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Fluorescent *p*-substituted-phenyl-imidazolo-cytidine analogues

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ABSTRACT

The synthesis and spectral properties of modified cytidine analogues are described here. To confer fluorescent properties, the cytidine fluorophore was extended by a cinnamyl moiety at the cytidine 5-position, (analogues **1a**–**c**), or extended by a phenyl-imidazolo ring, (analogues **3a**–**d**). Those compounds were synthesized via the Suzuki–Miyaura coupling reaction and via condensation of 5-amino-cytidine with *p*-substituted benzaldehydes, respectively. All analogues were fluorescent in the blue region (λ_{em}: 402–436 nm). Among the above mentioned analogues, *p*-CF_3–phenyl-imidazolo-cytidine, **3d**, was found to be a promising fluorescent probe, exhibiting a quantum yield 7000-fold larger than cytidine (Φ=0.617) and a red-shift of ca. 108 nm of maximum emission (411 nm). Its retained Watson–Crick hydrogen bonding face allows **3d** to base-pair specifically with guanosine, similar to cytidine. In addition, phenyl-imidazolo-cytidine analogues prefer *anti* conformation and the *C3′-endo* (*N*) sugar puckering. These properties make **3d** an attractive fluorescent probe for potential use in the various facets of nucleic acid chemistry.

1. Introduction

The fluorescence of natural nucleotide bases is weak, their fluorescent decay times are short, and the signals are averaged over all of the bases in the oligonucleotide, resulting in little useful information.[1–5] Cytidine, for example, displays a quantum yield of 0.00008, absorption around 261 nm, and an emission band centered around 303 nm.[3] Over the past decade, fluorescent nucleoside analogues have gained notable attention due to their important applications, particularly as pharmaceutical agents and biological probes or mimetics.[6–8] Applications of those analogues include DNA sequencing for SNP (single nucleotide polymorphism) identification,[9–14] study of nucleic acid dynamics, and nucleic acid—ligand or protein interactions.[7,15–18]

Fluorescent nucleoside probes may be divided into two general classes: those bearing an appended fluorophore, separated electronically and spatially from the base-paring face,[19–21] and those in which the nucleobase is integral to the fluorophore.[22–38] Members of the latter class are especially attractive because their fluorescent response can reveal the microenviroment of the nucleobase. This notion has been applied in fluorescent cytidine nucleosides analogues, such as pyrrolo-cytidine (pC),[22] phenyl-pyrrolo-cytidine (pC^{Phe}),[23] tricyclic 1,3-diaza-2-oxophenothiazinet (tC),[24] and 5-furyl deoxycytidine.[25,26] These fluorescent nucleosides were incorporated into an oligonucleotide, which in turn, was used as a probe for detection of genetic material in cells. The photophysical properties of the above mentioned fluorescent nucleosides were either slightly changed or remained unchanged upon their incorporation into the oligonucleotides.[22–35]

We have recently reported that appending various alkenyl–aryl moieties at the uridine 5-position, generates a family of responsive fluorescent nucleoside analogues exhibiting emission in the visible range and quantum yields up to 0.24.[36] Here, we report the design and synthesis of a novel series of cytidine-based fluorescent probes, **1**–**3** (Fig. 1), as well as their spectroscopic and conformational properties. In addition, we examined the formation of a Watson–Crick base pair between guanosine and the most promising fluorescent cytidine analogue **3d**.

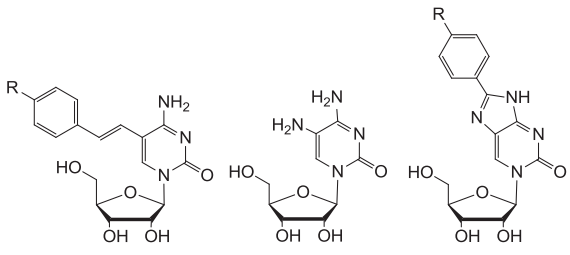

Fig. 1. Novel fluorescent cytidine analogues, **1**–**3** synthesized in this study.

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2. Results and discussion

2.1. Probe design

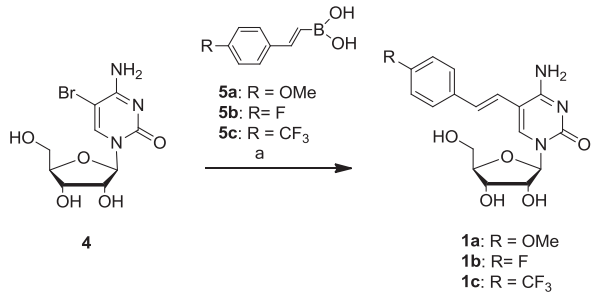
Our basic design for the development of novel intrinsically fluorescent cytidine relies on extension of the natural nucleobase by a conjugated system (e.g., a cinnamyl moiety) or by fusion to a heterocyclic system. To design fluorescent probes based on suitable extensions of the cytosine chromophore, we applied the following strategy: (1) Selection of minimal extensions of the natural nucleobase at positions not involved in H-bonding with a complementary base. (2) Selection of probes that may be excited and may emit at the longest possible wavelengths. (3) Selection of extensions of the cytosine base that can be achieved via a simple and short synthesis.

In a previous publication we reported four types of C5 substituted uridine analogues where the aromatic systems are: (a) directly coupled, (b) coupled via an alkenyl linker, (c) coupled via an alkynyl linker, and (d) coupled via a dienyl linker.[36] Analogues in which the uracil chromophore was extended by a cinnamyl moiety, exhibited the longest absorption and emission wavelengths and the highest quantum yields, (λ_{abs} 318 nm, λ_{em} 476 nm, Φ 0.24), as compared to uridine.[36]

Based on our previous findings for uridine fluorescent analogues, we have now designed and synthesized their cytidine counterparts. We targeted the preparation of push–pull cytidine containing systems to achieve a maximal enhancement of fluorescent properties.[37] Since we did not know whether cytidine plays the relative role of an electron rich or poor moiety, we have extended the cytosine scaffold by *p*-OMe, *p*-F, and *p*-CF_3 substituted cinnamyl moieties, to form analogues **1a**, **1b**, and **1c** (Fig. 1). We selected to couple the cinnamyl moiety at the cytosine C5 position, since substitution at C6 will drive the nucleoside into the undesired *syn* conformation. In addition, we designed bicyclic analogues of cytidine based on an imidazolo-cytidine scaffold, compounds **3**(**a**–**d**). In the case of phenyl-imidazolo-cytidine analogues, we prepared compounds bearing various substituents on the *para* position of the phenyl group, e.g., H, **3a**, *p*-Me, **3b**, *p*-OMe, **3c**, and *p*-CF_3, **3d**.

