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Microwave-assisted synthesis of base-modified fluorescent 1,4-dihydropyridine nucleosides: photophysical characterization and insights†

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A synthesis of a small library of fluorescent 1,4-dihydropyridine nucleoside analogues has been successfully carried out under solvent-free conditions *via* a one-pot three-component Hantzsch condensation reaction. The process involved a $Ba(NO_3)_2$ catalyzed solvent-free reaction between 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine, differently substituted β-keto ester and ammonium acetate under microwave irradiation. This facile methodology yielded the desired products with very high yields (86–96%) under solvent-free reaction conditions in a short reaction time, which was followed by a simple workup. Yields obtained under microwave and conventional heating were compared, with the microwave irradiation condition displaying superior results. The synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HETCOR, 2D NOESY NMR and HRMS analysis. These nucleoside analogues exhibited significant fluorescence, with a prominent emission band around 460 nm (excitation at 235 nm). Photophysical studies revealed strong fluorescence intensity, excellent Stokes shifts (70–162 nm), and high quantum yields (0.022–0.579), outperforming other pyrimidine-based fluorescent nucleosides. Notably, 5-(diethyl 2″,6″-propyl-1″,4″-dihydropyridine-3″,5″-dicarboxylate)-4″-yl-2′-deoxyuridine demonstrated a quantum yield as high as 0.579 in DMSO during solvatochromic studies, highlighting their potential for probing local nucleic acid structure and dynamics. Additionally, we demonstrated the scalability of the synthesis protocol by producing one of the compounds on a gram scale, confirming its practical viability for large-scale production. This study underscores the potential of these fluorescent nucleoside analogues for various biochemical applications.

1. Introduction

DNA, the "molecule of life", stores genetic instructions vital for biological processes such as cell division and viral replication.[1] DNA's structure is constituted by nucleotides, which contains nucleobase, carbohydrate and phosphate backbone, whereas nucleoside monomers are composed of a nucleobase and carbohydrate moiety. Over recent decades, chemists have synthesized numerous modified nucleosides to evaluate their biological activities, including antiviral,[2–5] anti-HIV,[6,7] anticancer,[8,9] antimetabolites,[10,11] antisense properties[12] and many more.[13,14] The worldwide clinical success of these nucleosides inspires scientists to advance the synthesis of modified nucleosides (Fig. 1).[15–20] Among the modified nucleosides, the synthesis of modified fluorescent nucleoside analogues has undergone rapid growth due to the non-emissive nature of the canonical bases in nucleic acids.[21]

Several base analogues, such as 2-aminopurine, pyrrolo-dC (pyrrolo-deoxycytidine), 1,3-diaza-2-oxophenoxazine (tCO), bodipy-labelled bases, and cyanine-labelled bases, which possess intrinsic fluorescence or can be chemically modified to exhibit fluorescent properties, have been used in the synthesis of fluorescent nucleosides.[22–25] When fluorescent nucleobases are incorporated synthetically into DNA and RNA, they serve as potent tools, providing new insights into DNA and RNA and leading to advancements in chemistry, biology, and medicine.[26] The fascination with base-modified fluorescent nucleosides lies in their exceptional sensitivity to the microenvironment. This characteristic feature positions them as potent tools for delving into the intricate structure and functions of nucleic acids, offering promising avenues for further research and applications.[27–30]

In the last few decades, structural modifications at the fifth position of the uracil base of uridine have gained attention for exploration of their photophysical properties (Fig. 2).[21,31–43] Modifications at this position exhibited Watson–Crick type hydrogen bonding as similar as their native

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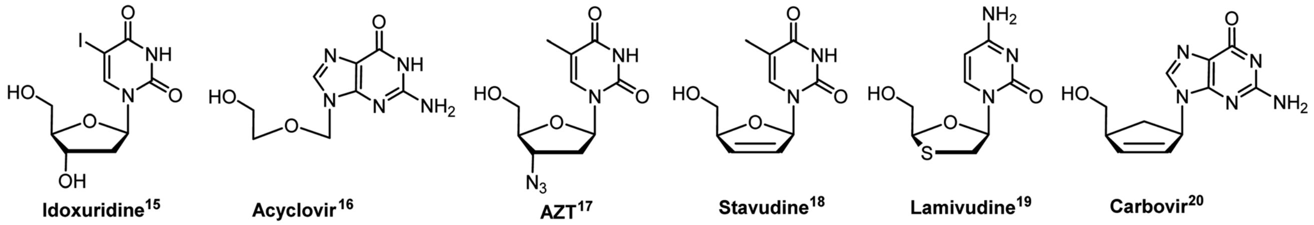


Fig. 1 Structures of some FDA approved sugar modified nucleosides.

