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Designing new isomorphic fluorescent nucleobase analogues: the thieno[3,2-*d*]pyrimidine core

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Abstract—A convergent approach for a family of fluorescent nucleosides is described. It relies on thieno[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)-dione that serves as a core heterocycle. This condensed pyrimidine is converted into an emissive pyrimidine nucleoside analogue by N-glycosylation and into an emissive purine nucleoside analogue by C-glycosidation at the thiophene's beta position. The design principles of this archetypical system are outlined together with the syntheses and photophysical properties of the resulting nucleosides.

1. Introduction

The core heterocycles commonly found in nucleic acids are practically non-emissive.[1] Numerous efforts to impart useful photophysical features upon these pyrimidines and purines have been reported;[2] many of them are represented in this issue of *Tetrahedron's Symposium-in-Print*. The various families of fluorescent nucleoside analogues can be broadly classified into the following categories:

(1) *Extended nucleobases*: Inspired by the naturally occurring and emissive tricyclic guanine derivatives such as the 'Y' bases,[3] Leonard has condensed an etheno bridge over the H-bonding face of the purines and pyrimidines.[4] Extending the conjugation of the natural bases in this way resulted in modified nucleosides with favorable fluorescence properties. One of the earliest examples is 1,N^6-ethenoadenine (Fig. 1).[5,6] Another approach, originally examined by Leonard,[7] and recently adopted by Kool,[8] involves the expansion of the heterocycle's size by introducing aromatic rings between the pyrimidine and the imidazole ring (e.g., Benzo-A, Fig. 1). Note that such structures deviate from the natural bases and cannot form canonical Watson–Crick base pairs, or be accommodated within a native Watson–Crick duplex.[6] A related approach involves condensing additional aromatic rings on the periphery of the heterocycle. Benzo-[*g*]quinazoline-2,4-(1*H*,3*H*)-dione (BgQ), represents a typical example (Fig. 1).[9,10] More recent examples include several structures of expanded, base-discriminating nucleosides, reported by Okamoto and Saito.[11]

(2) *Conjugated base analogues*: Linking the natural nucleobases to fluorescent chromophores, typically via conjugating linkers (e.g., ethynyl), has been shown to produce emissive nucleoside analogues (Fig. 2). Examples of attached chromophores include pyrene,[12] phenanthroline,[13] dimethylaminonaphthalene,[14] anthracene,[15] and fluorene.[16,17] Since the H-bonding face in many of these derivatives remains intact and the chromophore is projected into the major groove of a double helix, these nucleobase analogues typically maintain the hybridization properties of the parent nucleosides.

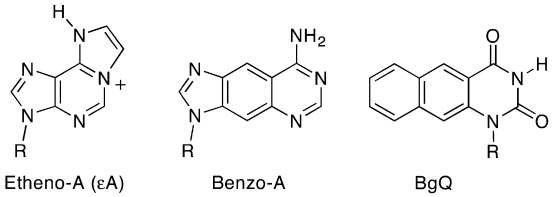

Figure 1. Fluorescent extended nucleobases. R stands for D-ribose or 2′-deoxy-D-ribose.

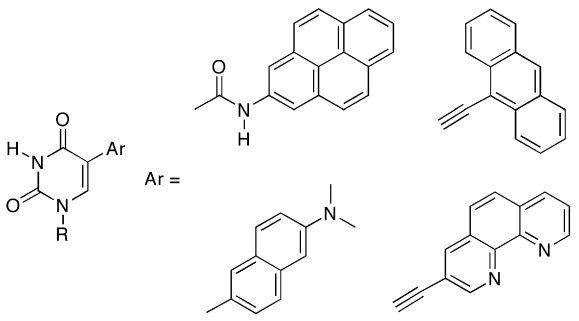

Figure 2. Examples for conjugated base analogues where a fluorophore is linked to a native heterocycle. R stands for D-ribose or 2′-deoxy-D-ribose.

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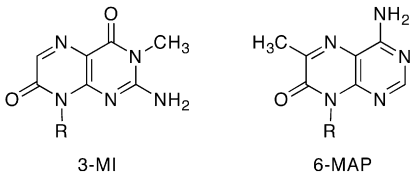


Figure 3. G and A pteridine-based analogues. R stands for D-ribose or 2′-deoxy-D-ribose.

(3) *Pteridine as purine analogues*: The pteridines represent fluorescent heterocycles that contain two condensed six membered rings (Fig. 3).[2c,18] Some of these very useful probes possess a H-bonding face that is identical to the purines found in DNA and RNA. Very little variation in emission wavelengths is observed among these intensely emissive derivatives.

(4) *Aromatic hydrocarbon and chromophoric base analogues*: In these novel structures, the natural heterocycle is replaced with a fluorescent aromatic residue (Fig. 4).[19–21] These analogues can be accommodated within oligonucleotides although they do not form canonical base pairs.