2.2. Synthesis of 5-(cinnamyl)-substituted-cytidine derivatives 1a–c

Suzuki coupling reactions are highly useful for the preparation of biaryl compounds. The Pd-catalyzed Suzuki–Miyaura reaction proved to be effective also for nucleosides cross-coupling[38] and was applied here for the preparation of derivatives **1a**–**c**, (Scheme 1).[39]

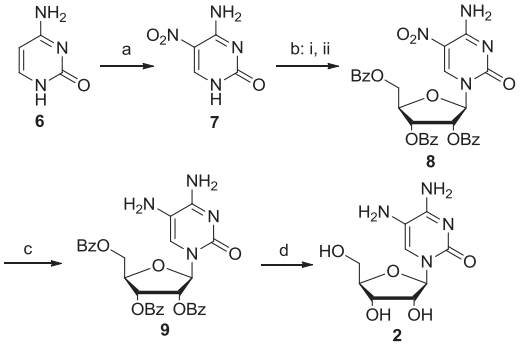


Scheme 1. Reaction conditions; (a) appropriate *trans*-vinylboronic acid **5**(**a**–**c**), 0.25 equiv TPPTS, 0.05 equiv $Pd(OAc)_2$, 3 equiv Na_2CO_3, H_2O/CH_3CN (2:1), reflux, 3 h.

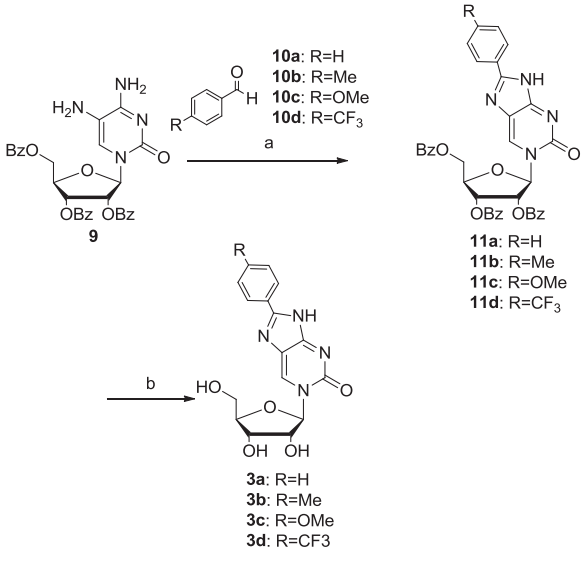
Thus, coupling of 5-Br–cytidine, **4** and the appropriate aryl boronic acid derivative, **5a**–**c**, was performed, using 5 mol% of $Pd(OAc)_2$, water-soluble tris(3-sulfonatophenyl)phosphane (TPPTS) ligand, and Na_2CO_3 in aqueous-phase conditions (water/acetonitrile mixtures). All the reactions proceeded reasonably well at 100 °C to reach full conversion in 3 h. The products were isolated in acceptable yields of 54–64% after column chromatography.[40,41]

2.3. Synthesis of imidazolo-cytidine derivatives 3a–d

For the synthesis of imidazolo-cytidine derivatives, cytosine, **6**, was converted to 5-nitro-cytosine, **7**, by HNO_3/H_2SO_4 in 64% yield (Scheme 2).[42] Nitration reaction was performed on the base, cytosine, and not on the nucleoside, cytidine, because of the drastic reaction conditions (pH=1–2), that would cleave the glycosidic bond. Next, 5-nitro-cytosine was coupled to protected ribose. Compound **7**, was reacted with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) to provide 5-nitro-cytosine trimethylsilyl derivative, which was directly reacted with, 1′-acetate-2′,3′,5′-tribenzoate-β-D-ribose with stannic tetrachloride ($SnCl_4$) in dichloromethane at room temperature to give **8** in 70% yield.[43] Reduction of **8** by hydrogenation with 10% Pd/C/H_2 in THF and AcOH provided **9** in 38% yield. Product **9** is used as a starting material for the preparation of cytidine derivatives **7**, **8** (Scheme 3). Deprotection of **8** with NaOH in aq EtOH, gave 5-amino cytidine **2** in 70% yield.[44]



Scheme 2. Reaction conditions; (a) concd H_2SO_4, fuming HNO_3; (b) i. HMDS, TMS–Cl, reflux, 36 h, ii. 1 M $SnCl_4$ in CH_2Cl_2, 1′-acetate-2′,3′,5′-tribenzoate-β-D-ribose, CH_3CN, rt, 3 h; (c) 10% Pd/C/H_2, THF, AcOH, 6 h; (d) NaOH in aq EtOH, rt, 3 h.



Scheme 3. Reaction conditions; (a) appropriate aldehyde **10a**–**d**, cat. *p*-TsOH, 80 °C; (b) 1 M NaOH in MeOH, rt, 0.5 h.

Synthesis of bicyclic phenyl-imidazolo-cytidine analogues was achieved by condensation of protected 5-amino-cytidine **9** with aryl aldehyde **10a**–**d**, in the presence of *p*-TsOH (20 mol%), in DMF at 80 °C, to give **11a**–**d** in 36–47% yield.[45] Addition of 1 M NaOH in

MeOH, at room temperature for 1 h, yielded **3a**–**d**, in 80–85% yield (Scheme 3).

2.4. Spectral properties of cytidine derivatives 1–3

We measured the absorption and emission of analogues **1a**–**c** and intermittent compound **2** in H_2O, and analogues **3a**–**d** in MeOH, due to solubility consideration. The spectral data for derivatives **1**–**3** are summarized in Table 1. All compounds were fluorescent in the blue region (λ_{em}: 402–436 nm). Although analogues **1a**, **1b**, and **1c**, *p*-substituted by OCH_3, F, and CF_3, respectively, showed relatively long emission wavelengths, their quantum yields were low, ranging from 0.017 to 0.052. However **3a**–**d**, in which the chromophore is extended by fusion to a phenyl-imidazolo-ring system, exhibited reasonably long absorption and emission wavelengths and up to 36-fold higher quantum yields as compared to those of **2** and **3a**–**d**. Specifically, compounds **3c** and **3d**, for which Φ=0.233 and 0.617, respectively, in MeOH.

Table 1
Fluorescent properties of analogues **6**–**8**

Compound	λ_{abs} (nm)	λ_{em} (nm)	Φ	Solvent
Cytidine	261	303	0.00008[a]	H_2O
1a	286	436	0.052[a]	H_2O
1b	285	430	0.019[a]	H_2O
1c	297	424	0.017[a]	H_2O
2	296	430	0.089[b]	H_2O
3a	342	402	0.215[b]	MeOH
3b	344	403	0.198[b]	MeOH
3c	347	405	0.233[b]	MeOH
3d	346	411	0.617[b]	MeOH

[a] Data obtained with tryptophan as a reference.
[b] Data obtained with quinine sulfate as a reference.

In the bicyclic phenyl-imidazolo nucleosides **3** series, the phenyl ring substituent caused a significant effect on the fluorescent properties. Thus, compounds **3b** and **3c** bearing OMe and Me at the *para* position of the phenyl ring, showed very similar fluorescent properties compared to **3a**. However, *p*-CF_3 substitution, in **3d** versus **3a**, resulted in enhancement of both λ_{em} and quantum yield, 411 nm and 0.617, respectively. Neither analogues **1**, nor analogues **3** exhibited characteristics of push–pull systems. In analogues **6** neither electron withdrawing (**1b**, **c**), nor electron donating substituents changed the photophysical behavior of the analogues. No clear dependence on the electronic character of the substituent was observed for analogues **3**.