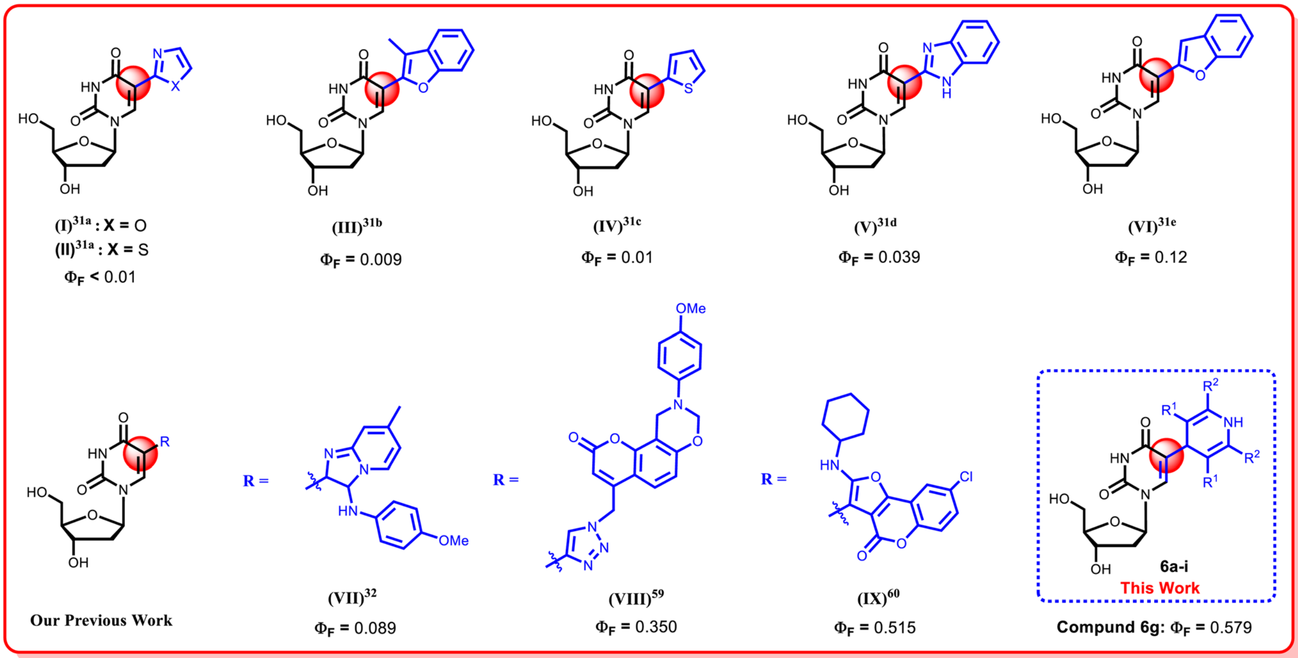
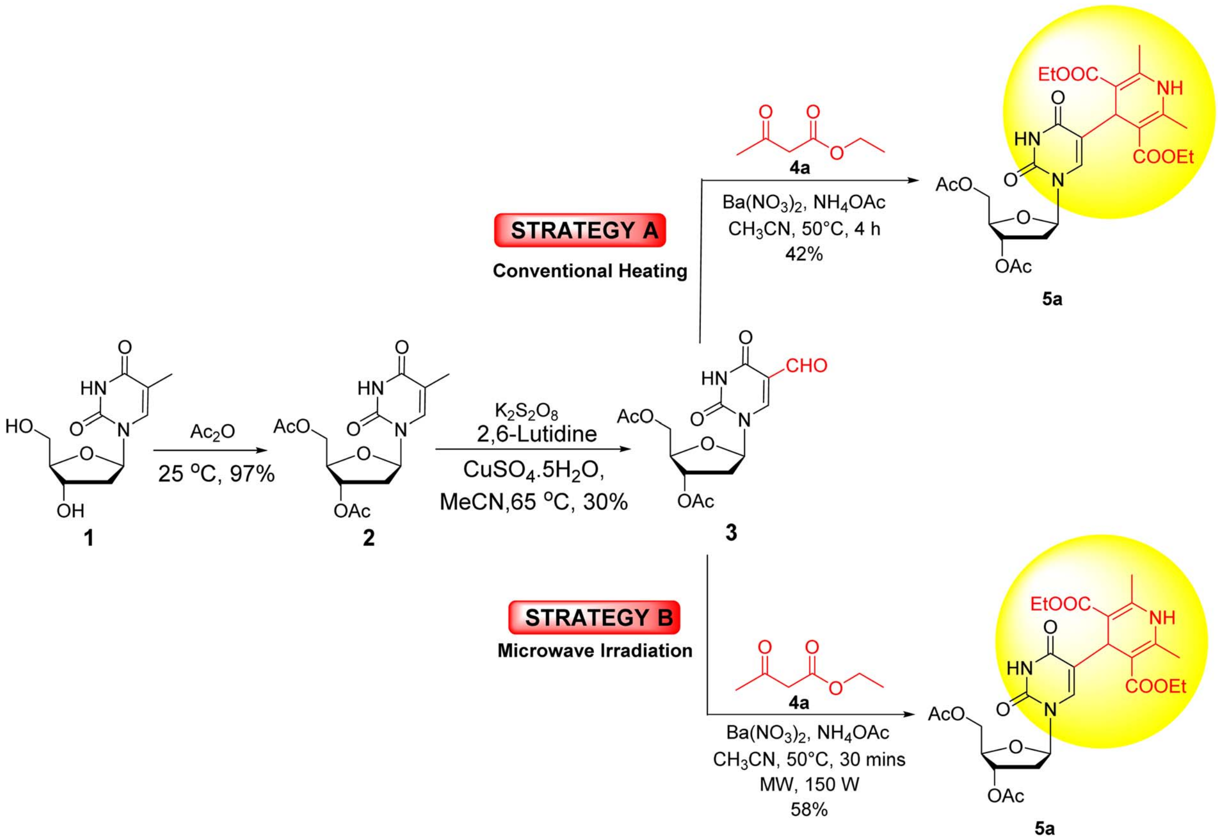