(5) *Isomorphic base analogues*: These fluorescent base analogues closely resemble the corresponding natural nucleobases with respect to their overall dimensions, hydrogen bonding face and ability to form isostructural Watson–Crick base pairs (Fig. 5). Possibly the most utilized nucleobase is 2-aminopurine (2-AP), a highly emissive isosteric adenosine analogue.[22] The exquisite sensitivity of 2-AP to its microenvironment has found numerous applications in biochemical studies and assay development.[23,24] 5-Methylpyrimidin-2-one (m^5K)[25] and pyrrolo-C[26] have been introduced as isomorphic pyrimidine analogues.[27,28]

Despite this diversity in structures and photophysical properties, the implementation of fluorescent nucleoside analogues in biophysical assays remains challenging, as there is no single analogue that can be universally employed. For example, in a recent study we compared ligand binding to fluorescent bacterial A-site RNA constructs.[29] Aminoglycoside antibiotics, a family of naturally occurring highly cationic bactericidal agents, target this autonomous RNA fold, also known as the ribosomal decoding site. Antibiotics discovery assays have been developed, where 2-AP replaces key adenosine residues in this small RNA sequence.[30,31] Intriguingly,

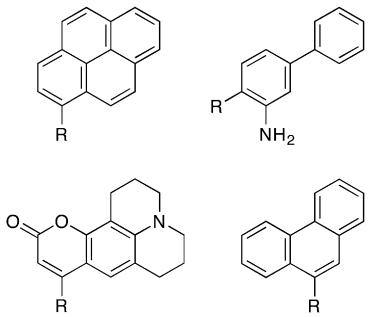


Figure 4. Fluorescent aromatic residues replace the heterocyclic bases. R stands for 2′-deoxy-D-ribose; some of these derivatives have the α configuration at the anomeric center.

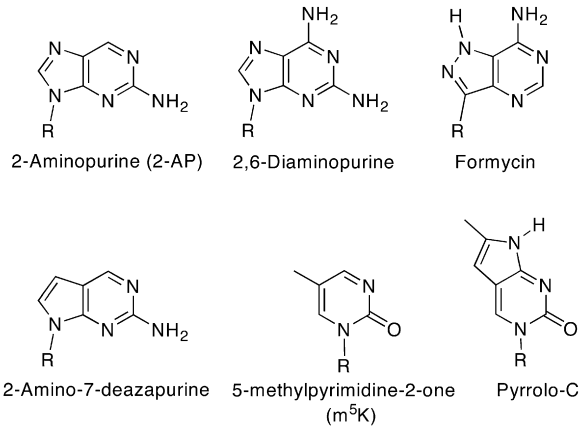


Figure 5. Isomorphic fluorescent purine and pyrimidine bases.

these otherwise extremely useful reporter oligonucleotides do not respond well to some aminoglycosides, particularly neomycin B, one of the most potent A-site binders. We have demonstrated that a fluorescent furan-modified U residue can be incorporated into an A-site construct and used effectively to monitor the binding of neomycin B.[29] This example illustrates the fundamental challenge articulated above and highlights the need for the development of new emissive nucleosides, in particular isosteric analogues of the naturally occurring pyrimidines and purines. Here we disclose a convergent approach for a family of fluorescent nucleosides under investigation in our laboratory. It relies on identifying an emissive condensed pyrimidine core and converting it into fluorescent pyrimidine and purine nucleosides. The design of an archetypical system, as well as its synthesis and photophysical characterization is outlined in this contribution.

2. Results and discussion

2.1. Design

Examination of the photophysical properties of simple condensed conjugated heterocycles such as benzofuran and benzothiophene reveals useful fluorescent properties.[32] Comparing the absorption and emission spectra of benzene and thiophene to that of the corresponding benzo[*b*]thiophene, clearly illustrates the favorable features of the condensed system (Fig. 6). While benzene and thiophene have relatively weak absorption bands above 250 nm and no apparent emission under these conditions, the 'hybrid' molecule, benzo[*b*]-thiophene, is significantly more emissive (Fig. 6).

Using this as a platform, we explored families of fluorescent nucleobases where thieno[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**1**) serves as the core heterocycle (Fig. 7). Glycosylation with D-ribose (or 2′-deoxy-D-ribose) at *N*-1 is expected to provide an expanded and emissive pyrimidine nucleoside analogue **A**, while C-glycosidation at the thiophene's beta position is anticipated to provide a viable route to a fluorescent purine nucleoside analogue **B** (Fig. 7). Elaboration of functional groups can then expand **A** and **B** to include various isosteric pyrimidine and purine analogues that mimic the naturally occurring nucleosides as schematically shown in Figure 7.

