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Efficient Synthesis of Ratiometric Fluorescent Nucleosides Featuring 3-Hydroxychromone Nucleobases

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ABSTRACT

The synthesis of a novel class of fluorescent nucleosides featuring 2-aryl-3-hydroxychromones (3-HC) as base analogues is described. Nucleoside **1a** bearing the 2-thienyl-3-HC nucleobase was prepared using sequential aryl–aldol condensation/cycloetherification or a Friedel–Crafts glycosylation as key steps. The synthesis of the triazolyl derivative **1b** was achieved using a convergent 1,3-dipolar cycloaddition strategy. Fluorescence studies show that 3-HC-thienyl-nucleoside **1a** displays high sensitivity of its dual emission to polarity changes and therefore is highly promising for nucleic acid labelling.

1. Introduction

Due to its exquisite sensitivity, fluorescence is one of the most sensitive techniques for investigating biological systems. In the context of nucleic acids, the common strategy consists to substitute a natural nucleoside by a fluorescent analogue because the canonical bases are poorly or non-fluorescent. Most of the fluorescent analogues used for nucleic acid labelling are single band emitter probes.[1] Among them, 2′-deoxyribosyl-2-aminopurine (2-AP), a mimic of 2′-deoxyadenosine, is the most popular fluorescent base.[2] However, 2-AP displays severe limitations related to the dramatic drop of its quantum yield when incorporated in oligonucleotides.[3] Moreover, when protein binding to nucleic acids does not induce a transition between stacked and unstacked conformations, the interactions are difficult to monitor due to the limited environment sensitivity of 2-AP. Recently, 8-vinyl-deoxyadenosine (8-vdA), a new fluorescent analogue of 2′-deoxyadenosine was reported by us.[4] Although 8-vdA displays improved sensitivity compared to 2-AP, its domain of application is similar. Other fluorescent nucleoside analogues have been developed but, when incorporated into ODNs, they are quenched, destabilizing or of limited sensitivity to environmental change. As a consequence, there is a strong demand for new fluorescent nucleoside analogues with improved spectroscopic properties and this is the purpose of intense research.[1]

On the other hand, 3-hydroxychromones (3-HC) have been shown to be powerful fluorescence probes for a large range of applications in model membranes, biomembranes and proteins.[5] Due to an excited state intramolecular proton transfer (ESIPT, Fig. 1),[6] these fluorophores exhibit two excited state forms: the initially excited normal (N*) and the tautomeric (T*) forms.[7] Since the ESIPT reaction in these dyes is strongly sensitive to polarity, [8] H-bonds[8b,9] and electric fields,[10] the ratio of the two emission bands could be used to sensitively monitor environmental changes. Moreover, the positions of the absorption and emission bands as well as the fluorescence intensity ratio in 3-HC dyes could be used to further characterize the properties of the probe environment.[8b,11] 3-HC fluorophores bearing a small heterocycle in 2-position are attractive analogues of the nucleic bases

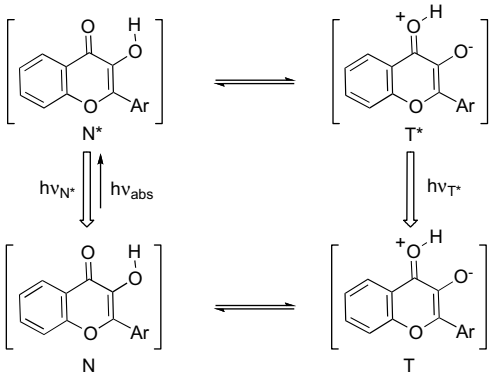

Figure 1. ESIPT reaction in 3-hydroxychromones.

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because their size corresponds well to the size of the two complementary (A–T or G–C) bases. Moreover, according to our recent studies, the 2-(2-furanyl)-3-hydroxychromone dye when conjugated to spermine is able to intercalate within the base pairs of double-stranded DNA, giving a strong variation of its dual emission and an increase in its fluorescence intensity.[12]

To explore the potentiality of 3-HC as fluorescent probes for DNA labelling, we report here the first synthesis and preliminary spectroscopic characterization of nucleoside analogues where a 3-HC substitutes a natural base. The structures of the targeted 3-HC-nucleosides **1a** and **1b** are shown in Figure 2. They were designed (i) to keep a 2′-deoxy structure and a β-anomeric configuration for incorporation into DNA oligonucleotides and (ii) to have an electron donating or an electron withdrawing group connected between the sugar and chromone moieties in order to modulate the photophysical properties of the nucleoside analogues.

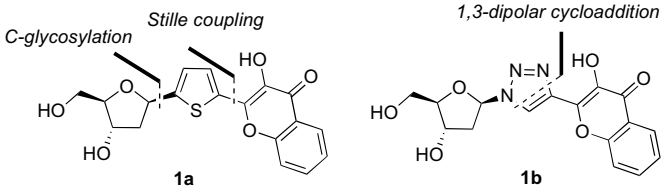


Figure 2. Targeted 2-aryl-3-HC-nucleosides.

2. Results and discussions

For the synthesis of **1a** and **1b** we used two convergent strategies based on C-glycosylation–Stille type coupling (for **1a**), and azide-alkyne 1,3-dipolar cycloaddition (for **1b**) as key steps (Fig. 2).

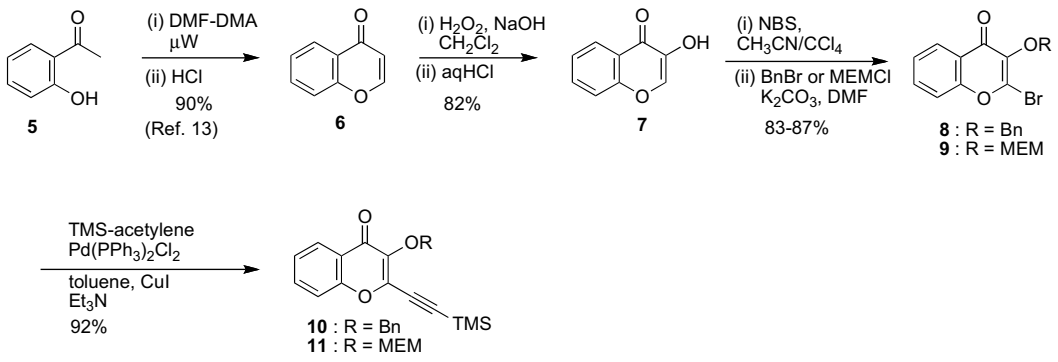
The preparation of 3-HC key intermediates **8**–**11** required for the synthesis of **1a** and **1b** is described in Scheme 1. Starting from 2-hydroxy-acetophenone **5**, condensation with *N*,*N*-dimethylformamide dimethylacetal followed by HCl-mediated in situ cyclization provided the chromone **6**.[13] Epoxidation of **6** using H_2O_2/NaOH in CH_2Cl_2 followed by acid-mediated ring-opening afforded **7** in 82% overall yield. The use of protic solvents (MeOH, EtOH) or other oxidizing agents such as *m*-CPBA and 2-butanone peroxide only gave low yields.[14] Bromination of **7** followed by successive protection with benzyl or MEM group gave **8** and **9**, respectively (83–87%, two steps). Sonogashira coupling carried out on **8** and **9** using TMS-acetylene provided the 3-HC **10** and **11**, respectively (90–93%). We found that the cleavage of the TMS group of compounds **10** and **11** resulted in unstable terminal alkynes. Therefore, the TMS-protected alkynes **10** and **11** were deprotected in situ and used without purification.