In conclusion, all tested cytidine analogues **1**–**3** showed significantly improved spectral properties as compared to cytidine. Analogues in which the cytosine was fused phenyl-imidazolo moiety exhibited the most promising spectral data, up to 7000-fold enhancement of the quantum yield of cytidine and red-shift of ca. 108 nm of maximum emission.

2.5. Conformational analysis of cytidine derivatives

Cytosine nucleosides are expected to possess conformational flexibility, due to possible rotations around the glycosidic bond and pseudorotation of the ribose ring as well as possible rotations around the C4′–C5′ bond angle (γ). However, it is well-established that most nucleosides adopt a predominant conformation in solution.[46] Thus, it has been shown that the majority of purine and pyrimidine nucleosides favor an *anti* conformation of the base ring with respect to the sugar ring.[47] Likewise, the ribose ring exhibits a puckered conformation in which either the C2′ or C3′ atom is furthest from the plane of the other atoms of the ribose ring, named as *south* (*S*) and *north* (*N*) conformations, respectively.[48] Finally, it has been shown that the ribose exocyclic group exists predominantly in a *gauche–gauche* (*gg*) conformation about the C4′–C5′ bond with the O5′ atom projecting over the furanose ring. Here, we employed ^{1}H, and ^{13}C NMR spectroscopy to analyze the solution conformations of representative nucleoside analogues, **1a** and **3b**.

2.5.1. Conformation around the glycosidic bond. Pyrimidine nucleosides can adopt two main conformations, *syn* or *anti*, in which the oxygen on C-2 of cytidine is projecting away or toward the sugar ring. The quantitative determination of the conformation around the glycosidic bond can be obtained by monitoring the vicinal coupling constants $^3J_{C6-H1'}$ and $^3J_{C2-H1'}$, which are extracted from ^{13}C NMR spectra. A practical rule for the orientation of the base relative to the ribose was formulated: a value of $^3J_{C2-H1'}<{}^3J_{C6-H1'}$ indicates that χ is in the *anti* conformation, whereas the reverse indicates that χ is in the *syn* conformation.[49]

Ippel et al. reparametrized and generalized the Karplus equations for the glycosidic bond conformation of purine and pyrimidine nucleosides and nucleotides.[49] Eqs. 1 and 2 were used in this study to calculate the glycosidic bond angle χ (O4′–C1′–N1–C2) in nucleoside **1a** based on $^3J_{C6-H1'}$ and $^3J_{C2-H1'}$ values.[50,51]

$$^3J_{C6-H1'} = 4.5\cos^2(\chi-60) - 0.6\cos(\chi-60) + 0.1 \quad (1)$$

$$^3J_{C2-H1'} = 4.7\cos^2(\chi-60) + 2.4\cos(\chi-60) + 0.1 \quad (2)$$

In the case of **3b** some of the NMR signals were broaden, probably because of the tautomeric equilibrium of the imidazole ring. Hence, small vicinal coupling constants $^3J_{C6-H1'}$ and $^3J_{C2-H1'}$, could not be observed (Fig. 2). NOE experiments are valuable for examining relative proton positions and deducing the *anti* or *syn* conformation of the nucleoside.[52] The conformation of analogue **3b** was determined by cross peaks between H-6 and H-1′/2′/3′ in NOESY spectrum.[53] NOE interactions in compounds **1a**, and **3b** were observed between H-6 and H-1′/2′/3′ (Fig. 3). These interactions suggest that the conformation of these compounds is *anti*, similar to the conformation of the natural cytidine. Moreover, calculated χ values for the C5-substitued nucleosides **1a** were within the range defined as *anti* (Table 2), which concurs with previously published data.[54]

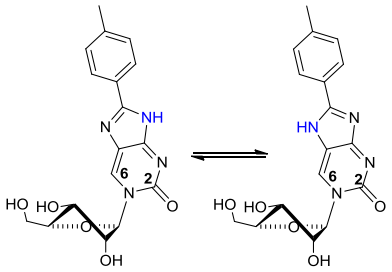


Fig. 2. Tautomers of compound **3b**.

2.5.2. Sugar puckering. The ribose moieties of nucleosides most often adopt either a C3′-*endo* (*north*, *N*) or a C2′-*endo* (*south*, *S*) orientation.[47] The conformation of the ribose ring of nucleosides **1a** and **3b** was analyzed in terms of a dynamic equilibrium between two favored puckered conformations: *North* conformer and *South* conformer.

N and *S* equilibrium populations were calculated from the observed $J_{1'2'}$ and $J_{3'4'}$ couplings.[51] According to this method, the observed vicinal couplings are related to the relative proportion of conformers by Eqs. 3–5:

$$J_{1'2'} = 9.3(1-\chi_N) = 9.3\chi_S \quad (3)$$

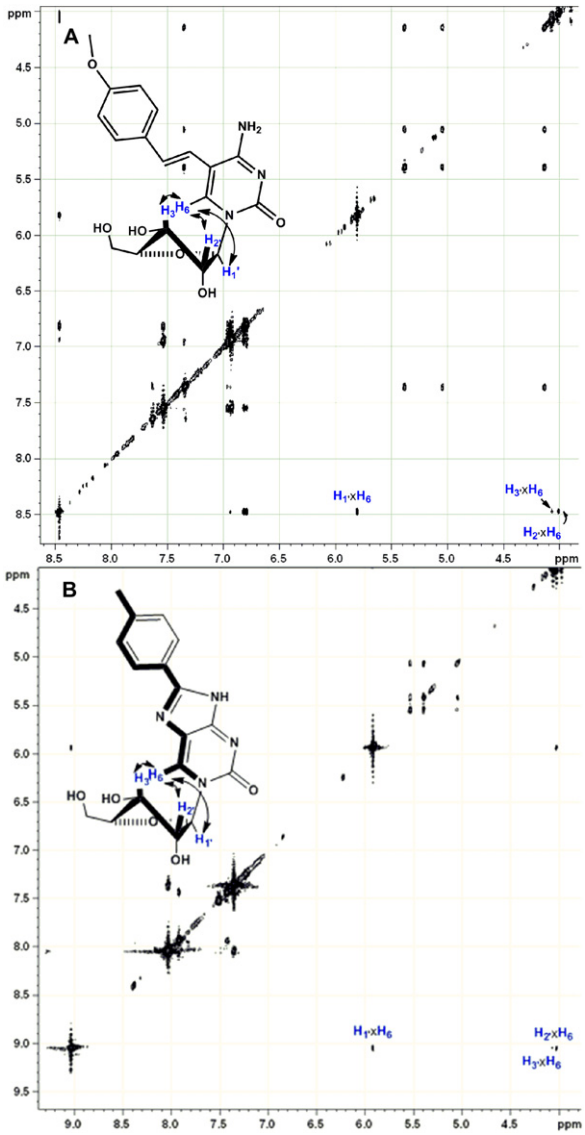


Fig. 3. NOE interactions within compounds (A) **1a**, (B) **3b**.