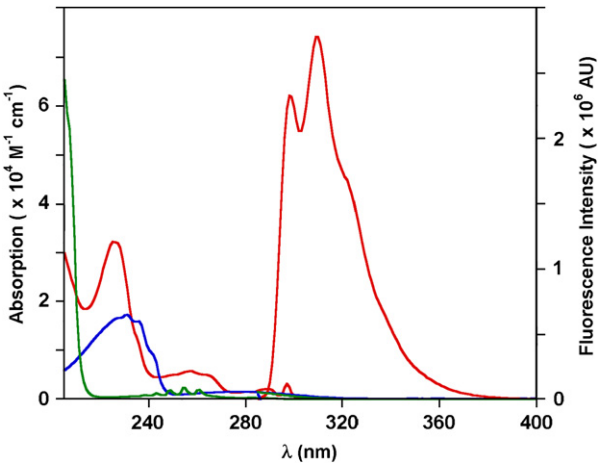


Figure 6. Absorption and emission spectra of benzothiophene (red) in comparison to thiophene (blue) and benzene (green); all taken in hexanes (1×10^{-6} M).

2.2. Synthesis

Not unexpectedly, nucleosides containing the thienopyrimidine core were previously prepared and examined for potential antiviral activity,[33] although their photophysical characteristics have not been reported. As illustrated in Scheme 1, constructing the parent heterocycle is accomplished by treating the commercially available methyl 3-aminothiophene-2-carboxylate with potassium cyanate. The resulting thienopyrimidine can be converted into ribonucleoside **2** using typical N-glycosylation conditions to exclusively provide the β-anomer (Scheme 1).[33] Barton-type deoxygenation of **2** to yield the corresponding 2′-deoxy derivative **3** turned out to be somewhat challenging, but was ultimately achieved using tris(trimethylsily)silane (TTMSS) instead of tri-*n*-butyltinhydride (Scheme 1; see Section 4).

The synthesis of the purine mimic **9** was accomplished as shown in Scheme 2. Bromination of the parent heterocycle

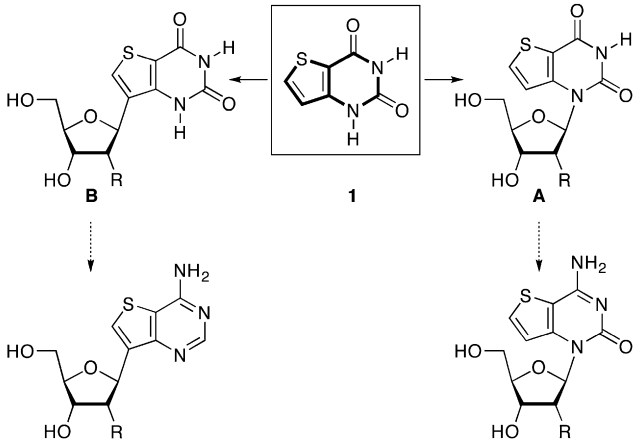
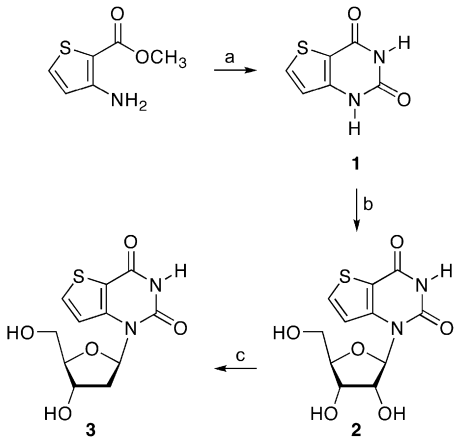
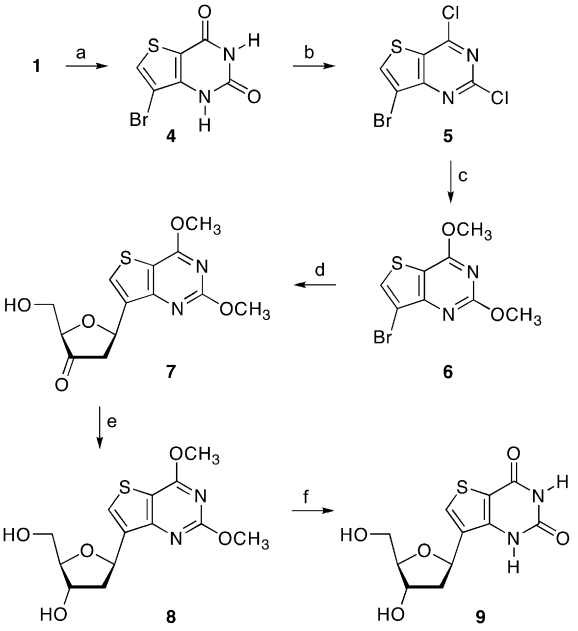


Figure 7. General design of new emissive nucleosides based on the thieno[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)-dione core (**1**). Glycosylation at *N*-1 is expected to provide an expanded pyrimidine analogue **A**, while C-glycosidation at the thiophene's beta position is anticipated to provide a purine analogue **B**. Elaboration of functional groups can then expand **A** and **B** to include various isosteric pyrimidine and purine analogues that mimic the naturally occurring nucleosides.