Fig. 2 Some representative base modified fluorescent nucleosides.

counterparts.[44] Additionally, 5-substituted 2′-deoxyuridines are known for their antiviral activity against a range of viruses, including *Herpes simplex virus* (HSV-1 and HSV-2),[45] *Hepatitis B virus* (HBV),[46] *Vaccinia virus* (VV),[47] *Varicella-zoster virus* (VZV),[48] and *Cowpox virus* (CV)[46] and these nucleoside monomers have also demonstrated notable antibacterial activities.[46,49,50]

Over the decades, our research group has developed numerous facile and fruitful methodologies to synthesize sugar-modified or nucleobase modified nucleosides.[32,51–60] Recently, we have focused our attention on modifying the C-5 position of the uracil base of uridine by introducing different heterocyclic functionalities at that position to develop nucleosides with enhanced fluorescence properties (Fig. 2).[32,59,60] As part of our ongoing efforts to enhance the fluorescence properties of the uridine moiety, a green methodology to incorporate 1,4-dihydropyridine (DHP) at the C-5 position of uridine has been reported herein. 1,4-Dihydropyridines (DHPs) stand out as crucial heterocyclic compounds with diverse pharmacological properties, commonly synthesized through the Hantzsch reaction,[61] which serves as a privileged pharmacophore in organic chemistry.[62] Additionally, the photophysical characteristics[63–66] of 1,4-dihydropyridine derivatives have been extensively investigated and documented in the literature. However, to the best of our knowledge, the incorporation of DHP moieties into the nucleoside chemistry has not been explored till date. In this article, we are reporting an environmentally friendly, efficient and solvent-free reaction to incorporate the 1,4-dihydropyridine moiety at the C-5 position of uridine for the first time *via* microwave irradiation. This research work includes the comparative studies of the conventional heating methodology and



Scheme 1 Method A: conventional Heating; Method B: microwave Irradiation.

microwave irradiation procedures, where the later one provided an excellent solution as a non-conventional energy source, offering uniform internal heating and significantly reducing the reaction time, followed by an easier workup.[67] A comprehensive study of the photophysical properties of the synthesized nucleoside analogues is also included in this article.

2. Results and discussion

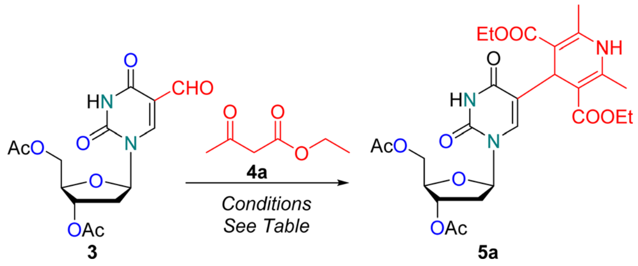
2.1. Chemistry

The primary goal of this research work was to incorporate 1,4-dihydropyridines into the uridine moiety. For this purpose, a one-pot, three-component, Hantzsch condensation reaction was attempted with 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**3**) under both conventional as well as microwave heating conditions (Scheme 1).

At the outset, thymidine **1** was acetylated with acetic anhydride to produce 3′,5′-di-*O*-acetylthymidine (**2**). Subsequent oxidation with potassium persulfate, 2,6-lutidine, and cupric sulfate in acetonitrile yielded 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**3**).[68] Initially, both microwave and conventional methods were used for synthesizing the target compound **5a** starting from nucleoside **3**. In Method A (conventional heating), 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**3**) was treated with ethyl acetoacetate (**4a**) and ammonium acetate in the presence of $Ba(NO_3)_2$ as a catalyst in CH_3CN at 50 °C. It yielded the desired compound **5a** in 42% yield. Whereas, in Method B (microwave heating), under identical reaction conditions, 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**3**) produced the desired product **5a** in 58% yield in 30 minutes (Scheme 1).