Table 2
Conformational parameters of analogues **1a** and **3b** in DMSO-d_6 solution

Compound	Sugar puckering %*N*	Conformer population around C4′–C5′ bond			Conformation around glycosidic bond χ
		% *gg*	% *tg*	% *gt*	
1a	65	88	8	4	*anti*
3b	73	84	8	8	*anti*

$$J_{2'3'} = 4.6\chi_N + 5.3(1-\chi_N) \quad (4)$$

$$J_{3'4'} = 9.3\chi_N \quad (5)$$

The mole fraction of conformers *S* and *N* can be calculated directly from the observed values of $J_{1'2'}$ and $J_{3'4'}$. Using the assigned *J*-coupling constants and the above equations, the mole fractions of conformers *S* and *N* for nucleoside **1a** and **3b** were calculated. The data reveal that analogues **1a** and **3b**, similar to cytidine[55] all prefer the *C3′-endo* (*N*) conformation and consist 65 and 73% of the total population, respectively (Table 2).

2.5.3. Conformations of the exocyclic CH_2OH group. The nucleoside coupling constants $J_{4'5'}$ and $J_{4'5''}$ can be interpreted in terms of three classical staggered rotamers, with a preferred *gauche–gauche* (*gg*) conformation. The mole fractions of each staggered rotamer of C4′–C5′ were calculated from the following expressions (Eqs. 6–8):

$$\rho gg = [(Jt + Jg) - (J4'5' + J4'5'')]/(Jt - Jg) \quad (6)$$

$$\rho tg = (J4'5' - Jg)/(Jt - Jg) \quad (7)$$

$$\rho gt = (J4'5'' - Jg)/(Jt - Jg) \quad (8)$$

The observed proton signals, labeled H5′ and H5″, refer to downfield and upfield signals, respectively. The percentages of *gg*, *gt*, and *tg* conformers are presented in Table 2. For analogues **1a** and **3b** there is a clear preference for the *gg* rotamer around the C4′–C5′ bond, 88 and 84%, respectively.

2.6. Compound 3d preserves natural H-bonding pattern with guanosine

Natural base pairing of nucleic acids is essential for hybridization-based diagnostics.[56] Therefore, we examined here the formation of a Watson–Crick base pair between guanosine and *p*-CF_3–phenyl-imidazolo-cytidine, **3d**.

Hydrogen bonding results in the formation of planar base pairs and hence NH_2 and NH protons are deshielded due to the presence of ring currents in the same plane. Since we could not obtain ^{1}H NMR data on the N^3–H of compound **3d**, due to tautomerization of this proton, we studied the interaction of guanosine with **3d** by high-field ^{1}H NMR (700 MHz) and focused on the NH_2 and NH protons of G. Previous NMR studies have shown hydrogen-bonded association between guanosine and cytidine in DMSO-d_6 solution.[57] Here, we applied the same technique with our cytidine analogue **3d**. We have obtained data at a range of concentrations of **3d** with constant concentration of guanosine (0.02 M) in DMSO-d_6 solution, up to 1.5:1 ratio. The monitoring of spectral changes due to H-bonding was carried out on the N^2–NH_2 and N^1–NH protons of guanosine. Upon hydrogen bonding, N^2–amino and N^1–NH guanosine protons are deshielded due to the weakening of the N–H covalent bond. Indeed, downfield shifts of the these protons were observed with increasing concentrations of analogue **3d** with maximum $\Delta\delta$ of 16.61 and 19.53 Hz, respectively, indicating hydrogen bonding between guanosine and compound **3d** (Fig. 4).

3. Conclusions

In summary, a novel family of fluorescent cytosine nucleoside analogues has been prepared and their photophysical and base-pairing properties have been evaluated. Phenyl-imidazolo-cytidine compounds **3a**–**d** showed high quantum yields. Specifically, **3d** exhibited the highest quantum yield, 0.617 and excitation and emission maxima of 346 and 411 nm, making it a suitable probe for study of nucleic acid structure, dynamics, and interactions. Analogues **3** retain the conformation of cytidine. Futhermore, its unperturbed Watson–Crick face allows **3d** to base-pair specifically with guanosine, similar to an unmodified cytidine. Hence, we suggest **3d** as promising cytidine-based fluorescent probe.

4. Experimental section

4.1. General procedure for the synthesis of 5-(cinnamyl)-substituted-cytidine derivatives (1a–c): 4-amino-1-(3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-(4-methoxystyryl)pyrimidin-2(1*H*)-one (1a)

Water/acetonitrile (2:1, 1.6 mL) was added through a septum to a nitrogen-purged round bottom flask containing 5-Br–cytidine,

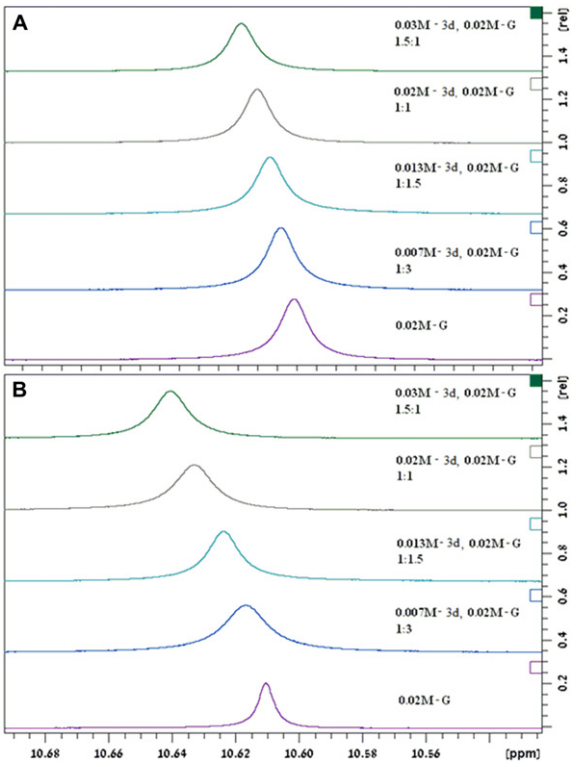


Fig. 4. Changes in the ^{1}H NMR spectra of guanosine upon addition of analogue **3d** in DMSO-d_6 solution (700 MHz, 25 °C). A. ^{1}H NMR spectra of N^2–NH_2 protons of guanosine (ppm). B. ^{1}H NMR spectra of N^1–NH protons of guanosine (ppm).