Scheme 1. Synthesis of thienopyrimidine-based N-nucleosides **2** and **3**. *Reagents and conditions*: (a) KOCN, AcOH/H_2O (80%); (b) (i) Me_3SiCl, $Me_3SiNHSiMe_3$, (ii) 1-*O*-acetyl-2,3,5-tribenzoyl-D-ribofuranoside, CH_2Cl_2, 58% for two steps, (iii) NH_3, MeOH, 76%; (c) (i) imidazole, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, 72%, (ii) PhCSCl, DMAP, CH_3CN followed by AIBN, TTMSS, dioxane, 71%, (iii) TBAF, THF, 71%.

1 with bromine in acetic acid provided the substituted derivative **4** in almost quantitative yield. Treatment with $POCl_3$ yielded the dichloro derivative **5**. Note this reaction has to be carefully monitored to avoid over chlorination. Derivative **5** was then converted into the dimethoxy derivative **6** using sodium methoxide in dry methanol. Palladium mediated coupling with the known glycal, 1,2-dehydro-3-*O*-(*t*-butyldiphenylsilyl)-5-hydroxymethyl-furan, gave the Heck-coupled product, which underwent desilylation under the reaction conditions to provide the keto derivative **7**. Stereospecific reduction using sodium triacetoxyborohydride then yielded **8**. Final de-methylation of the heterocycle using NaI in acetic acid gave the desired C-nucleoside **9** (Scheme 2; see Section 4).



Scheme 2. Synthesis of thienopyrimidine-based C-nucleoside **9**. *Reagents and conditions*: (a) Br_2, AcOH, 97%; (b) $POCl_3$, *N*,*N*-dimethylaniline, 64%; (c) NaOMe, MeOH, 97%; (d) $Pd(OAc)_2$, Bu_4NCl, $NaHCO_3$, 1,2-dehydro-3-*O*-(*t*-butyldiphenylsilyl)-5-hydroxymethyl-furan, DMF, 61%; (e) $NaB(OAc)_3H$, ACN/AcOH 88%; (f) NaI, AcOH, 74%.

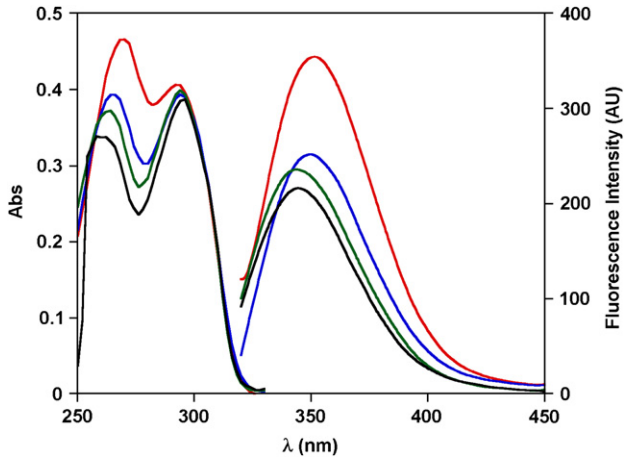


Figure 8. Absorption (5.0×10^{-5} M) and emission (5.0×10^{-6} M) spectra of nucleoside **2** in water (red), methanol (blue), acetonitrile (green), and ethyl acetate (black). Stock solutions for absorption and emission spectra contained 5% and 0.5% DMSO, respectively.

2.3. Photophysical characteristics

Absorption spectra of the free ribonucleoside **2** show, in addition to a high energy transition at around 265 nm, a longer wavelength absorption at ca. 300 nm. Note that, while the former shows slight sensitivity to solvent polarity, the latter, lower energy absorption band is practically insensitive to changes in medium polarity (Fig. 8).[34] Excitation at the lower energy band results in a relatively strong emission centered at around 350 nm (Fig. 8). Quantum yield measurements in water, using tryptophan as a standard, reveal a moderate value of 0.058. Unlike many other modified nucleosides, the emission of **2** is relatively insensitive to solvent polarity. A slight bathochromic shift (ca. 6 nm) and a moderate hyperchromic effect (1.7 fold) are observed when the emission in ethyl acetate is compared to the emission in water (Figs. 8 and 9 and Table 1). Steady-state Stern–Volmer titrations of nucleoside **2** with the native nucleobase monophosphates, show significant quenching by GMP, CMP and TMP and marginal quenching by AMP (Fig. 10). The

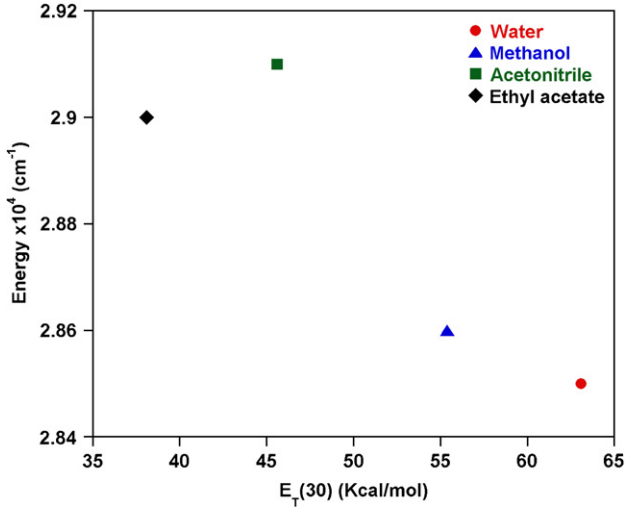


Figure 9. Emission energy plotted against $E_T(30)$, a microscopic solvent polarity scale. Note the trend for the lower emission energy in more polar solvent.