With these key intermediates in hand, the thienyl-derived 3-HC-C-nucleoside **1a** bearing the β-C–C linkage was prepared starting from the aldehyde **2** (Path A)[15] or the acetyl-deoxyribose (**3a** or **3b**, Path B) as illustrated in Scheme 2.

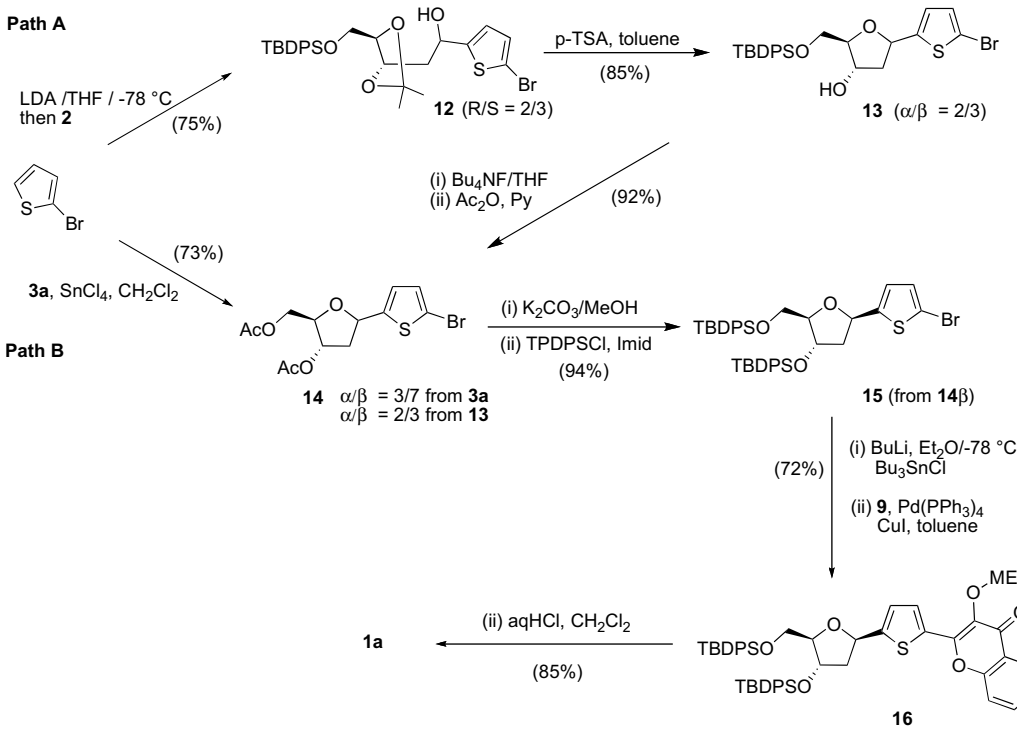
Thus, regioselective *ortho*-lithiation of 2-bromothiophene using LDA in THF at −78 °C followed by addition of the protected aldehyde **2** led to the alcohol **12** in 75% yield as a mixture of inseparable *R*/*S* isomers (40:60). C-Nucleoside **13** (α/β mixture) was then obtained from **12** (*R*/*S*) following isopropylidene cleavage and subsequent C4′–C1′ cycloetherification (85%). However, the separation of α/β anomers required conversion of **13** to its acetyl-protected analogue **14**. Interestingly and according to path B, the direct glycosylation leading to **14** was cleanly achieved in one step using the catalytic Friedel–Crafts reaction.[16] Indeed, treatment of 2-bromothiophene and acetyl-ribose **3a** (or **3b**) with $SnCl_4$ in CH_2Cl_2 provided good yield and diastereoselectivity in favour of the desired β-anomer **14**β (73% yield, β/α=70:30). Furthermore, the anomeric configuration was clearly evidenced by the observed NOE correlations between H1′ and H4′ for **14**β, and between H1′–H3′ and H1′–H5' for **14**α (Scheme 2). The synthesis of **1a** was then pursued from **14**β following subsequent acetyl cleavage/TBDPS protection to provide **15** (94%).[16f] Halogen–metal exchange/stannylation followed by Pd-catalyzed Stille type coupling between **15** and 2-bromo-3-HC **9** led to the protected C-nucleoside **16** (72%), which was quantitatively converted, after MEM and TBDPS cleavage, to the 2-thienyl-3-HC C-nucleoside **1a**.

The synthesis of compound **1b** was achieved straightforwardly. As shown in Scheme 3, after in situ cleavage of the TMS group, the 1,3-dipolar cycloaddition between **10** and azido-sugar **3**[17a] was carried out both in solution using AcOH as a co-catalyst and on SiO_2 under solvent-free and microwave activation, according to our recent published work.[17] Both procedures provided the cycloadduct **17** in high yields (89–95%). Finally, methanolysis of **17** followed by catalytic hydrogenolysis afforded the triazolyl-3-HC nucleoside **1b** in 86% yield (two steps). Following the same strategy, but using the 3-HC **11** rather than **10**, the deprotection of the MEM group (HCl or TFA) failed since a concomitant cleavage of the glycosidic bond was observed.

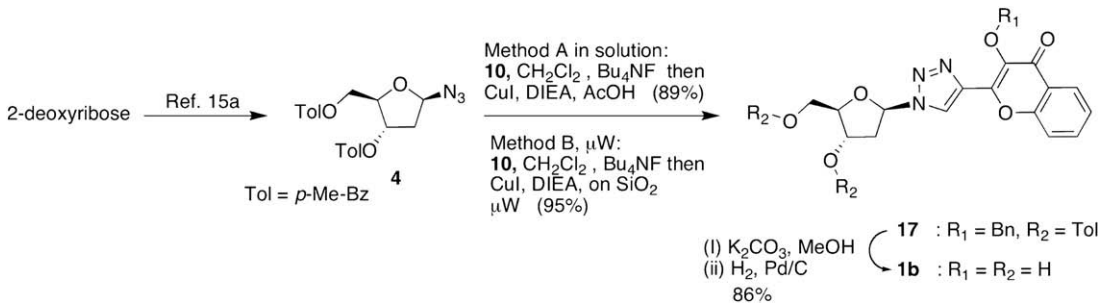
The photophysical properties of compounds **1a** and **1b** were investigated in solvents of different polarity. To scale the polarity, we used the $E_T(30)$ index. This empirical parameter accounts for the dielectric constant of the solvent and its H-bond donor ability,[18] both of which influence strongly the fluorescence properties of the 3-HC dyes.[8b,11] The results are given in Table 1 and Figures 3 and 4. In the studied solvents, compound **1a** shows an absorption band centred around 361–369 nm, whereas **1b** exhibits a significantly blue shifted absorption having two distinct maxima at 299–304 and 332–338 nm (Fig. 3). The position of the absorption maximum slightly increases with $E_T(30)$ (Table 1). In all studied solvents, compound **1a**, featuring the electron-rich thienyl heterocycle, presents a well-resolved dual emission (Fig. 4A), where the short- and the long-wavelength bands could be assigned to the emission



Scheme 1. Synthesis of protected 3-HC **10** and **11**.