4 (280 mg, 0.87 mmol), *trans*-2-(4-methoxyphenyl)-vinylboronic acid **5a** (194 mg, 1.09 mmol), $Pd(OAc)_2$ (10 mg, 0.04 mmol), TPPTS (124 mg, 0.22 mmol), and Na_2CO_3 (277 mg, 2.61 mmol). The mixture was stirred under reflux for 3 h and monitored by TLC (7:3 $CHCl_3$/MeOH). The solvent was evaporated and water was added. The resulting solution was freeze dried. The residue was separated on a silica gel column (70:30 $CHCl_3$/MeOH). Product **1a** was obtained as a white solid in a 54% yield (176 mg). Mp 125–127 °C. $[\alpha]_D^{23}$ −9.5 (*c* 1.12, DMSO). IR (ZnSe): ν 3545, 3401, 2976, 1716, 1636, 1613, 1509, 1482, 1451, 1249, 1176, 1097, 1026, 961, 852, 780, 699 cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.43 (s, 1H), 7.51 (d, J=7.8 Hz, 2H), 7.32 (s, 2H), 6.92 (d, J=15.1 Hz, 1 H), 6.90 (d, J=7.8 Hz, 2H), 6.75 (d, J=15.1 Hz, 1H), 5.78 (d, J=3.2 Hz, 1H), 5.36–5.34 (m, 2H), 5.03–5.01(m, 1H), 4.09–4.03 (m, 1H), 3.99–3.97 (m, 1H), 3.87–3.86 (m, 1H), 3.78–3.76 (m, 1H), 3.77 (s, 3H), 3.63–3.60 (m, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 163.5, 158.7, 154.6, 136.9, 129.9, 127.8, 126.7, 117.1, 113.9, 104.3, 89.6, 83.7, 74.4, 68.6, 59.7, 55.2 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{18}H_{21}N_3NaO_6^+$: 398.132 (MNa^+). Found: 398.134 (MNa^+).

4.2. 4-Amino-1-(3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-(4-fruorostyryl)pyrimidin-2(1*H*)-one (1b)

Product **1b** was obtained from **4** (103 mg, 0.32 mmol) and **5b** (67 mg, 0.40 mmol) according to the general procedure. Product **1b** was obtained as a white solid in 64% yield (77 mg). Mp 161–163 °C. $[\alpha]_D^{23}$ −22.3 (*c* 0.98, DMSO). IR (ZnSe): ν 3326, 2855, 1718, 1636, 1599, 1507, 1477, 1249, 1160, 1101, 1059, 1023, 992, 962, 827, 763, 700, 671 cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.52 (s, 1H), 7.63–7.61 (m, 2H), 7.42 (s, 2H), 7.18–7.15 (m, 2H), 7.40 (d, J=16.1 Hz, 1H), 6.82 (d, J=16.1 Hz, 1H), 5.77 (d, J=3.3 Hz, 1H), 5.37–5.38 (m, 2H), 5.01–5.00 (m, 1H), 4.05–4.04 (m, 1H), 3.99–3.98 (m, 1H), 3.88–3.87 (m, 1H), 3.78–3.76 (m, 1H), 3.63–3.62 (s, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 163.6, 162.6, 161.0, 154.6, 137.9, 143.2, 134.2, 128.7, 128.7, 126.2, 119.7, 115.8, 115.6, 104.2, 90.1, 84.1, 74.8, 68.8, 59.9 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{17}H_{18}FN_3NaO_5^+$: 386.112 (MNa^+). Found: 386.109 (MNa^+).

4.3. 4-Amino-1-(3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-(4-(trifluoromethyl)styryl)pyrimidin-2(1*H*)-one (1c)

Product **1c** was obtained from **4** (143 mg, 0.48 mmol) and **5c** (130 mg, 0.60 mmol) according to the general procedure. Product **1c** was obtained as a white solid in 60% yield (118 mg). Mp 115–117 °C. $[\alpha]_D^{22}$ −25 (*c* 1.15, DMSO). IR (ZnSe): ν 3334, 2248, 2126, 1644, 1485, 1325, 1193, 1109, 1047, 1023, 996, 826, 765, 618 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.62 (s, 1H), 7.76–7.66 (m, 4H), 7.39 (s, 2H), 7.26 (d, J=15.8 Hz, 1H), 6.89 (d, J=15.8 Hz, 1H), 5.75 (d, J=2.3 Hz, 1H), 5.44–5.40 (m, 2H), 4.99–5.97 (m, 1H), 4.06–4.01 (m, 1H), 3.96–3.95 (m, 1H), 3.89–3.86 (m, 1H), 3.82–3.78 (m, 1H), 3.65–3.62 (m, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 163.3, 154.2, 148.3, 148.2, 141.3, 138.3, 132.1, 131.6, 131.5, 129.2, 128.4, 126.8, 125.2, 124.7, 122.6, 103.2, 89.7, 83.5, 74.5, 68.2, 59.4 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{18}H_{18}F_3N_3NaO_5^+$: 436.109 (MNa^+). Found: 436.109 (MNa^+).

4.4. 4-Amino-5-nitropyrimidin-2(1*H*)-one (7)

Cytosine, **6** (100 mg, 0.9 mmol) was added after 45 min to a stirred mixture of fuming HNO_3 (0.62 mL) and concd sulfuric acid (0.124 mL). The resulting mixture was heated at 80 °C for 18 h. A new spot was seen on TLC (MeOH/$CHCl_3$ 6:4) with R_f=0.64, indicating the formation of the product. The mixture was poured onto ice (5.25 g) and neutralized with satd NaOH. The solid was filtered off and washed with ice-cold water, ice-cold abs EtOH, and ice-cold diethyl ether, and dried in vacuum, yielding 77 mg (60%). Mp>250 °C decomp. IR (ZnSe): ν 3381, 3168, 3077, 2418, 2050, 1951, 1877, 1681, 1650, 1599, 1567, 1511, 1435, 1343, 1318, 1238, 1137, 1102, 1031, 955, 852, 775, 618 cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.88 (br s, 1H), 8.86 (s, 1H), 8.30 (br s, 1H), 8.10 (s, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 157.5, 154.2, 151.9, 118.5 ppm. HRMS (CI/CH_4) *m/z*: calculated for $C_4H_4N_4O_3$: 157.036 (MH^+). Found: 157.036 (MH^+).

4.5. 2-(4-Amino-5-nitro-2-oxopyrimidin-1(2*H*)-yl)-5((benzoyloxy) methyl)tetrahydrofuran-3,4-diyl dibenzoate (8)

5-Nitrocytosine, **7** (3 g, 19.23 mmol) was suspended in HMDS (40 mL, 191.8 mmol) containing TMS–Cl (1 mL, 7.82 mmol). The mixture was heated under reflux for 36 h and then concentrated in vacuo. To this crude material was added β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (9.737 g, 19.30 mmol) and the material was suspended in MeCN (125 mL). The suspension was degassed with N_2, then $SnCl_4$ (23 mL in 1 M solution in CH_2Cl_2) was added. The suspension quickly turned clear and then the solution was stirred for 3 h at room temperature. Distilled water (100 mL) was added with vigorous stirring to hydrolyze the remaining $SnCl_4$. This facilitated the precipitation of the product. The solid was collected, suspended in EtOAc (500 mL) and washed with aq Na_2CO_3 (600 mL×4). The organic layer was diluted with EtOAc and washed with satd NaCl (700 mL×1) and H_2O (700 mL×2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting solid was triturated with $CHCl_3$ and dried in vacuo yielding 8 g of the product (70%). Mp 218–220 °C decomp. (Reported mp 211–213 °C decomp.).[43] $[\alpha]_D^{23}$ −101.3 (*c* 1.02, $CHCl_3$). IR (ZnSe): ν 3480, 3366, 3060, 1716, 1684, 1653, 1601, 1589, 1583, 1491, 1119, 1418, 1363, 1281, 1265, 1130, 1110, 1093, 1039, 1069, 1048, 1024, 927, 891, 851, 819, 761, 736, 703 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.08 (s, 1H), 8.27 (br s, 1H), 8.07 (d, J=6.2 Hz, 2H), 7.95 (s, 1H), 7.92–7.89 (m, 4H), 7.55–7.52 (m, 3H) 7.45–7.31 (m, 6H),