Table 1. Photophysical data of nucleoside **2**[a]

Solvent	λ_{max}[b] (nm)	λ_{em} (nm)	I_{rel}[c]
Water	292	351	1.7
Methanol	294	350	1.3
Acetonitrile	294	344	1.2
Ethyl acetate	296	345	1.0

[a] Conditions for absorption and emission spectra: 50 and 5.0×10^{-6} M, respectively.
[b] The lowest energy maximum is given. Note, the nucleoside shows another intense absorption at higher energies, at around 263–270 nm.
[c] Relative emission intensity with respect to intensity in ethyl acetate.

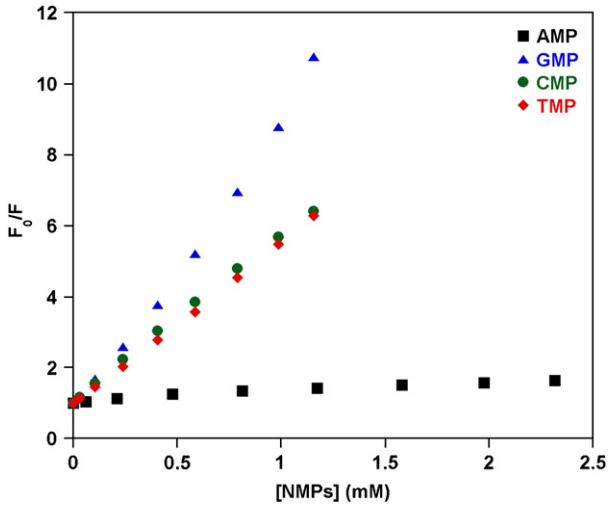


Figure 10. Steady-state Stern–Volmer plot for the titration of nucleoside **2** with AMP, GMP, CMP, and TMP. Quenching constant, K_{sv} for GMP, CMP, and TMP were determined to be 7.72×10^{-3} M^{-1}, 4.83×10^{-3} M^{-1}, and 4.56×10^{-3} M^{-1}, respectively.

2′-deoxy nucleoside **3** shows analogous photophysical features.[35]

The photophysical characteristics of the C-nucleoside **9** are similar to the corresponding N-nucleoside **2**, with small differences in absorption and emission maxima (Fig. 11 and Table 2). The emission quantum yield for **9** is, however,

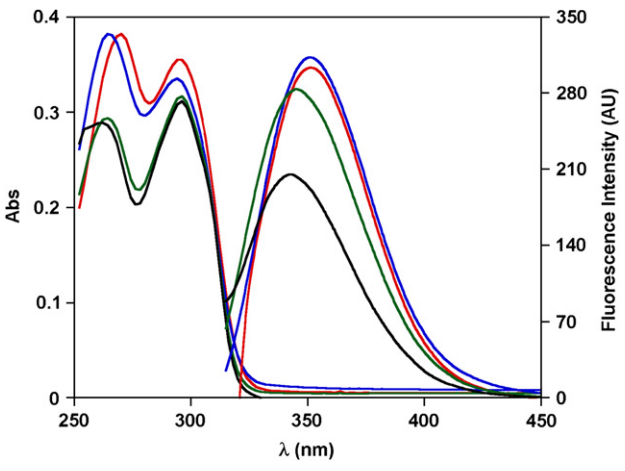


Figure 11. Absorption (5.0×10^{-5} M) and emission spectra (5.0×10^{-6} M) of nucleoside **9** in water (red), methanol (blue), acetonitrile (green), and ethyl acetate (black). Stock solutions for absorption and emission spectra contained 5% and 0.5% DMSO, respectively.

Table 2. Photophysical data of nucleoside **9**[a]

Solvent	λ_{max}[b] (nm)	λ_{em} (nm)	I_{rel}[c]
Water	294	351	1.4
Methanol	294	351	1.5
Acetonitrile	296	345	1.3
Ethyl acetate	296	343	1.0

[a] Conditions for absorption and emission spectra: 50 and 5.0×10^{-6} M, respectively.
[b] The lowest energy maximum is given. Note, the nucleoside shows another intense absorption at higher energies, at around 262–270 nm.
[c] Relative emission intensity with respect to intensity in ethyl acetate.

slightly lower (0.037) compared to the value obtained for **2**. As with **2**, the emission of **9** is relatively insensitive to solvent polarity. A slight bathochromic shift (ca. 8 nm) and a very modest hyperchromic effect (1.4 fold) are observed when the emission in ethyl acetate is compared to the emission in water (Fig. 11, Table 2). These results confirm our working hypothesis and illustrate that emissive pyrimidine and purine analogues can indeed be generated from the thienopyrimidine core **1**.