Scheme 2. Synthesis of 3-HC C-nucleoside **1a**.



Scheme 3. Synthesis of 3-HC nucleoside **1b**.

Table 1
Spectroscopic data of nucleosides **1a** and **1b** in different solvents[a]

Nucl.	Solv.	$E_T(30)$	λ_{abs}	ε	λ_{N^*}	λ_{T^*}	N^*/T^*	QY
1a	Buffer	63.1	367	13,000	440	515	1.72	0.046
	MeOH	55.4	368	12,500	434	545	1.34	0.075
	EtOH	51.9	369	12,300	433	548	0.68	0.077
	BuOH	49.7	369	—	431	546	0.52	0.091
	DMSO	45.1	368	—	431	554	0.30	0.197
	MeCN	45.6	362	—	421	546	0.13	0.179
	Acetone	42.2	362	—	421	552	0.22	0.053
	CH_2Cl_2	40.7	363	—	418	543	0.04	0.141
	EtOAc	38.1	361	—	415	551	0.09	0.100
1b	Buffer	63.1	338	11,500	401	500	0.06	0.022
	MeOH	55.4	336	12,000	398	516	0.17	0.024
	EtOH	51.9	335	12,000	397	517	0.13	0.027
	BuOH	49.7	333	—	393	518	0.16	0.033
	DMSO	45.1	341	—	390	520	0.06	0.052
	MeCN	45.6	332	—	387	511	0.02	0.189
	Acetone	42.2	334	—	387	515	0.07	0.037
	CH_2Cl_2	40.7	333	—	—	506	<0.01	0.183
	EtOAc	38.1	332	—	387	512	0.03	0.074

[a] λ_{abs} is the position of the absorption maxima, λ_{N^*} and λ_{T^*} are the positions of the fluorescence maxima of the N^* and T^* bands, respectively; N^*/T^* is the intensity ratio of the two emission bands at their maxima; QY is the fluorescence quantum yield calculated using quinine sulfate (QY=0.577 in 0.5 M H_2SO_4) as a reference; ε ($M^{-1}cm^{-1}$) is the extinction coefficient at the maximum of absorption; 10 mM phosphate buffer (pH 6.5) was used.

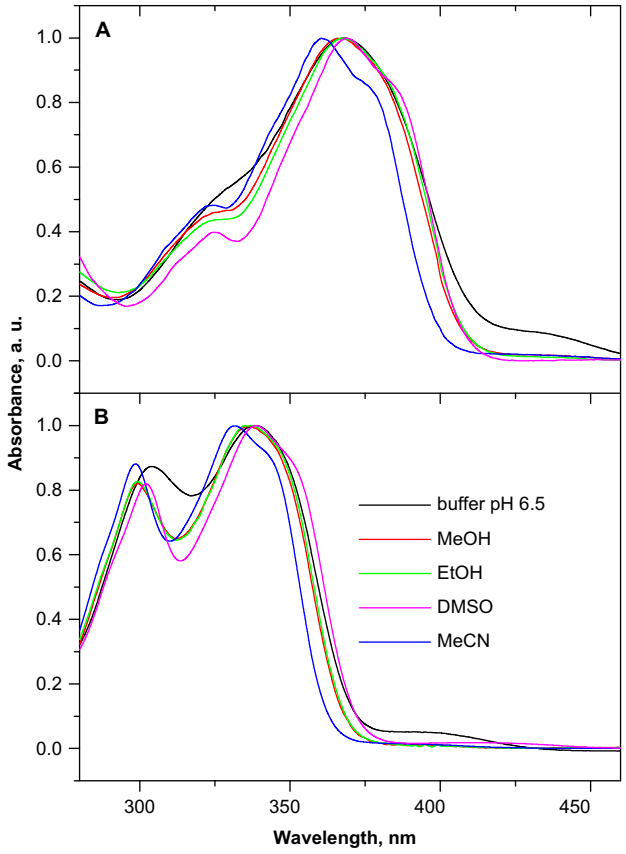


Figure 3. Normalized absorption spectra of nucleosides **1a** (A) and **1b** (B) in different solvents.

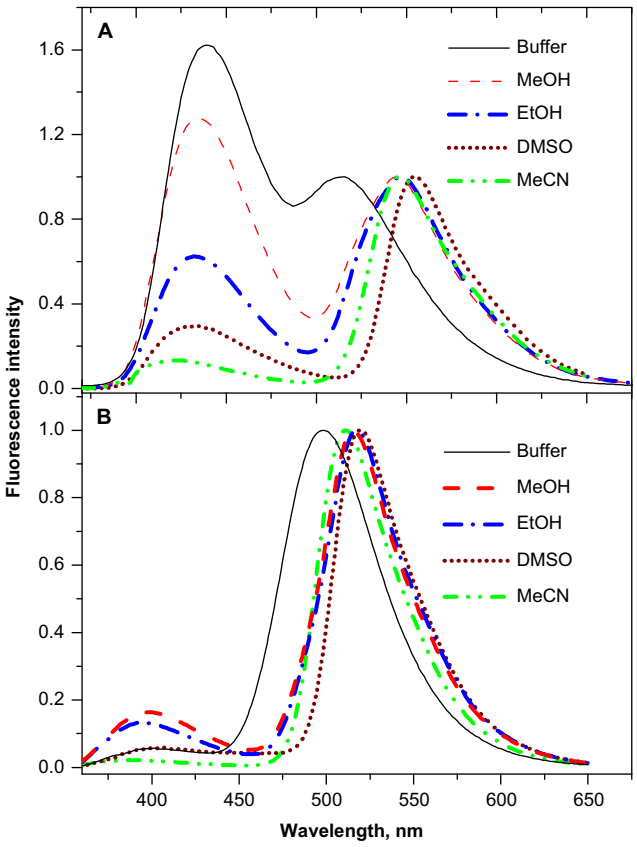


Figure 4. Fluorescence spectra of **1a** (A) and **1b** (B) in different solvents. The spectra were normalized at the long-wavelength maximum. Excitation wavelength was 360 and 340 nm for **1a** and **1b**, respectively.

of the N* and T* species, respectively. The N*/T* intensity ratio of this nucleoside increases gradually with increasing $E_T(30)$ values from 0.04 to 0.09 in dichloromethane and ethyl acetate up to 1.72 in water (Table 1 and Fig. 4A). Figure 5 shows that the N*/T* ratio correlates well with the $E_T(30)$ values. This correlation suggests that the ESIPT reaction and thus, the formation of the T* form in **1a** is hampered especially in polar protic solvents, as for its analogue 2-(2-furyl)-3-hydroxychromone.[11a] The hampering of the ESIPT reaction by protic solvents in 3-HC is connected with the formation of intermolecular H-bonds that weaken or even disrupt the intramolecular H-bond, needed for the ESIPT reaction.[8b,9] Moreover, the increase in $E_T(30)$ leads to a large blue shift of the T* band, especially in water (Table 1), due to its high H-bond donor ability.[19] In contrast, the N* band maximum shifts to the red on $E_T(30)$ increase, similarly to the absorption band. The fluorescence quantum yield of **1a** varies considerably with solvent, showing the highest value in polar aprotic DMSO (0.197) and the lowest one in polar protic water (0.046).

