2011, **23**, 4599-4605.
13. A. M. Hossion, R. Islam, R. K. Kandahary, T. Nagamatsu, *Synthesis*, 2009, **16**, 2689-2696.
14. J. P. Ferris, V. R. Rao, T. A. Newton, *J. Org. Chem.,* 1979, **44**, 4378-4381.
15. C. Ribes, E. Falomir, J. Murga, *Tetrahedron,* 2006, **62**, 1239-1244.
16. Y.-H. Gong, P. Audebert, G. Clavier, F. Miomandre, J. Tang, S. Badré, R. Méallet-Renault, E. Naidus, *New J. Chem.,* 2008, **32**, 1235-1242.
17. G. Wang, K. Sakthivel, V. Rajappan, T. W. Bruice, K. Tucker, P. Fagan, J. Brooks, T. Hurd, J. M. Leeds, P. D. Cook, *Nucleosides Nucleotides Nucleic Acids,* 2004, **23**, 317-337.
18. A. Procopio, M. Gaspari, M. Nardi, M. Oliverio, A. Tagarelli, G. Sindona, *Tetrahedron Lett.,* 2007, **48**, 8623-8627.
19. Z. Novák, B. Bostai, M. Csékei, K. Lőrincz, A. Kotschy, *Heterocycles,* 2003, **60**, 2653-2668.
20. a) R. Lucas, R. Zerrouki, R. Granet, P. Krausz, Y. Champavier, *Tetrahedron,* 2008, **64**, 5467-5471; b) X. Chi, P. Pahari, K. Nonaka, S. G. Van Lanen, *J. Am. Chem. Soc.*, 2011, **133**, 14452–14459.
21. a) N. Yotapan, C. Charoenpakdee, P. Wathanathavorn, B. Ditmangklo, H.-A. Wagenknecht, T. Vilaivan, *Belstein J. Org. Chem.*, 2014, **10**, 2166-2174; b) M. Börgardts, K. Verlinken, M. Neidhardt, T. Wöhrle, A. Herbst, S. Laschat, C. Janiak, T. J. J. Müller, *RSC Adv.,* 2016, **6**, 6209-6222; c) M. S. J. Briggs, I. Bruce, J. N. Miller, C. J. Moody, A. C. Simmonds, E. Swann, *J. Chem. Soc, Perkin Trans. 1,* 1997, 1051-1058.
22. P. M. Chaudhary, S. R. Chavan, F. Shirazi, M. Razdan, P. Nimkar, S. P. Maybhate, A. P. Likhite, R. Gonnade, B. G. Hazara, M. V. Deshpande, S. R. Deshpande, *Bioorg. Med. Chem.,* 2009, **17**, 2433-2440.
23. J. Isaad, M. Rolla, R. Bianchini, *Eur. J. Org. Chem*., 2009, 2748-2764.
24. L. Li, X. Shen, Q.-H. Xu, S. Q. Yao, *Angew. Chem., Int. Ed.,* 2013, **52**, 424-428.
25. (a) S. Vertuani, A. Baldisserotto, K. Varani, P. A. Borea, B. De Marcos Maria Cruz, L. Ferraro, S. Manfredini, A. Dalpiaz, *Eur. J. Med. Chem.,* 2012, **54**, 202-209. (b) L. R. Comstock, S. R. Rajski, *Tetrahedron,* 2002, **58**, 6019-6026.

View Article Online
DOI: 10.1039/C8OB01606B

A novel set of visibly coloured dye-labeled 5'-nucleosides including 1,2,4,5-tetrazine (orange), dicyanomethylene-4H-pyran (yellow), benzophenoxazinone (pink), 9,10-anthraquinone (blue) and azobenzene (red) chromophores, were prepared, and their absorption and fluorescence spectra recorded. Such nucleodyes have great potential for future competitive lateral flow test MIP-based strips.