3. Summary

As a proof of concept, we have demonstrated the synthesis of emissive nucleosides, all derived from a single heterocycle, thieno[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)-dione. N-glycosylation provided an expanded pyrimidine analogue, whereas C-glycosidation at the thiophene's beta position generated a C-nucleoside that can be viewed as an isosteric purine mimic. Rewardingly, all nucleosides were found to be fluorescent with emission centered at around 350 nm and quantum efficiency between 4 and 6%. Further functional group elaboration is anticipated to generate additional emissive derivatives that can mimic the natural nucleobases and contribute to the growing repertoire of biophysically useful and minimally perturbing fluorescent nucleoside analogues.

4. Experimental

4.1. General considerations

All chemicals were obtained commercially and used without further purification. *O*-(*t*-Butyldiphenylsilyl)-5-hydroxymethyl-furan was synthesized from thymidine according to a reported procedure.[36] To increase the reproducibility of the procedure, we have introduced an additional purification step. Specifically, in the last base-induced elimination step, the silylated intermediate was first passed through a short silica gel column using chloroform as an eluent, before being subjected to potassium carbonate in MeOH. Reactions were monitored by thin layer chromatography (TLC) using 0.25 mm thick pre-coated UV sensitive silica gel plates. Compounds were visualized with short-wave ultraviolet light at 254 nm and/or by immersion in staining solution (cerric ammonium molybdate). Flash chromatography was carried out using Kieselgel 60 (230–400 mesh). NMR spectra were recorded on a Varian Mercury 300 MHz or Varian Mercury 400 MHz spectrometers. Mass spectra were measured at the UCSD Mass Spectrometry Facility. UV–vis spectra were recorded on a Hewlett Packard 8452 A Diode Array Spectrophotometer. Emission spectra were recorded using Perkin Elmer LS 50B and FluoroMax-3, Horiba Jobin Yvon luminescence spectrometer.

4.2. Synthesis of nucleoside 2

The parent heterocycle **1** was synthesized from methyl 3-aminothiophene-2-carboxylate and potassium cyanate and was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose to afford 1-(β-D-ribofuranosyl)thieno[3,2-*d*]pyrimidine-2,4-dione (**2**) as reported.[33]

4.3. Synthesis of nucleoside 3

4.3.1. 1-(2-Deoxy-β-D-erythropentosyl)thieno[3,2-*d*]-pyrimidine-2,4-dione (3). Imidazole (1.8 g, 26.6 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.8 mL, 6.66 mmol) were added successively to a solution of the deprotected ribonucleoside **2** (2 g, 6.66 mmol) in anhydrous DMF (27 mL) under an inert atmosphere. The mixture was stirred at room temperature for 1 h, and partitioned between H_2O and $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ solution followed by H_2O, dried over Na_2SO_4, filtered, and concentrated to give a clear syrup, which was purified by flash chromatography (10–50% EtOAc/ hexanes) to give 2.6 g of a white foam (72% yield). (TLC CH_2Cl_2:CH_3OH 98:2, R_f=0.29); IR (KBr) cm^{-1}: 3600–3440 (OH), 1710–1660 (CO), 1490, 1390, 1110, 1050; ^1H NMR (DMSO-d_6): δ 11.62 (1H, NH), 8.12 (d, 1H, H-6, J=5.4 Hz), 7.37 (d, 1H, H-7, J=5.4 Hz), 6.15 (1H, OH), 5.81 (d, 1H, H-1′), 5.17 (m, 1H, H-2′), 4.68 (m, 1H, H-3′), 4.54 (m, 1H, H-4′), 3.97 (m, 2H, CH_2-5′), 1.05 (s, 24H, CH_3); ^{13}C NMR (DMSO-d_6): δ 157.5, 150.3, 145.5, 135.4, 117.6, 113.5, 92.6, 80.9, 71.4, 70.2, 61.4, 17.0; ESI-MS *m/z* calcd for $C_{23}H_{38}N_2O_7SSi_2$ $[M–H]^-$ 541, found 541.