For derivative **1b** bearing the triazole electron-deficient system, the emission maxima are blue shifted in respect to **1a** (Table 1, Fig. 4B). The N*/T* band ratio in all the solvents is much lower than for **1a**, suggesting a faster ESIPT reaction that results in a predominant emission of the T* form. This observation is in line with the fast ESIPT previously reported for 3-HC dyes bearing 2-aryl group with low electron donor ability.[20] Remarkably, the N*/T* ratio is very low in buffer (0.06), probably due to specific solvation of the triazole ring. Moreover, compound **1b** exhibits a relatively low fluorescence quantum yield in most solvents, except in dichloromethane and acetonitrile (Table 1).

From the comparison of these two nucleosides, it is clear that **1a** is much more advantageous than **1b** in terms of spectroscopic properties and sensitivity to solvent polarity. The strong variation of the N*/T* ratio of **1a**, especially in polar solvents makes it attractive for applications in DNA research, where the local environment is expected to be relatively polar.

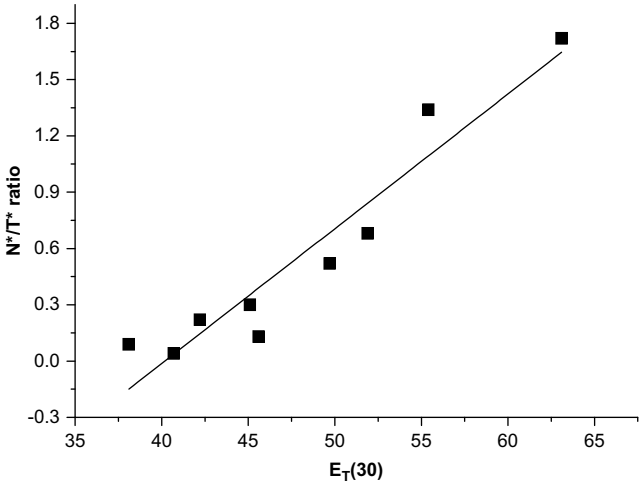


Figure 5. Correlation between the N*/T* intensity ratio of **1a** and the $E_T(30)$ polarity index of the different solvents used in this study (see Table 1). The line represents the linear fit to the experimental data.

3. Conclusion

In conclusion, we developed an efficient synthesis of unprecedented environment-sensitive ratiometric fluorescent

nucleosides bearing 2-aryl-3-HC nucleobases. The synthesis of **1a** involved as key steps an aryl–aldol condensation followed by regiocontrolled C4′–C1' cycloetherification or Friedel–Crafts type glycosylation. In the case of **1b** we used the azide-alkyne 1,3-dipolar cycloaddition, conveniently achieved under the cooperative effects of microwave activation and Cu(I)/SiO_2 catalysis. Fluorescence studies showed that **1a** and to a lesser extent **1b** retain the dual emission highly sensitive to the environment of the 3-HC fluorophore. Thus, a decrease of solvent polarity induces a significant change of the N*/T* ratio in **1a**, together with red and blue shifts of the T* and N* bands, respectively.

Due to the large polarity differences between the interior and the surface of the DNA helix, incorporation of **1a** in DNA should allow monitoring the microenvironmental changes and DNA dynamics at the probe site. Moreover, the N* and T* bands of **1a** at 440 and 515 nm, respectively, are well separated from each other and from the absorption/emission of the natural nucleobases and amino acids. Therefore, **1a** can be selectively excited and studied even in the presence of other nucleic acids and proteins. Incorporation of such ratiometric nucleosides into synthetic oligonucleotides is under way to explore their applications as nucleic acid labels.

4. Experimental section

4.1. General

All reactions were run under nitrogen atmosphere in dried glassware. Solvents were dried and distilled by standard procedures. Toluene was purchased from commercial sources and dried over 4 Å molecular sieves before use. Reagents were purchased and used without further purification. All reactions were monitored by thin layer chromatography (TLC) plates (0.2 mm, silica gel 60 with fluorescent indicator UV_{254}). Flash chromatography was performed using silica gel (60, 0.040–0.063 mm). ^{1}H NMR and ^{13}C NMR were recorded on 200 and 500 instruments (200 and 500 MHz for ^{1}H, 50 and 125 MHz for ^{13}C). Chemical shifts (δ) were reported in parts per million and the coupling constants were reported in hertz (Hz). Analytic High Performance Liquid Chromatography (HPLC) was recorded using a RP-C18 column (300A, 5 μm particle size). Absorption and fluorescence spectra were recorded on Cary 4 spectrophotometer (Varian) and FluoroMax 3.0 spectrofluorometer (Jobin Yvon, Horiba), respectively. Fluorescence quantum yields were determined by taking quinine sulfate in 0.5 M sulfuric acid (quantum yield, QY = 0.577)[21] as a reference.

4.2. Synthesis of the ethynyl-3-hydroxy-chromone 10 and 11

4.2.1. (E)-3-(Dimethylamino)-1-(2-hydroxyphenyl)-prop-2-en-1-one (enamide intermediate)

A mixture of 2′-hydroxyacetophenone (5 mmol, 0.6 mL) and *N*,*N*-dimethylformamide-dimethylacetal (1 equiv, 0.66 mL) was irradiated under microwave for 15 s (300 W max, T=115 °C). The resulting mixture was cooled at room temperature and crystallized in pentane to give the enamine intermediate (red crystals, 955 mg, 100%). Mp (ether)=130–131 °C (lit. 132–134, Ref. 1). R_f=0.2 (cyclohexane/ethyl acetate: 60:40). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=2.95 (s, 3H, NMe), 3.17 (s, 3H, NMe), 5.76 (d, 1H, J=12.1 Hz), 6.81 (dt, 1H, J=1.0 and 7.1 Hz), 6.92 (dd, 1H, J=0.9 and 8.4 Hz), 7.30 (dt, 1H, J=1.5 and 8.4 Hz), 7.69 (dd, 1H, J=1.4 and 8.0 Hz), 7.87 (d, 1H, J=12.0 Hz), 13.99 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=37.5, 45.5, 90.1, 118.1, 118.3, 120.4, 128.3, 134.0, 154.9, 163.0, 191.6. MS (ESI, MeOH) m/z: 213.70 $[M+Na]^+$.