6.33 (d, J=2.1 Hz, 1H), 5.91–5.91 (m, 2H), 4.83–4.76 (m, 3H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.1, 165.2, 157.7, 151.8, 146.4, 133.7, 133.7, 133.5, 129.9, 129.8, 129.7, 128.5, 120.2, 90.5, 81.2, 74.9, 70.9, 63.4 ppm. HRMS (MALDI-TOF) m/z: calculated for $C_{30}H_{24}N_4NaO_{10}^+$: 623.138 (MNa^+). Found: 623.140 (MNa^+).

4.6. 2-((Benzoyloxy)methyl)-5-(4,5-diamino-2-oxopyrimidin-1(2*H*)yl)tetrahydrofuran-3,4-diyl dibenzoate (9)

To a solution of **8** (1.037 g, 1.72 mmol) in THF (65 mL) and glacial AcOH (5 mL) was added Pd/C (10%, 377.5 mg). The flask was charged with H_2 (1.5 atm), and stirred for 7 h at room temperature. The reaction mixture was filtered through a pad of Celite, washed with excess MeOH, and concentrated in vacuo. The crude material was purified by automated column chromatography (10% MeOH in EtOAc) yielding pure **9** (363 mg, 38%). Mp 150–153 °C (Reported mp 153–157 °C).[43] $[\alpha]_D^{23}$ −61 (c 1.10, $CHCl_3$). IR (ZnSe): ν 3064, 1719, 1672, 1600, 1488, 1450, 1377, 1315, 1263, 1176, 1092, 1069, 1024, 1024, 803, 776, 701 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, J=6.2 Hz, 2H), 7.99 (d, J=6.2 Hz, 4H), 7.63 (t, J=5.3 Hz, 3H), 7.51–7.41 (m, 6H), 7.11 (s, 1H), 6.11 (d, J=5.4 Hz, 1H), 5.90–5.86 (m, 2H), 4.70–4.64 (m, 3H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.5, 164.6, 164.6, 161.4, 146.4, 153.7, 133.8, 133.8, 133.5, 129.3, 128.7, 123.2, 116.6, 90.3, 78.3, 78.3, 73.5, 71.1, 64.1 ppm. HRMS (MALDI-TOF) m/z: calculated for $C_{30}H_{26}N_4NaO_8^+$: 593.164 (MNa^+). Found: 593.167 (MNa^+).

4.7. 4,5-Diamino-1-(3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-on (2)

Compound **9** (820 mg, 1.45 mmol) was suspended in aq EtOH (40 mL, 4:1 EtOH/H_2O) at room temperature. 1 M NaOH (3.5 mL, 6 mmol) was added at 20 min intervals and the solution was stirred for 3 h. The reaction was then acidified to pH ca. 2 with 1 M HCl and EtOH was removed in vacuo. The water layer was extracted with $CHCl_3$, neutralized to pH ca. 8 with 10% NH_4OH, and then concentrated in vacuo. The crude material was dissolved in H_2O (4 mL) followed by an addition of EtOH (40 mL), the solution was cooled to −20 °C and the resulting solid was collected, and washed with excess Et_2O. The resulting solid was dissolved in H_2O and freeze-dried to dryness yielding **2** as a light yellow solid (260 mg, 70% yield). Mp 190–193 °C decomp. (Reported 191–192 °C).[43] $[\alpha]_D^{23}$ −14.5 (c 0.88, H_2O). IR (ZnSe): ν 3189, 2921, 1716, 1659, 1574, 1488, 1404, 1312, 1252, 1093, 1046, 935, 856, 741, 617 cm^{-1}. ^{1}H NMR (600 MHz, D_2O): δ 7.51 (s, 1H), 5.88 (d, J=5.3 Hz, 1H), 4.28–4.26 (m, 1H), 4.19–4.17 (m, 1H), 4.11–4.10 (m, 1H) 3.91–3.78 (m, 2H) ppm. ^{13}C NMR (150 MHz, D_2O) δ: 159.9, 153.1, 127.7, 115.2, 89.7, 84.0, 73.8, 69.2, 60.6 ppm. HRMS (MALDI-TOF) m/z: calculated for $C_9H_{14}N_4NaO_5^+$: 281.086 (MNa^+). Found: 281.087 (MNa^+).

4.8. General procedure for synthesis of protected imidazolocytidine derivatives (11a–d): 2-(benzoyloxymethyl)-5-(2-oxo-8-phenyl-2,9-dihydro-1*H*-purin-1-yl)tetrahydrofuran-3,4-diyl dibenzoate (11a)

Benzaldehyde **10a** (20 μL, 0.17 mmol) and **9** (100 mg, 0.17 mmol) were thoroughly mixed in DMF (2 mL), then p-TsOH (7 mg, 0.034 mmol) was added, and the solution heated and stirred at 80 °C for 2–4 h and monitored by TLC ($CHCl_3$/MeOH 9.5:0.5). When the reaction was completed, the solution was cooled to room temperature. The reaction mixture was added dropwise with vigorous stirring into a mixture of Na_2CO_3 (0.034 mmol) and H_2O (10 mL). The crude product was extracted into EtOAc, the organic phase was washed with H_2O, brine and dried (Na_2SO_4). Evaporation of solvent gave the crude product, which was purified by column chromatography over silica gel (9:1 $CHCl_3$/EtOH) yielding 40 mg (36%). Mp 152–155 °C. $[\alpha]_D^{23}$ −81.5 (c 0.97, $CHCl_3$). IR (ZnSe): ν 3181, 2928, 1721, 1673, 1583, 1531, 1488, 1451, 1407, 1314, 1262, 1176, 1092, 1068, 1025, 935, 776, 703, 617 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.38 (m, 2H), 8.12 (d, J=7.7 Hz, 2H), 8.01 (d, J=7.52 Hz, 2H), 7.94 (d, J=7.52 Hz, 2H), 7.58–7.53 (m, 4H), 7.48–7.47 (m, 2H), 7.43–7.35 (m, 6H), 6.45–6.44 (m, 1H), 6.07 (br s, 1H), 5.98–5.96 (m, 1H), 4.91–4.89 (m, 1H), 4.86–4.84 (m, 3H) ppm. ^{13}C NMR (175 MHz, $CDCl_3$) δ: 166.3, 165.2, 165.1, 163.1, 157.62, 154.5, 134.2, 133.6, 133.4, 131.8, 130.1, 130.0, 129.9, 129.8, 129.5, 129.1, 128.9, 128.5, 127.9, 126.3, 93.1, 81.0, 76.1, 71.2, 64.2 ppm. HRMS (MALDI-TOF) m/z: calculated for $C_{37}H_{28}N_4NaO_8^+$: 679.180 (MNa^+). Found: 679.179 (MNa^+).