The silyl protected ribonucleoside (800 mg, 1.48 mmol) and DMAP (558 mg, 4.6 mmol) were flushed with argon and dissolved in anhydrous CH_3CN (48 mL). Phenylthionylchloride (307 μL, 2.21 mmol) was added to the mixture under argon, and the reaction mixture was stirred at room temperature for 4 h. The mixture was concentrated, taken up in CH_2Cl_2, washed with ice water, 0.5 M HCl, then with ice water again. The organic layer was dried over Na_2SO_4, and concentrated to produce a tan foam. The foam was dried under vacuum and freeze-pump-thawed several times. AIBN (73 mg, 0.44 mmol) was added to the flask and the mixture was dissolved in anhydrous dioxane (15 mL) and transferred to a three-neck flask that was fitted with an addition funnel and condenser. TTMSS (1.2 mL, 3.84 mmol) was added dropwise, and the mixture was heated to 100 °C. After 2 h, the dioxane was removed under reduced pressure to give an oil, which was purified over a silica column (0–1% MeOH/CH_2Cl_2) to give 552 mg of a yellow foam (71% yield). (TLC EtOAc/hexanes R_f=0.41). ^1H NMR ($CDCl_3$): δ 8.03 (d, 1H, H-6), 7.71 (d, 1H, H-7, J=5.37 Hz), 7.24 (d, 1H, H-6, J=5.37 Hz), 6.50 (s, 1H, H-1′), 4.84 (m, 1H, H-3′), 4.04 (m, 1H, H-4′), 3.82 (m, 2H, CH_2-5′), 2.53 (m, 2H, CH_2-2′), 1.10 (s, 24H, CH_3); ^{13}C NMR ($CDCl_3$): δ 157.2, 149.9, 145.8, 134.9, 118.1, 94.9, 80.5, 74.6, 69.5, 60.9, 27.9, 17.6; ESI-MS *m/z*

calcd for $C_{23}H_{38}N_2O_6SSi_2$ $[M+H]^+$ 527, found 527, $[M+Na]^+$ calcd 549, found 549.

The siloxane protected nucleoside (500 mg, 0.95 mmol) was dissolved in anhydrous THF (19 mL) and treated with a solution of tetrabutylammonium fluoride in THF (1 M, 1.9 mL). The reaction mixture was stirred at room temperature for 1 h and then concentrated to give the crude product. The resulting oil was purified by flash chromatography (5–10% CH_3OH/CH_2Cl_2) to give 242 mg of a white solid (71% yield). This compound was crystallized from water. (TLC CH_2Cl_2:CH_3OH 9:1, R_f=0.20); IR (KBr) cm^{-1}: 3500–3420 (OH), 3180 (NH), 1675 (CO), 1490, 1420, 1090, 1010; ^1H NMR (DMSO-d_6): δ 11.52 (1H, NH), 8.06 (d, 1H, H-6), 7.67 (d, 1H, H-7), 6.52 (d, 1H, H-1′), 5.00–5.24 (OH), 4.63 (m, 1H, H-3′), 3.73 (m, 1H, H-4′), 3.65 (m, 2H, CH_2-5′), 2.42 (m, 2H, H-2′); ^{13}C NMR (DMSO-d_6): δ 157.7, 150.7, 144.2, 134.7, 119.4, 114.0, 86.7, 83.7, 69.4, 60.6, 37.2; ESI-MS *m/z* calcd for $C_{11}H_{12}N_2O_5S$ $[M-H]^-$ 283, found 283. An X-ray crystal structure further confirmed the structure and the anoneric configuration of this nucleoside.

4.4. Synthesis of nucleoside 9

4.4.1. 7-Bromothieno[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4). To compound **1** (1.0 g, 5.9 mmol) in AcOH (40 mL) was added bromine (0.91 mL, 17.8 mmol) and the mixture was kept at 110 °C inside a pressure tube for 48 h. The solution was cooled; the solvent removed under vacuum and cold water was added. The precipitate was filtered, washed with water, and dried under vacuum to give **4** (1.43 g, 97%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 11.42 (s, 1H), 8.22 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.4, 151.5, 144.4, 132.9, 111.6, 99.0; ESI-MS *m/z* calcd for $C_6H_4BrN_2O_2S$ $[M+H]^+$ 246.91, found 247.04.

4.4.2. 7-Bromo-2,4-dichlorothieno[3,2-*d*]pyrimidine (5). To compound **4** (2.2 g, 8.9 mmol) and $POCl_3$ (8.2 mL, 89 mmol) was added *N*,*N*-diethylaniline (5.7 mL, 35.6 mmol) and the solution was refluxed for 3 h. It was cooled and volatiles were removed under vacuum. The residue was diluted with $CHCl_3$ and washed with ice-cold H_2O (twice). The organic layers were dried (Na_2SO_4), the solvent removed, and the residue purified by flash chromatography (5% EtOAc/hexane). The product **5** (1.63 g, 64%) was isolated as a white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9, 157.3, 156.1, 135.4, 128.5, 109.4; ESI-MS *m/z* calcd for $C_6H_2BrCl_2N_2S$ $[M+H]^+$ 282.84, found 283.05.

4.4.3. 7-Bromo-2,4-dimethoxythieno[3,2-*d*]pyrimidine (6). To compound **5** (0.27 g, 0.9 mmol) in dry MeOH (30 mL) was added NaOMe (0.20 g, 3.8 mmol) and the mixture refluxed for 22 h. The reaction was cooled, HCl (1 M, 1.9 mL) added, and extracted with CH_2Cl_2. The organic layers were dried (Na_2SO_4) and the solvent removed to yield **6** (0.25 g, 97%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 4.13 (s, 3H), 4.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 164.5, 159.5, 130.9, 111.0, 108.4, 55.1, 54.5; ESI-MS *m/z* calcd for $C_8H_8BrN_2O_2S$ $[M+H]^+$ 274.94, found 275.04.