*4.2.2. Chromone **6***

To a solution of the enamide previously obtained (1 g, 5.24 mmol) in methylene chloride (40 mL) was added concentrated HCl (4 mL). The resulting mixture was refluxed for 1 h. After cooling, the mixture was extracted with methylene chloride (3×40 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution, then with brine, dried over $MgSO_4$, filtered and concentrated to afford chromone **6** (red crystals, 700 mg, 90%). Mp (ether)=52–54 °C (lit. 52 °C, Ref. 1). R_f=0.45 (cyclohexane/ethyl acetate: 60:40). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=6.31 (d, 1H, J=6.0 Hz), 7.33–7.44 (m, 2H), 7.63 (ddd, 1H, J=1.8, 7.1 and 8.4 Hz), 7.83 (d, 1H, J=6.0 Hz), 8.18 (dd, 1H, J=1.5 and 7.9 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=113.1, 118.3, 125.0, 125.3, 125.9, 133.8, 155.4, 156.6, 177.7. MS (ESI, MeOH) m/z: 168.9 $[M+Na]^+$.

4.2.3. 2,3-Epoxy-chromone (intermediate)

To a solution of chromone **6** (740 mg, 5 mmol) in methylene chloride (7.5 mL), was slowly added hydrogen peroxide (2 equiv, 35% solution, 1.4 mL) and NaOH (1.5 equiv, 407 mg) at 0 °C. The mixture was stirred 3 h at 0 °C, quenched with water and extracted with methylene chloride (3×20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to give a crude amorphous solid (668 mg, 82%), which was used in the next step without purification. Mp (ether)=64–66 °C. R_f=0.8 (cyclohexane/ethyl acetate: 60:40). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=3.69 (d, 1H, J=2.4 Hz), 5.66 (d, 1H, J=2.5 Hz), 7.05 (dd, 1H, J=0.7 and 8.5 Hz), 7.14 (td, 1H, J=1.0 and 8.0 Hz), 7.55 (ddd, 1H, J=1.8, 7.2 and 8.5 Hz), 7.85 (dd, 1H, J=1.8 and 8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=55.4, 77.3, 118.1, 119.9, 123.4, 127.2, 136.4, 155.5, 188.2. MS (ESI, MeOH) m/z: 160.7 $[M-H]^-$.

*4.2.4. 3-Hydroxy-chromone **7***

To the epoxide previously obtained (570 mg, 3.5 mmol) was added concd HCl (20 mL) and the resulting mixture was heated at 70 °C for 1 h. After cooling, water was added (20 mL) and the mixture was extracted with methylene chloride (3×30 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered and concentrated to afford 3-hydroxychromone **7** (brown powder, 568 mg, 100%). Mp (ether)=179–181 °C. R_f=0.5 (cyclohexane/ethyl acetate: 50:50). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=6.33 (s, 1H), 7.41 (ddd, 1H, J=1.1, 7.0 and 8.0 Hz), 7.50 (dd, 1H, J=0.6 and 8.6 Hz), 7.69 (ddd, 1H, J=1.8 and 7.0 and 8.6 Hz), 8.01 (s, 1H), 8.26 (dd, 1H, J=1.3 and 8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=117.3, 123.4, 124.3, 132.3, 137.2, 140.5, 147.9, 155.1, 172.2. MS (ESI, MeOH) m/z: 184.9 $[M+Na]^+$. HRMS (ESI) calcd for $C_9H_7O_3$ $[M+H]^+$, 163.0395; found, 163.0389.

4.2.5. 2-Bromo-3-hydroxy-chromone (intermediate)

To a solution of 3-HC **7** (540 mg, 3.34 mmol, 0.65 equiv) in acetonitrile (10 mL) was added NBS (1 equiv, 940 mg) and 2,2′-azobis(2-methylpropionitrile) (vazo® 67, 0.1 equiv, 100 mg), and the mixture was refluxed for 8 h (0.1 equiv of vazo® 67 was added each 2 h). The mixture was cooled and the solvent evaporated in vacuo. The crude product was purified on silica gel (cyclohexane/ethyl acetate: 90:10) to give the corresponding 2-Br-3-HC derivative (colourless powder, 730 mg, 90%). Mp (ether)=178–180 °C. R_f=0.57 (cyclohexane/ethyl acetate: 50:50). ^{1}H NMR (MeOD, 200 MHz) δ=7.37 (ddd, 1H, J=1.0, 7.1 and 8.1 Hz), 7.47 (dd, 1H, J=0.6 and 8.6 Hz), 7.66 (ddd, 1H, J=1.6, 7.0 and 8.6 Hz), 8.06 (dd, 1H, J=1.7 and 8.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=118.1, 121.0, 125.4, 126.0, 129.8, 134.0, 140.3, 156.7, 171.3. MS (ESI, MeOH) m/z: 238.9–240.9 $[M-H]^-$. HRMS (ESI) calcd for $C_9H_4BrO_3$ $[M-H]^-$, 238.9349; found, 238.9353.

*4.2.6. 2-Bromo-3-benzyloxy-chromone **8***

To a solution of 2-bromo-3-HC previously obtained (222 mg, 0.92 mmol) in DMF (3 mL) was added K_2CO_3 (2 equiv, 254 mg) and benzylbromide (2 equiv, 0.22 mL). The mixture was stirred overnight under N_2 atmosphere, then quenched by addition of water

and extracted with methylene chloride. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography using 10% of ethyl acetate in cyclohexane to afford compound **8** (yellow oil, 275 mg, 90%). R_f=0.37 (cyclohexane/ethyl acetate: 90:10). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=5.26 (s, 2H, CH_2), 7.34–7.55 (m, 7H), 7.65 (ddd, 1H, J=1.8, 7.1 and 8.6 Hz), 8.25 (dd, 1H, J=1.8 and 8.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=74.6, 117.8, 124.1, 125.7, 126.4, 128.5, 129.1, 133.9, 136.4, 142.4, 156.2, 172.9. MS (ESI, MeOH) m/z: 352.8–354.8 $[M+Na]^+$. HRMS (ESI) calcd for $C_{16}H_{12}O_3Br$ $[M+H]^+$, 330.9969; found, 330.9975.

4.2.7. 2-Bromo-3-(2-methoxyethoxy)methoxy-chromone **9**

To a solution of 2-bromo-3-HC previously obtained (566 mg, 2.33 mmol) in DMF (6 mL) was successively added K_2CO_3 (2 equiv, 644 mg) and MEMCl (2 equiv, 0.53 mL). The mixture was stirred overnight under N_2 atmosphere, then quenched by addition of water and extracted three times with methylene chloride. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography using 30% of ethyl acetate in cyclohexane to give compound **9** (white resin, 725 mg, 95%). R_f=0.47 (cyclohexane/ethyl acetate: 50:50). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=3.37 (s, 3H, CH_3), 3.57–3.62 (m, 2H, CH_2), 4.00–4.06 (m, 2H, CH_2), 5.39 (s, 2H, CH_2), 7.38–7.49 (m, 2H), 7.68 (ddd, 1H, J=1.8, 7.1 and 8.6 Hz), 8.20 (dd, 1H, J=1.5 and 8.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=59.2, 69.5, 71.8, 96.5, 117.7, 124.0, 125.7, 126.4, 134.0, 142.2, 172.6. MS (ESI, MeOH) m/z: 351–353 $[M+Na]^+$. HRMS (ESI) calcd for $C_{13}H_{14}O_5Br$ $[M+H]^+$, 329.0024; found, 329.0020.