4.9. 2-((Benzoyloxy)methyl)-5-(2-oxo-8-(*p*-tolyl)-2,9-dihydro-1*H*-purin-1-yl)tetrahydrofuran-3,4-diyl dibenzoate (11b)

Product **10b** was obtained by condensation of **9** (200 mg, 0.35 mmol) with p-tolyl aldehyde **10b** (38 μL, 0.35 mmol) with p-TsOH (13.5 mg, 0.07 mmol) as a catalyst, according to the general procedure. Compound **11b** was obtained as a white solid in 66% yield (157 mg). Mp 141–144 °C. $[\alpha]_D^{23}$ −113.2 (c 0.98, $CHCl_3$). IR (ZnSe): ν 2978, 1720, 1675, 1584, 1261, 1091, 1068, 1024, 778, 704, 617 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (s, 1H), 8.24 (d, J=8.2 Hz, 2H), 8.12–8.11 (m, 2H), 8.02–7.99 (m, 2H), 7.95–7.92 (m, 2H), 7.61–7.50 (m, 3H), 7.42–7.37 (m, 6H), 2.26 (d, J=8.2 Hz, 2H), 6.43 (d, J=3.13 Hz, 1H), 6.10–6.08 (m, 1H), 6.01–5.97 (m, 1H), 4.90–4.83 (m, 3H), 2.32 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.3, 165.2, 165.2, 163.2, 158.0, 154.6, 142.3, 133.6, 133.4, 129.9, 129.9, 129.8, 129.3, 128.9, 128.7, 128.7, 128.5, 128.5, 127.9, 125.1, 92.6, 80.5, 75.4, 70.7, 63.6, 21.5 ppm. HRMS (MALDI-TOF) m/z: calculated for $C_{38}H_{30}N_4NaO_8^+$: 693.196 (MNa^+). Found: 693.193 (MNa^+).

4.10. 2-((Benzoyloxy)methyl)-5-(8-(4-methoxyphenyl)-2-oxo-2,9-dihydro-1*H*-purin-1-yl)tetrahydrofuran-3,4-diyl dibenzoate (11c)

Product **11c** was obtained by condensation of **9** (200 mg, 0.35 mmol) with anisaldehyde **10c** (40 μL, 0.35 mmol) with p-TsOH (13.5 mg, 0.07 mmol) as a catalyst, according to the general procedure. Compound **3c** was obtained as a white solid in 42% yield (103 mg). Mp 151–153 °C. $[\alpha]_D^{23}$ −110.2 (c 1.05, $CHCl_3$). IR (ZnSe): ν 2993, 1725, 1680, 1615, 1545, 1260, 1094, 1054, 1036, 1022, 778, 734, 614 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48 (s, 1H), 8.31 (d, J=8.7 Hz, 2H), 8.09 (d, J=7.53 Hz, 2H), 7.98 (d, J=7.53 Hz, 2H), 7.90 (d, J=7.53 Hz, 2H), 7.54–7.50 (m, 3H), 7.38–7.25 (m, 6H), 6.96 (d, J=8.7 Hz, 2H), 6.35 (br s, 1H), 6.14–6.15 (m, 1H), 6.03–6.01 (m, 1H), 4.81–4.45 (m, 3H), 3.78 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.3, 165.2, 163.2, 162.7, 154.7, 133.6, 133.4, 129.8, 129.3, 128.9, 128.6, 128.5, 128.5, 120.3, 114.5, 114.1, 93.1, 81.0, 76.0, 71.2, 64.2 ppm. HRMS (MALDI-TOF) m/z: calculated for $C_{38}H_{30}N_4NaO_9^+$: 709.191 (MNa^+). Found: 709.194 (MNa^+).

4.11. 2-((Benzoyloxy)methyl)-5-(2-oxo-8-(4-(trifluoromethyl)phenyl)-2,9-dihydro-1*H*-purin-1-yl)tetrahydrofuran-3,4-diyl dibenzoate (11d)

Product **3d** was obtained by condensation of **9** (200 mg, 0.35 mmol) with p-trifluoromethylbenzaldehyde **10e** (60 μL, 0.35 mmol) with p-TsOH (13.5 mg, 0.07 mmol) as a catalyst, according to the general procedure. Compound **11d** was obtained as a white solid in 47% yield (120 mg). Mp 160–163 °C. $[\alpha]_D^{23}$ −75 (c 1.13, $CHCl_3$). IR (ZnSe): ν 2987, 1720, 1644, 1584, 1451, 1316, 1261, 1175, 1091, 1064, 1025, 778, 705, 616 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69 (s, 1H), 8.48–8.45 (m, 2H), 8.11–8.09 (m, 2H), 8.02–7.99 (m, 2H), 7.93–7.90 (m, 2H), 7.70–7.68 (m, 2H), 7.54–7.52 (m, 3H), 7.40–7.34 (m, 6H), 6.40 (br s, 1H), 6.15 (br s, 1H), 5.94–5.90 (m, 1H), 4.93–4.86 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.0, 166.2, 165.2, 165.1, 162.7, 158.8, 156.1, 154.8, 134.9, 133.8, 133.7, 133.1,

129.9, 129.2, 128.7, 128.6, 128.6, 128.5, 128.3, 128.0, 126.0, 125.9, 125.6, 125.0, 92.1, 80.4, 75.2, 70.2, 63.2 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{38}H_{27}F_3N_4NaO_8^+$: 747.167 (MNa^+). Found: 747.164 (MNa^+).

4.12. General procedure for synthesis of imidazolo-cytidine derivatives (3a–d): 1-(3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-phenyl-1*H*-purin-2(9*H*)-one (3a)

Protected nucleoside **11a** (100 mg, 0.15 mmol) was added to a solution of NaOH (9 equiv) in MeOH (2 mL) and the resulting mixture was stirred at room temperature for 2 h. After evaporation of the MeOH the product was purified by an automated medium pressure column chromatography (8:2 $CHCl_3$/MeOH) yielding 31 mg (60%) of a white solid. Mp 207–210 °C decomp. $[\alpha]_D^{23}$ +3.2 (*c* 0.94, DMSO). IR (ZnSe): ν 3290, 2978, 1701, 1676, 1645, 1619, 1584, 1476, 1324, 1263, 1168, 1100, 1015, 935, 834, 1091, 778, 704, 702 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 9.09 (s, 1H, H6), 8.13–8.12 (m, 2H), 7.55–7.54 (m, 3H), 5.91 (d, *J*=2.40 Hz, 1H), 5.53 (br s, 1H) 5.39 (br s, 1H), 5.01 (br s, 1H) 4.07–4.06 (m, 1H), 4.03–4.02 (m, 1H), 3.97–3.96 (m, 1H), 3.85–3.84 (m, 1H) 3.69–3.68 (m, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 161.9, 134.66, 131.0, 129.2, 128.9, 128.9, 126.7, 91.2, 83.9, 75.1, 68.2, 59.37 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{16}H_{16}N_4NaO_5^+$: 367.101 (MNa^+). Found: 367.103 (MNa^+).