After establishing the superiority of microwave heating over conventional heating for the synthesis of our desired compound **5a**, optimization of the reaction conditions was carried out (Table 1). Initially, performing the reaction in acetonitrile (CH_3CN) at 50 °C yielded the desired 1,4-dihydropyridine nucleoside **5a** in 58% yield in 30 minutes (entry 1, Table 1). Next, various solvents, such as 1,4-dioxane, MeOH, ethanol, THF, AcOH, EtOAc, H_2O, DMF, isopropyl alcohol, and toluene (entries 2–11, Table 1), were screened. The higher yield of 72% was obtained in 1,4-dioxane, while other solvents resulted in inferior yields. Interestingly, significant improvement in the yield was achieved under

Table 1 Optimization of the reaction of 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3** with ethyl acetoacetate **4a** and ammonium acetate in the presence of $Ba(NO_3)_2$ as a catalyst to obtain 1,4-dihydropyridine nucleoside analogue **5a**[ad]

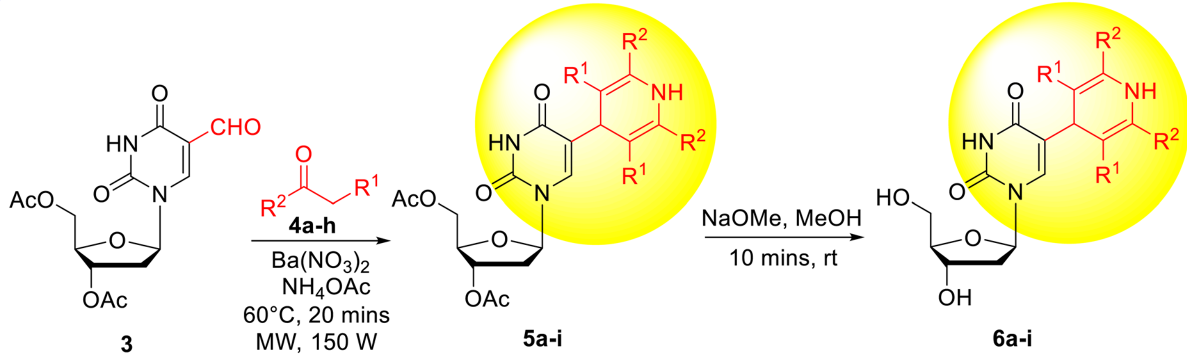


S. no.	Solvent	Temperature (°C)	Time (min)	Reaction yield (percent yield)[b]
1	Acetonitrile	50	30	58
2	1,4-Dioxane	50	30	72
3	MeOH	50	30	62
4	EtOH	50	30	54
5	THF	50	30	46
6	AcOH	50	30	38
7	EtOAc	50	30	40
8	H_2O	50	30	44
9	DMF	50	30	35
10	*i*-PrOH	50	30	38
11	Toluene	50	30	65
12	Solvent-free	50	20	86
13	**Solvent-free**	**60**	**20**	**93**
14	Solvent-free	70	20	88
15	Solvent-free	80	20	68
16	Solvent-free	100	20	32
17	Solvent-free	120	20	25
18	Solvent-free	60	30	20[c]

[a] Reaction conditions: 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3** (0.34 g, 1 mmol), ethyl acetoacetate (0.39 g, 3 mmol), NH_4OAc (92.3 mg, 1.2 mmol) and barium nitrate (26.2 mg, 0.1 mmol). [b] Isolated yield. [c] Without catalyst: reaction remained incomplete within 30 min and only 20 percent of the final product was obtained. [d] When we attempted to extend the optimized reaction conditions to synthesize unsymmetrical DHP using 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3** (0.34 g, 1 mmol), ethyl acetoacetate (0.13 g, 1 mmol), allyl acetoacetate (0.14 g, 1 mmol), NH_4OAc (92.3 mg, 1.2 mmol) and barium nitrate (26.2 mg, 0.1 mmol), the reaction produced a un-separable diastereomeric mixture, as indicated by un-separable spots on TLC. Unfortunately, we were unable to separate these diastereomeric mixture product (see ESI, Fig. S43 and S44) using column chromatography.

solvent-free reaction conditions, with 86% yield at 50 °C in just 20 minutes (entry 12, Table 1). This outcome encouraged us to conduct the next reactions under solvent free reaction conditions. Hence, the following reactions were carried out at different temperatures, where the highest yield (93%) was obtained at 60 °C (entry 13, Table 1). Higher temperatures like 70 °C, 80 °C, 100 °C, and 120 °C resulted in relatively inferior yields of 88%, 68%, 32%, and 25%, respectively (entries 14–17, Table 1). When the reaction was performed in the absence of catalyst $Ba(NO_3)_2$ under solvent-free conditions at 60 °C for 30 minutes under microwave irradiation, the reaction remained incomplete, yielding the desired product **5a** in 20% yield only (entry 18, Table 1). Among the conditions explored, microwave irradiation under solvent-free conditions at 60 °C was found to be the most favourable for synthesizing the 1,4-dihydropyridine nucleoside analogue **5a** (entry 13, Table 1).