4.2.8. 3-Benzyloxy-2-(trimethysilyl-ethynyl)-chromone **10**

Note that the cleavage of TMS group of pure compounds **10** and **11** was observed. In addition, the corresponding terminal alkynes of **10** and **11** were unstable and underwent slow degradation in solution. Therefore, these compounds were stored at low temperature under N_2 atmosphere and deprotected and used in situ in the next coupling step.

To a solution of compound **8** (245 mg, 0.74 mmol), trimethylsilyl-acetylene (0.615 mL, 6 equiv), TEA (2 mL, 13.86 mmol, 20 equiv) in toluene (12 mL) was added $PdCl_2(PPh_3)_2$ (52 mg, 0.1 equiv), CuI (26 mg, 0.2 equiv) under N_2 atmosphere. The mixture was stirred at 120 °C for 1 h, filtered through Celite and washed several times with ethyl acetate. The filtrate was evaporated and the obtained residue was purified by flash chromatography on silica gel, using 5% of ethyl acetate in cyclohexane, to afford **10** as yellow oil (235 mg, 92%). Compound **11** was prepared in similar manner. Analysis of compound **10** and the corresponding terminal alkyne (partial TMS-cleavage in solution). R_f=0.26 (cyclohexane/ethyl acetate: 90:10). ^{1}H NMR of a mixture of **10** and its acetylenic derivative ($CDCl_3$, 200 MHz) δ=0.31 (s, 5H, TMS), 3.79 (s, 0.3H, H-alkyne), 5.32 and 5.34 (2s, 2H, CH_2), 7.32–7.54 (m, 7H), 7.61–7.70 (m, 1H), 8.20 (dd, 1H, J=1.0 and 8.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=0.0, 74.8, 89.8, 93.9, 109.8, 118.1, 124.7, 124.9, 125.1, 125.9, 126.0, 128.3, 128.4, 128.5, 128.8, 129.0, 130.6, 130.8, 132.1, 132.3, 133.8, 134.0, 134.3, 134.6, 136.6, 136.9, 155.6, 174.3. MS (ESI, MeOH) m/z: 370.8 $[M+Na]^+$.

4.3. Synthesis of the thienyl 3-hydroxychromone nucleoside 1a

Path A. Aryl–aldol condensation.

4.3.1. 1-(5-Bromothiophen-2-yl)-2-((4R,5R)-5-((tert-butyldiphenylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanol **12**

To a solution of 2-bromothiophene (4 mmol), in THF (30 mL) was slowly added LDA (6 mmol) at −78 °C. After 45 min, the aldehyde **2** was added dropwise (1.5 mmol in 2 mL of THF). After completion of the reaction (TLC monitoring), the mixture was quenched with saturated aq NH_4Cl solution, extracted with methylene chloride, dried over $MgSO_4$, filtered and concentrated in vacuo. The obtained residue was purified by flash chromatography on silica gel (eluting with cyclohexane/ethyl acetate: 90:10) to give compound **12** (yellow oil, *R*/*S*=40:60, 75%). R_f=0.53 (cyclohexane/ethyl acetate: 70:30). Major isomer: ^{1}H NMR ($CDCl_3$, 200 MHz) δ=1.08 (s, 9H, *t*-Bu), 1.35 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.14–2.20 (m, 2H, H-2′), 3.41 (br d, 1H, J=6.3 Hz, OH), 3.70–3.74 (m, 2H, 2H-5′), 4.26 (dd, 1H, J=5.9 and 12.8 Hz, H-4′), 4.53 (dd, 1H, J=6.0 and 13.1 Hz, H-3′), 5.12 (dd, 1H, J=5.5 and 11.1 Hz, H-1′), 6.68 (dd, J=0.9 and 3.7 Hz, H-thiophene), 6.91–6.93 (d, J=3.7 Hz, H-thiophene), 7.39–7.43 (m, 6H), 7.63–7.69 (m, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=19.3, 25.6, 27.0, 28.1, 29.8, 30.3, 37.6, 39.0, 62.4, 68.1, 70.4, 74.2, 76.8, 77.5, 108.4, 111.1, 123.3, 127.9, 129.6, 130.0, 133.0, 133.1, 135.6, 135.7, 150.8. MS (ESI, MeOH) m/z: 596.7–598.7 $[M+Na]^+$.

4.3.2. α and β-1-(5-Bromothiophen-2-yl)-3-hydroxy-5-O-tert-butyldiphenylsilyl-2-deoxy-D-ribofuranose **13**

To a solution of **12** (*R*/*S*=40:60) (1.51 mmol) in toluene (40 mL) was added *p*-toluenesulfonic acid (0.2 equiv, 0.3 mmol). The mixture was stirred at 50 °C for 1 h then quenched with a saturated solution of sodium hydrogencarbonate and extracted with methylene chloride. The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give a crude oil. Silica gel column chromatography purification (cyclohexane/ethyl acetate: 90:10 to 50:50) afforded **13** as a yellow oil (740 mg, 85%, α/β=40:60). R_f=0.35 (cyclohexane/ethyl acetate: 70:30). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=1.02 (s, 9H, *t*-Bu), 2.00–2.32 (m, 2H, 2H-2′), 3.63–4.08 (m, 3H, 2H-5′and H-4′), 4.53–4.60 (m, 1H, H-3′), 5.26–5.34 (m, 1H, H-1′), 6.72 (dd, J=0.8 and 3.7 Hz, H-thiophene), 6.87 (d, J=3.7 Hz, H-thiophene), 7.35–4.10 (m, 6H), 7.67–7.80 (m, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=19.4, 27.0, 43.0, 43.7, 64.7, 64.9, 74.4, 74.6, 76.2, 85.6, 87.4, 111.7 (C–Br), 124.5, 124.7, 127.9, 127.9, 127.9, 129.4, 129.6, 129.9, 130.0, 133.2, 134.9, 135.6, 135.7, 135.8, 147.1. MS (ESI, MeOH) m/z: 539.2–541.2 $[M+Na]^+$. HRMS (ESI) calcd for $C_{25}H_{28}BrO_3SSi$ $[M-H]^-$, 515.0717; found, 515.0723.

Path B. Friedel–Crafts glycosylation.