4.4.4. 2,4-Dimethoxy-7-(β-D-*glycero*-pentofuran-3′-ulos-1′yl)thieno[3,2-*d*]pyrimidine (7). Compound **6** (0.11 g, 0.4 mmol) and tetrabutylammonium chloride (0.81 g, 2.9 mmol) were co-evaporated with CH_3CN and dried under vacuum for 1 h. Separately, 1,2-dehydro-3-*O*-(*t*-butyldiphenylsilyl)-5-hydroxymethyl-furan (0.27 g, 0.8 mmol; see Section 4.1) was also co-evaporated with CH_3CN and dried under vacuum. Compound **4**/Bu_4NCl was dissolved in dry DMF (5 mL) and purged under argon for 5 min. $Pd(OAc)_2$ (0.03 g, 0.1 mmol) and $NaHCO_3$ (0.05 g, 0.7 mmol) were added followed by the furan in DMF (4 mL). The solution was heated at 40 °C for 36 h, cooled, and the solvent was removed. The residue was purified by flash chromatography (50% EtOAc/hexane), yielding **7** (0.07 g, 61%) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.79 (s, 1H), 5.50 (q, 1H, H-1′, *J*=4.8 Hz, *J*=11.2 Hz), 4.88 (d, 1H, OH, *J*=7.2 Hz), 4.14 (s, 3H), 4.09 (t, 1H, H-4′, *J*=2.8 Hz), 4.01 (s, 3H), 3.96 (s, 2H, H-5′), 3.21 (q, 1H, H-2′, *J*=6.8 Hz, *J*=18 Hz), 2.81 (dd, 1H, H-2′, *J*=6 Hz); ^{13}C NMR (100 MHz, CD_3CN): δ 214.1, 166.0, 163.9, 160.6, 135.2, 133.0, 112.7, 82.5, 73.2, 61.9, 55.0, 54.4, 43.1; ESI-MS *m/z* calcd for $C_{13}H_{14}N_2O_5S$ $[M+Na]^+$ 333.06, found 333.03.

4.4.5. 1′-β-[7-(2,4-Dimethoxythieno[3,2-*d*]pyrimidine)]-2′-deoxyribofuranose (8). Sodium triacetoxyborohydride (0.11 g, 0.5 mmol) was added to a solution of **7** (0.12 g, 0.4 mmol) in CH_3CN/AcOH (1:1, 20 mL) at −15 °C and the mixture stirred for 1 h. It was warmed to room temperature, the solvent removed, and the residue purified by flash chromatography (5% MeOH/EtOAc), to afford **8** (0.11 g, 88%) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.70 (s, 1H), 5.43 (q, 1H, H-1′, *J*=5.6 Hz, *J*=11.2 Hz), 4.67 (d, 1H, H-3′, *J*=5.2 Hz), 4.12 (s, 3H), 4.01 (s, 3H), 3.94 (d, 1H, H-5′, *J*=12 Hz), 3.77 (d, 1H, H-5′, *J*=12 Hz), 2.74 (m, 1H, H-2′), 2.09 (m, 1H, H-2′), 2.05 (m, 1H, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 163.3, 160.3, 134.7, 132.8, 112.9, 94.2, 88.7, 75.0, 63.8, 55.3, 54.3, 41.8; ESI-MS *m/z* calcd for $C_{13}H_{16}N_2O_5S$ $[M+Na]^+$ 335.08, found 335.07.

4.4.6. 1′-β-[7-(Thieno[3,2-*d*]pyrimidine-2,4-dione)]-2′-deoxyribofuranose (9). To compound **8** (0.02 g, 0.06 mmol) in AcOH (2 mL) was added NaI (0.05 g, 0.3 mmol) and the reaction stirred at 60 °C for 2 h. The solution was cooled, the solvent removed, and the residue diluted with EtOAc. It was washed with $NaHCO_3$, the organic layer dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (10% MeOH/CH_2Cl_2), to give **9** (0.01 g, 74%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 2H), 7.98 (s, 1H), 5.81 (s, 1H, OH-5′), 5.22 (dd, 1H, H-1′, *J*=3.6 Hz, *J*=10 Hz), 5.13 (d, 1H, OH-3′, *J*=3.2 Hz), 4.26 (m, 1H, H-3′), 3.85 (m, 1H, H-4′), 3.60 (m, 2H, H-5′), 1.98 (m, 2H, H-2′); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.0, 151.4, 144.0, 131.6, 131.5, 112.5, 87.7, 75.2, 72.9, 61.8, 41.8; ESI-MS *m/z* calcd for $C_{11}H_{13}N_2O_5S$ $[M+H]^+$ 285.05, found 285.08.

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