The optimized reaction condition was employed for the condensation of 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3** with eight differently substituted β-keto ester **4a–h** or acetyl acetone **4i** in the presence of NH_4OAc, yielding base-modified 1,4-dihydropyridine nucleoside analogues **5a–i** in 86–96% yields (Scheme 2). Deacetylation of the diacetylated nucleosides **5a–i** was performed using NaOMe in MeOH at 25 °C, resulting in the desired 1,4-dihydropyridine nucleoside analogues **6a–i** in quantitative yields (Scheme 2).



Compds. 5a-h/6a-h	R^1	R^2	Compds. 5a-h/6a-h	R^1	R^2
5a/6a	$COOCH_2CH_3$	CH_3	**5f/6f**	$COOCH_2CH(CH_3)_2$	CH_3
5b/6b	$COOCH_3$	CH_3	**5g/6g**	$COOCH_2CH_3$	$CH_2CH_2CH_3$
5c/6c	$COO(CH_3)_3$	CH_3	**5h/6h**	$COOCH_2CH_3$	CHF_2
5d/6d	$COOCH(CH_3)_2$	CH_3	**5i/6i**	$COCH_3$	CH_3
5e/6e	$COOCH_2CH{=}CH_2$	CH_3			

Scheme 2 Synthesis of fluorescent 1,4-dihydropyridine nucleoside analogues.

The structures of all the synthesized compounds, including 3′,5′-di-*O*-acetyl-thymidine **2**, 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3**, base modified 1,4-dihydropyridine nucleoside analogues **5a–i** and **6a–i**, were unambiguously established by spectral data analysis (IR, ^1H, ^{13}C-NMR, ^1H–^1H COSY NMR, ^1H–^{13}C HETCOR NMR, NOESY NMR, and HRMS). The structures of the known compounds **2** and **3** were confirmed by comparing their melting point and spectral data with literature reports.[32,59,60]

2.2. Gram scale synthesis of 3′,5′-di-*O*-acetyl-5-(diethyl 2″,6″-dimethyl 1″,4″-dihydropyridine-3″,5″-dicarboxylate)-2′-deoxyuridine (5a)

To showcase the applicability and broaden the applicative range of our methodology, we carried out the gram scale synthesis of 3′,5′-di-*O*-acetyl-5-(diethyl 2″,6″-dimethyl-1″,4″-dihydropyridine-3″,5″-dicarboxylate)-2′-deoxyuridine **5a**, which highlights the practicality and effectiveness of the methodology for large-scale reactions. A reaction mixture comprising of 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3** (1.02 g, 3 mmol), ethyl acetoacetate **4a** (1.17 g, 9 mmol),

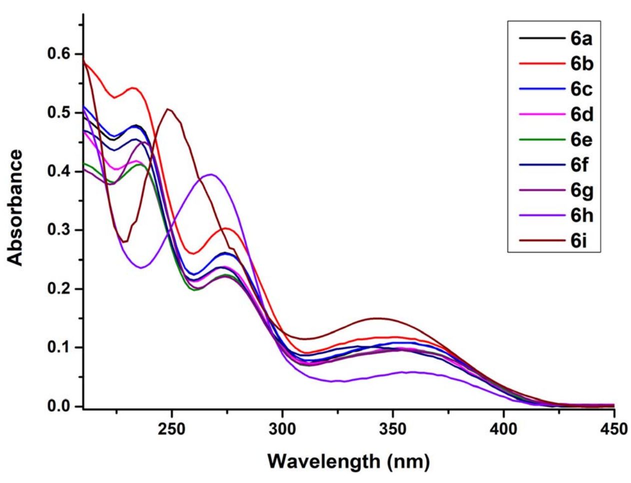


Fig. 3 UV/vis absorption spectra of compounds **6a–i** in acetonitrile at 2.5×10^{-5} M concentration.

NH_4OAc (0.277 g, 3.6 mmol) and $Ba(NO_3)_2$ (78.5 mg, 0.3 mmol) was subjected to microwave irradiation under solvent-free conditions. The reaction proceeded smoothly resulting in

86% yield (1.45 g), highlighting the efficacy of this protocol for large-scale reactions.