4.3.3. β-1-(5-Bromothiophen-2-yl)-3,5-di-O-acetyl-2-deoxy-D-ribofuranose **14β**

To a stirred solution of acetyl-deoxyribose **3a** or **3b** (8.6 mmol) in CH_2Cl_2 (50 mL) and 2-bromothiophene (2 equiv) was added dropwise $SnCl_4$ (1 equiv) at 0 °C. The mixture was stirred 30 min, then quenched with saturated aqueous $NaHCO_3$ solution and extracted with methylene chloride (3×50 mL). The combined organic layers were dried over $MgSO_4$, concentrated and the crude product was purified on silica gel chromatography (cyclohexane/ethyl acetate: 95:5 to 80:20) to give **14** (2.78 g, α/β=30:70, 73% combined yield). Compound **14-β**: R_f=0.55 (cyclohexane/ethyl acetate: 50:50). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=2.09 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.17 (ddd, 1H, J=13.8, 10.7 and 6.0 Hz, $H_{2'}$), 2.36 (dd, 1H, J=13.8, and 5.8 Hz, $H_{2'}$), 4.18 (m, 2H, $H_{4'}$ and $H_{5'}$), 4.32 (dd, 1H, J=13.2 and 5.2 Hz, $H_{5'}$), 5.22 (d, J=6.0 Hz, 1H, $H_{3'}$), 5.26 (dd, 1H, J=10.7 and 5.2 Hz, $H_{1'}$), 6.75 (dd, 1H, J=3.7 and 0.6 Hz, H-thiophene), 6.89 (d, 1H, J=3.7 Hz, H-thiophene). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=21.0, 21.2, 41.5, 64.3, 76.5, 76.7, 82.8, 112.3, 125.3, 129.5, 145.7, 170.6, 170.8. MS (ESI, MeOH) m/z: 384.9–386.9 $[M+Na]^+$, 400.8–402.8 $[M+K]^+$. HRMS (ESI) calcd for $C_{13}H_{14}BrO_5S$ $[M-H]^-$, 360.9750; found, 360.9740.

4.3.4. β-1-(5-Bromothiophen-2-yl)-2-deoxy-D-ribofuranose (diol intermediate)

To a solution of **14-β** (835 mg, 2.3 mmol) in MeOH (12 mL) was added K_2CO_3 (3 equiv, 953 mg) and the mixture was stirred at room

temperature for 1 h. The solution was evaporated in vacuo and the residue was purified by flash chromatography (methylene chloride/methanol: 90:10) to give the diol as a foam (630 mg, 98%). The spectral data of this product are in accordance with those recently reported by M. Hocek et al. (Ref. 2) (see ^{1}H and ^{13}C spectral data). HRMS (ESI) calcd for $C_9H_{10}BrO_3S$ $[M-H]^-$, 276.9539; found, 276.9528.

4.3.5. β-1-(5-Bromothiophen-2-yl)-3,5-di-O-tert-butyldiphenylsilyl-2-deoxy-D-ribofuranose **15**

To a solution of diol previously obtained (610 mg, 2.19 mmol) in dry DMF (11 mL) were successively added imidazole (3.5 equiv) and TBDPSCl (3.5 equiv, 1.97 mL) under N_2 atmosphere. After stirring for 24 h, the reaction mixture was quenched with a saturated solution of NH_4Cl and then extracted three times with methylene chloride. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel column (cyclohexane/ethyl acetate: 90:10) to afford **15** as a colourless oil (1.57 g, 95%). R_f=0.8 (cyclohexane/ethyl acetate: 80:20). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=0.85 (s, 9H, *t*-Bu), 1.01 (s, 9H, *t*-Bu), 1.81 (ddd, 1H, J=12.6, 10.9 and 5.2 Hz, $H_{2'}$), 1.81 (dd, 1H, J=11.4 and 5.1 Hz, $H_{2''}$), 3.24 (dd, 1H, J=11.0 and 4.0 Hz, $H_{5'}$), 3.40 (dd, 1H, J=11.0 and 4.0 Hz, $H_{5''}$), 4.00 (dt, 1H, J=3.7 and 1.1 Hz, $H_{4'}$), 4.32 (br d, 1H, J=5.0 Hz, $H_{3'}$), 4.96 (dd, 1H, J=10.9 and 5.1 Hz, 1H, $H_{1'}$), 6.64 (dd, 1H, J=3.7 and 0.6 Hz, H-thiophene), 6.77 (d, 1H, J=3.7, H-thiophene), 7.115–7.65 (m, 20H, Ar). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=19.2, 26.9, 27.1, 44.7, 64.3, 75.6, 88.5, 127.7, 127.7, 127.8, 127.9, 129.3, 129.8, 130.0, 133.2, 133.7, 133.8, 135.9, 147.2. MS (ESI, MeOH) *m*/*z*: 777.4–779.4 $[M+Na]^+$. HRMS (ESI) calcd for $C_{41}H_{46}BrO_3SSi_2$ $[M-H]^-$, 753.1895; found, 753.1884.

4.3.6. β-1-(5-Tributylstannylthiophen-2-yl)-3,5-di-O-tert-butyldiphenylsilyl-2-deoxy-D-ribofuranose (intermediate)

To a solution of **15** (2.08 mmol) in dry ether (8 mL) was added dropwise *n*-BuLi (1.1 equiv, 1.6 M solution) at 0 °C, under N_2 atmosphere. After 1 h, the mixture was cooled to −78 °C and tributyltin chloride (1.1 equiv) was slowly added and the mixture was stirred overnight. The reaction mixture was quenched with saturated NH_4Cl solution and extracted with ether. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The obtained crude product was used in the next step without further purification.

4.3.7. β-1-(5-(3-Hydroxy-chromone-2)-thiophen-2-yl)-3,5-di-O-tert-butyldiphenylsilyl-2-deoxy-D-ribofuranose **16**

To a stirred solution of 2-bromo-3-HC **9** (284 mg, 0.86 mmol) and tin derivative (2 mmol) in toluene (10 mL) under N_2 atmosphere were successively added $Pd(PPh_3)_4$ (50 mg) and CuI (16 mg). The mixture was stirred at 120 °C for 2 h, filtered through Celite and concentrated in vacuo. The crude residue was then purified by flash chromatography on silica gel (eluting with cyclohexane/ethyl acetate: 90:10) to afford **16** as a yellow oil (573 mg, 72%). R_f=0.30 (cyclohexane/ethyl acetate: 70:30). ^{1}H NMR (acetone-d_6, 200 MHz) δ=0.87 (s, 9H, *t*-Bu), 1.02 (s, 9H, *t*-Bu), 1.97 (m, 1H, $2H_{2'}$), 2.29 (dd, 1H, J=12.6 and 5.0 Hz, $H_{2''}$), 3.02 (s, 3H, CH_3), 3.19 (t, 2H, J=4.6 Hz, CH_2 MEM), 3.30 (dd, 1H, J=11.0 and 3.7 Hz, $H_{5'}$), 3.47 (dd, 1H, J=11.1 and 3.8 Hz, $H_{5''}$), 3.62 (t, 2H, J=4.6 Hz, CH_2 MEM), 4.05 (dt, 1H, J=3.5 and 1.3 Hz, $H_{4'}$), 4.58 (d, 1H, J=5.1 Hz, $H_{3'}$), 5.37 (s, 2H, OCH_2O), 5.47 (dd, 1H, J=10.4 and 5.0 Hz, $H_{1''}$), 7.08 (d, 1H, J=3.9, H-thiophene), 7.15–7.70 (m, 23H, Ar), 7.85 (d, 1H, J=3.9 Hz, H-thiophene), 8.02 (dd, 1H, J=7.8 and 1.4 Hz, H-chromone). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=20.0, 20.1, 27.6, 27.8, 46.2, 59.2, 65.5, 71.0, 72.7, 76.9, 77.7, 89.8, 97.0, 119.1, 125.3, 126.0, 126.0, 126.5, 129.0, 129.1, 129.2, 131.0, 131.1, 131.3, 131.5, 132.0, 134.2, 134.3, 134.8, 134.9, 136.4, 136.7, 136.8, 137.0, 151.7, 154.8, 158.1, 174.0. MS (ESI, MeOH) *m*/*z*: 947.4 $[M+Na]^+$.