4.13. 1-(3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-(*p*-tolyl)-1*H*-purin-2(9*H*)-one (3b)

Product **3b** was obtained by treating the protected nucleoside **11b** (100 mg, 0.15 mmol) with NaOH in MeOH according to the general procedure, yielding 45 mg (85%) of a white solid. Mp>250 °C decomp. $[\alpha]_D^{23}$ +5.5 (*c* 1.12, DMSO). IR (ZnSe): ν 3281, 2977, 1720, 1674, 1584, 1476, 1414, 1356, 1324, 1261, 1171, 1091, 1068, 1034, 1015, 965, 854, 778, 704, 617 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.02 (d, *J*=8.2 Hz, 2H), 7.34 (d, *J*=8.2 Hz, 2H), 5.91 (d, *J*=2.5 Hz, 1H), 5.53 (br s, 1H), 5.39 (br s, 1H), 5.04 (br s, 1H), 4.06–4.05 (m, 1H), 4.02–4.01 (m, 1H), 3.96–3.95 (m, 1H), 3.83–3.82 (m, 1H), 3.68–3.67 (m, 1H), 2.38 (s, 3H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 162.4, 157.3, 153.8, 140.5, 133.5, 129.1, 128.8, 126.6, 91.1, 84.1, 74.9, 68.3, 59.6, 20.7 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{17}H_{18}N_4NaO_5^+$: 381.117 (MNa^+). Found: 381.118 (MNa^+).

4.14. 1-(3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-(4-methoxyphenyl)-1*H*-purin-2(9*H*)-one (3c)

Product **8c** was obtained by treating protected nucleoside **11c** (100 mg, 0.15 mmol) with NaOH in MeOH according to the general procedure, yielding 35 mg (65%) of a white solid. Mp 220–223 °C decomp. $[\alpha]_D^{23}$ +5.6 (*c* 0.99, DMSO). IR (ZnSe): ν 3324, 2992, 1725, 1680, 1615, 1547, 1467, 1434, 1389, 1324, 1261, 1167, 1110, 1094, 1055, 1036, 1021, 998, 943, 778, 736, cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 8.91 (s, 1H), 8.08 (d, *J*=8.1 Hz, 2H), 7.07 (d, *J*=8.1 Hz, 2H), 5.91 (d, *J*=2.3 Hz, 1H), 5.51 (br s, 1H), 5.36 (br s, 1H), 5.02 (br s, 1H), 4.02–4.01 (m, 1H), 3.96–3.95 (m, 1H), 3.95–3.94 (m, 1H), 3.83–3.82 (m, 4H), 3.68–3.68 (m, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 161.3, 128.5, 114.3, 91.2, 83.9, 75.1, 68.3, 59.6, 55.4 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{17}H_{18}N_4NaO_6^+$: 397.112 (MNa^+). Found: 397.111 (MNa^+).

4.15. 1-(3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-(4-(trifluoromethyl)phenyl)-1*H*-purin-2(9*H*)-one (3d)

Product **8d** was obtained by treating protected nucleoside **11d** (120 mg, 0.17 mmol) with NaOH in MeOH according to the general procedure, yielding 56 mg (80%) of a yellowish solid. Mp 195–200 °C decomp. $[\alpha]_D^{23}$ +7.9 (*c* 1.16, DMSO). IR (ZnSe): ν 3298, 1664, 1619, 1584, 1499, 1446, 1403, 1373, 1324, 1262, 1170, 1102, 1065, 1046, 1016, 944, 848, 780, 701 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.02 (d, *J*=8.2 Hz, 2H), 7.34 (d, *J*=8.2 Hz, 2H), 5.91 (d, *J*=2.5 Hz, 1H), 5.53 (br s, 1H), 5.39 (br s, 1H), 5.04 (br s, 1H), 4.07–4.06 (m, 1H), 4.04–4.03 (m, 1H), 3.99–3.97 (m, 1H), 3.88–3.84 (m, 1H), 3.70–3.67 (m, 1H), 2.38 (s, 3H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ: 162.4, 157.3, 153.8, 140.5, 133.5, 129.1, 128.8, 126.6, 91.1, 84.1, 74.9, 68.3, 59.6, 20.7 ppm. HRMS (MALDI-TOF) *m/z*: calculated for $C_{17}H_{15}F_3N_4NaO_5^+$: 435.089 (MNa^+). Found: 435.086 (MNa^+).

4.16. NMR base-paring experiments

Approximate amounts of guanosine and **3d** were put into volumetric flasks and stored under vacuum overnight to remove adsorbed water. All ^1H NMR spectra were measured in DMSO-d_6 at 700 MHz. The data were collected at 300 K. ^1H NMR spectra were obtained at a range of concentrations of **8d** (0.007 M, 0.013 M, 0.020 M, and 0.030 M) with constant concentration of guanosine (0.02 M) in DMSO-d_6 solution.

4.17. Absorbance and fluorescence measurements

Absorption spectra were determined in H_2O (pH=7) for compounds **1a–c**, **2** and MeOH for analogues **3a–d**. The concentrations of samples were 2–5 μM. Absorbance was kept less than 0.05 AU in order to avoid inner filter distortion. Samples were measured in a 10 mm quartz cell. For accurate measurements of the fluorescence, the concentrations of the samples were in the linear range in which the Beer–Lambert law applies, the optical density was less than 0.05 to avoid the inner filter effect.[58] Emission spectra of compounds **1c**, **2** were determined in H_2O (pH 7) and in MeOH for compounds **1a**,**b**, and **3a–d**. Measurement conditions of analogues **6–8** included 740 V sensitivity and a 4 nm slit. The concentration of the samples was 3 μM. Samples were measured in a 10 mm quartz cell.

4.18. Quantum yield measurements

The quantum yield of each compound was calculated from the observed absorbance and the area of the fluorescence emission band. For accurate measurements of the fluorescence, the concentrations of the samples were in the linear range in which the Beer–Lambert law applies, and the optical density was less than 0.05 to avoid the inner filter effect. The fluorescence quantum yield (Φ_F) was calculated based on Φ of a known reference, tryptophan (Φ 0.14, λ_{abs} 280 nm, λ_{em} 350 nm in H_2O)[59] or quinine sulfate (Φ 0.54, λ_{abs} 350 nm, λ_{em} 446 nm in 0.1 M H_2SO_4),[60] according to Eq. 9. λ_{abs} and λ_{em} values of the selected reference compounds were in the same range as those of the evaluated compounds.

$$\Phi_F = \Phi_R I/I_R^* OD_R/OD^* \eta^2/\eta_R \quad (9)$$

where R=Reference, I=Integration of the peak and η=refractive index of the solvent.

Supplementary data

Figures of absorbance (UV–vis), fluorescence spectra, and NMR experiments spectra, of **1a–c**, **2**, **3a–d** (G·**8d**) are available. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.03.005.

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