3. Photophysical characterization

Over the last few decades, several modified nucleosides with fluorescent properties have been synthesized and studied for their application for studying the structure and dynamics of nucleic acid. The photophysical characterization of the thus synthesized 5-(1″,4″-dihydropyridine)-2′-deoxyuridine nucleoside analogues **6a–i**, consisting of a highly extended conjugation system, was carried out in acetonitrile at 2.5×10^{-5} M concentration.

3.1. Absorption spectra

The absorption spectra of the synthesized 5-(1″,4″-dihydropyridine)-2′-deoxyuridine nucleoside analogues **6a–i** was recorded in acetonitrile at 2.5×10^{-5} M concentration at 25 °C and all the compounds exhibited three absorption bands in the region 200–250 nm, 250–300 nm and 300–400 nm (Fig. 3). The presence of multiple peaks in the absorption spectra can be attributed to the extended conjugated system at the C-5 position of the nucleoside, corresponding to n → π* and π → π* transitions in the UV-vis region. Further, no significant effect on the absorption spectra was observed on changing the substituents at the C-2″, C-3″, C-5″ or C-6″ positions of the 1″,4″-dihydropyridine ring for compounds **6a–6h**. However, a significant hypsochromic shift for compound **6i** was observed.

3.2. Emission spectra

The emission spectra of the 5-(1″,4″-dihydropyridine)-2′-deoxyuridine nucleoside analogues **6a–i** was also recorded in acetonitrile at the same concentration of 2.5×10^{-5} M with λ_{ex} = 235 nm as the excitation wavelength (Fig. 4). All the compounds exhibited a strong red-shifted band in the region 446–477 nm with two weak bands at around 310 and 350 nm. The highest fluorescence intensity was observed for compounds **6a** and **6h.** No significant substituent effect was observed on the emission spectra for compounds **6a–6h**. However, a significant

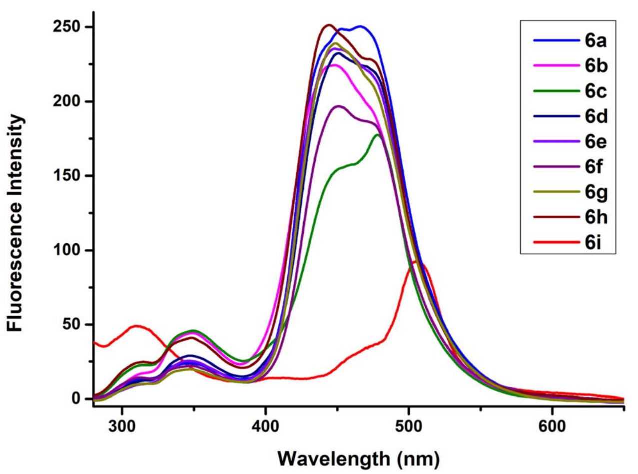


Fig. 4 Emission spectra of compounds **6a–i** in acetonitrile at 2.5×10^{-5} M concentration (λ_{ex} = 235 nm).

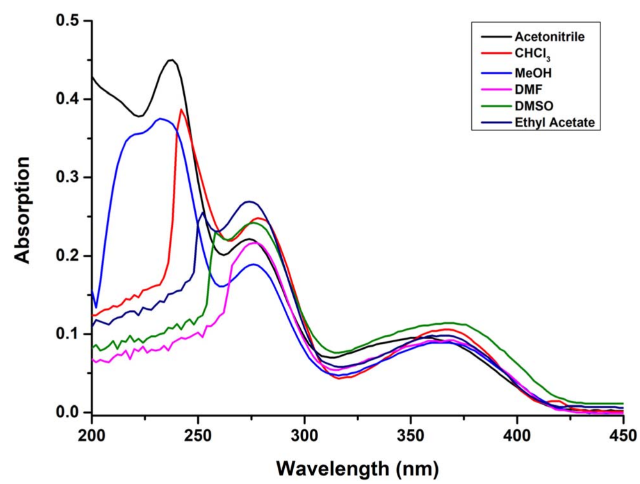


Fig. 5 UV/vis absorption spectra of compound **6g** in different organic solvents; acetonitrile, $CHCl_3$, MeOH, DMF, DMSO, and ethyl acetate at 2.5×10^{-5} M concentration.

Table 2 Photophysical properties of 5-(1″,4″-dihydropyridine)-2′-deoxyuridine nucleoside analogues **6a–i** in acetonitrile

Compound no.	Absorbance λ_{abs} (nm)	Emission λ_{em} (nm)	Stokes shift (nm)	Quantum yield (Φ_F)	Molar extinction coefficient ε (M^{-1} cm^{-1})	Brightness $\varepsilon \times \Phi_F$ (M^{-1} cm^{-1})
6a	356	464	108	0.077	1.9×10^4	1.5×10^3
6b	352	446	94	0.080	2.1×10^4	1.7×10^3
6c	356	477	121	0.119	1.9×10^4	2.2×10^3
6d	354	472	118	0.091	1.7×10^4	1.5×10^3
6e	356	449	93	0.089	1.6×10^4	1.5×10^3
6f	334	474	140	0.078	1.8×10^4	1.4×10^3
6g	360	474	114	0.123	9.4×10^3	1.1×10^3
6h	354	449	95	0.074	1.8×10^4	1.3×10^3
6i	342	504	162	0.022	2.0×10^4	4.4×10^2

bathochromic shift was observed for compound **6i** with a red-shifted band at 504 nm. Also, a weak blue-shifted band appeared at 310 nm for compound **6i**.

3.3. Quantum yield calculations and Stokes shift analysis

The quantum yields Φ_F for compounds **6a–i** were calculated using the following equation:

$$\Phi_F = \Phi_{st} \times S_u/S_{st} \times A_{st}/A_u \times {n_{Du}}^2/{n_{Dst}}^2$$

with quinine sulphate ($\Phi_{st} = 0.54$ in 0.1 M H_2SO_4) as the reference standard. Wherein, Φ_F = Emission quantum yield for the synthesized compounds **6a–i**, Φ_{st} = emission quantum yield of the standard, S_{st} = integrated emission band area of the standard S_u = integrated emission band area of the samples A_{st} = absorbance of the standard at the excitation wavelength A_u = absorbance of the samples at the excitation wavelength η_{Dst} = solvent refractive index of the standard η_{Du} = solvent refractive index of the sample.

The subscripts 'u' and 'st' are used to denote the unknown samples and the standard, respectively. The quantum yields thus calculated are tabulated in Table 2.

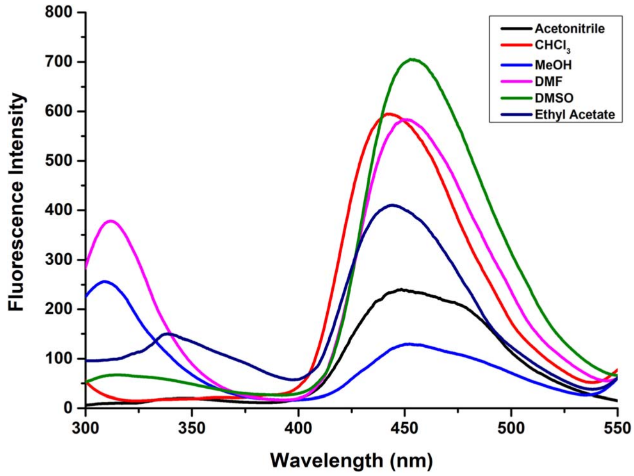


Fig. 6 Emission spectra of compound **6g** in different organic solvents; acetonitrile, $CHCl_3$, MeOH, DMF, DMSO, and ethyl acetate at 2.5 × 10^{-5} M concentration.

The quantum yield values (Φ_F) were found to be in the range 0.022–0.123 with the highest being 0.123 for compound **6g**. High values of Stokes shift were observed ranging from 93–162 nm. The brightness of all modified nucleosides **6a–i** was calculated and enlisted in Table 2.

3.4. Solvatochromism

Now, in order to study the effect of solvent polarity on the photophysical properties of the synthesized nucleoside analogues **6a–i**, the solvatochromic characterization of one of the compounds **6g** was performed in six different organic solvents; acetonitrile, $CHCl_3$, MeOH, DMF, DMSO and ethyl acetate.

Minor bathochromic/hypsochromic shifts in the absorption spectra were observed on changing the solvent from acetonitrile to $CHCl_3$, MeOH, DMF, DMSO or ethyl acetate (Fig. 5). A dual emission was observed in MeOH (310 nm and 456 nm), DMF (313 nm and 450 nm), DMSO (315 nm and 454 nm) and ethyl acetate (338 nm and 444 nm) while a single emission band was observed in acetonitrile (474 nm) and $CHCl_3$ (442 nm) (Fig. 6). The highest fluorescence intensity as well as highest quantum yield (0.579) was observed in DMSO. The highest Stokes shift value (114 nm) was observed in case of acetonitrile while the lowest value (70 nm) was recorded in $CHCl_3$. The emission wavelength, quantum yield, Stokes shift values as well as the molar absorption coefficient of compound **6g** in different solvents is tabulated in Table 3.

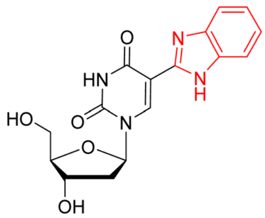
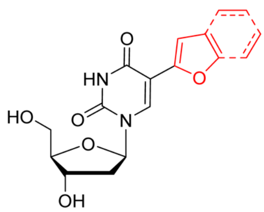
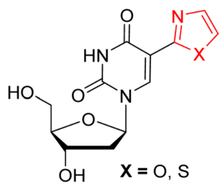
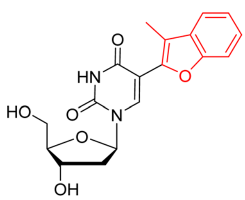
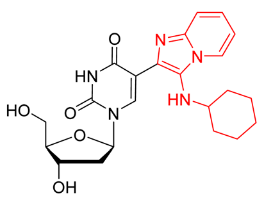
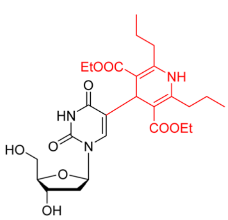
3.5. Comparative discussion

The synthesized fluorescent 1,4-dihydropyridine nucleoside analogues **6a–i** demonstrated superior photophysical properties compared to previously reported C-5 modified pyrimidine nucleosides. Specifically, these compounds exhibited higher quantum yields (0.022–0.579) and Stokes shift values (70–162 nm), as detailed in Table 4. This indicated that these modified nucleosides not only surpassed the quantum yield values obtained in previous reports but also showed competitive Stokes shift values, emphasizing their potential in fluorescence applications.

Table 3 Photophysical properties of 5-(1″,4″-dihydropyridine)-2′-deoxyuridine **6g** in acetonitrile, $CHCl_3$, MeOH, DMF, DMSO, and ethyl acetate

	Solvent					
Properties	Acetonitrile	$CHCl_3$	MeOH	DMF	DMSO	Ethyl acetate
Absorbance λ_{abs} (nm)	360	372	370	368	375	369
Emission λ_{em} (nm)	474	442	456	450	454	444
Stokes shift (nm)	114	70	86	82	79	75
Quantum yield (Φ_F)	0.123	0.331	0.441	0.468	0.579	0.197
Molar extinction coefficient ε (M^{-1} cm^{-1})	9.4×10^3	1.5×10^4	1.4×10^4	8.8×10^3	9.9×10^3	1.2×10^4
Brightness $\varepsilon \times \Phi_F$ (M^{-1} cm^{-1})	1.1×10^3	4.9×10^3	6.2×10^3	4.1×10^3	5.7×10^3	2.3×10^3

Table 4 Comparison of various photophysical parameters of compound **6g** with reported C-5 modified pyrimidine nucleosides[a]

Ref.	Structure	Absorbance λ_{abs} (nm)	Emission λ_{em} (nm)	Stokes shift (nm)	Quantum yield (Φ_F)	Extinction coefficient ε (M^{-1} cm^{-1})	Solvent
Ref. 31*d*		320	405	85	0.039	Nr	100 mM HCl
Ref. 31*e* & 34	 **I: Benzofuran** **II: Furan**	(I) 322 (II) 316	(I) 423 (II) 431	(I) 101 (II) 115	(I) 0.12 (II) 0.03	(I) nr (II) nr	(I) MeOH (II) H_2O
Ref. 31*a*		(X = O) 296 (X = S) 316	(X = O) 400 (X = S) 404	(X = O) 104 (X = S) 88	(X = O) < 0.01 (X = S) < 0.01	(X = O) nr (X = S) nr	(X = O) H_2O (X = S) H_2O
Ref. 31*b*		306	457	151	0.009	Nr	MeOH
Ref. 32		242	346	107	0.072	1.46×10^4	MeOH
This work (**6g**)		370 375	456 454	86 79	0.441 0.579	1.4×10^4 9.9×10^3	Acetonitrile DMSO

[a] Nr: not reported.

4. Conclusion

In this study, we have successfully developed an environmentally-friendly, economical and highly efficient methodology for synthesizing a small library of novel fluorescent 1,4-dihydropyridine nucleoside analogues, **5a–i** and **6a–i**, *via* a one-pot three-component Hantzsch condensation reaction under solvent-free conditions. This process involved reaction between 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine **3**, β-keto ester **4a–h** or acetyl acetone **4i**, and ammonium acetate, catalyzed by $Ba(NO_3)_2$ under microwave irradiation. This method offered high efficiency, producing the desired products in high yields up to 96%. Comparative studies demonstrated the superior performance of microwave irradiation over conventional heating. The protocol demonstrated practicability and broad applicability through successful gram-scale synthesis. The synthesized compounds displayed strong fluorescence at around 442–477 nm and exhibited significantly higher absorption and emission bands compared to thymidine, attributed to extended conjugation. Photophysical investigations revealed a noteworthy fluorescence intensity, excellent Stokes shift values (70–162 nm), and superior quantum yields (0.022–0.579), particularly notable in DMSO (0.579). These highly fluorescent nucleoside derivatives are suitable for their incorporation into oligonucleotides for carrying out hybridization studies with complementary DNA strands, facilitating the investigation of DNA local structure.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its ESI materials.†

Conflicts of interest

The authors declare no competing financial interest.

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