4.3.8. β-1-(5-(3-Hydroxy-chromone-2)-thiophen-2-yl)-2-deoxy-D-ribofuranose **1a**

To a solution of MEM-protected nucleoside **16** previously obtained (140 mg, 0.15 mmol) in dioxane (3 mL) was added HCl 6 N (1.5 mL). The mixture was stirred at room temperature overnight then neutralized by saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3×10 mL). The organic layers were evaporated in vacuo and the residue was purified by silica gel column chromatography (methylene chloride/methanol: 95:5 to 90:10) to give the free 3-HC-nucleoside **1a** as a pale resin (46 mg, 85%). R_f=0.26 (9:1 CH_2Cl_2/MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz) δ=1.85 (ddd, 1H, J=2.1, 11.3 and 13.7 Hz, $H_{2'}$), 2.12 (ddd, 1H, J=2.1 and 3.8 and 13.7 Hz, $H_{2''}$), 3.30–3.62 (m, 1H, $H_{5'}$), 3.65–3.67 (m, 2H, $H_{5''}$and $H_{4'}$), 3.99 (br m, 1H, $H_{3'}$), 4.97 (dd, 1H, J=2.1 Hz and 11.3 Hz, $H_{1'}$), 7.15 (d, 1H, J=3.9 Hz, H-thiophene), 7.47 (t, 1H, J=7.4 Hz, H-6), 7.71 (d, 1H, J=8.3 Hz, H-8), 7.81 (m, 1H, H-7), 7.82 (d, 1H, J=3.9 Hz, H-thiophene), 8.11 (dd, 1H, J=1.2 and 7.4 Hz, H-5). ^{13}C NMR (DMSO-d_6, 125 MHz) δ=42.4, 67.8, 68.2, 68.7, 71.0, 120.0, 123.7, 126.0, 126.5, 126.7, 129.9, 132.8, 135.5, 138.5, 145.2, 152.8, 156.1, 173.9. MS (ESI, MeOH) *m*/*z*: 358.9 $[M-H]^-$. HRMS (ESI) calcd for $C_{18}H_{17}O_6S$ $[M+H]^+$, 361.0745; found, 361.0741. IR (KBr) ν: 3432, 1651 cm^{-1}.

4.4. Synthesis of the triazolyl-3-hydroxy-chromone 1b

4.4.1. β-1-(4-(3-Benzyloxy-chromone-2)-triazol-1-yl)-3,5-di-O-tolouyl-2-deoxy-D-ribofuranose **17**

Method A. To a stirred solution of azido-sugar **4** (1 mmol), alkyne **10** (1.1 equiv) and *n*-Bu_4NF (1.1 equiv) in methylene chloride (5 mL) were successively added CuI (164 mg, 2 equiv), DIEA (0.37 mL, 5 equiv) and acetic acid (1 equiv). The mixture was stirred 4 h at room temperature, filtered and the solvent removed in vacuo. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 80:20) to give **17** (598 mg, 89%).

Method B. A mixture of azido-sugar **4** (1 mmol), alkyne **10** (1.1 equiv), *n*-Bu_4NF (1.1 equiv), CuI (164 mg, 2 equiv), DIEA (0.37 mL, 5 equiv) was adsorbed on silica gel (1 g) using methylene chloride. After evaporation, the resulting yellow powder was placed into a microwave and irradiated for 2 min. The mixture was eluted twice with ethyl acetate and the solvent evaporated under reduced pressure to give a crude product, which was subjected to a simple filtration over silica gel (cyclohexane/ethyl acetate: 80:20) to give **17** (638 mg, 95%). Mp (methylene chloride/ether)=183–185 °C. R_f=0.56 (cyclohexane/ethyl acetate: 50:50). ^{1}H NMR ($CDCl_3$, 200 MHz) δ=2.25 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.82–2.92 (m, 1H, $H_{2'}$), 3.11–3.24 (m, 1H, $H_{2''}$), 4.50 (d, 2H, J=4.4 Hz, $2H_{5'}$), 4.63–4.67 (m, 1H, $H_{4'}$), 5.21 and 5.32 (2d, 2H, J=11.0 Hz, CH_2), 5.69–5.74 (m, 1H, $H_{3'}$), 6.42 (t, 1H, J=5.7 Hz, $H_{1'}$), 7.13 (d, 2H, J=8.0 Hz), 7.30–7.47 (m, 8H), 7.75 (m, 2H), 7.90 (d, 2H, J=8.2 Hz), 7.95 (d, 2H, J=8.2 Hz), 8.30 (d, 2H, J=8.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ=21.7, 21.9, 38.4, 63.7, 74.4, 74.6, 83.9, 89.2, 118.7, 124.5, 125.0, 125.8, 126.5, 128.7, 129.0, 129.3, 129.4, 129.8, 129.9, 133.8, 136.8, 139.2, 144.2, 144.7, 155.3, 165.9, 166.2, 174.4. MS (ESI, MeOH) *m*/*z*=709.8 $[M+K]^+$. HRMS (ESI) calcd for $C_{39}H_{33}N_3O_8$ $[M+H]^+$, 672.2345; found, 672.2341.

4.4.2. β-1-(4-(3-Benzyloxy-chromone-2)-triazol-1-yl)-2-deoxy-D-ribofuranose (intermediate)

By the same procedure as described above for the synthesis of **14**, compound **17** (100 mg, 0.15 mmol) was deprotected using K_2CO_3 in MeOH to afford the pure product (62 mg, 95%). Mp (methylene chloride/ether)=94–96 °C. R_f=0.24 (9:1 CH_2Cl_2/MeOH). ^{1}H NMR (MeOD, 200 MHz) δ=2.47–2.78 (m, 2H, $2H_{2'}$), 3.60 (dd, 1H, J=4.6 and 11.9 Hz, $1H_{5'}$), 3.70 (dd, 1H, J=3.8 and 11.9 Hz, $1H_{5''}$), 4.03 (dd, 1H, J=4.2 and 8.2 Hz, $H_{4'}$), 4.48–4.56 (dd, 1H, J=5.5 and 10.0 Hz, $H_{3'}$), 5.28 (s, 2H, CH_2), 6.42 (t, 1H, J=5.9 Hz, $H_{1'}$), 7.20–7.35 (m, 4H), 7.44 (td, 1H, J=1.1 and 8.1 Hz), 7.64 (d, 1H, J=8.4 Hz), 7.75 (ddd, 1H,

J=1.5, 7.0 and 8.4 Hz), 7.85 (d, 1H, J=8.2 Hz), 8.15 (dd, 1H, J=1.3 and 8.1 Hz), 8.71 (s, 1H, H-5). ^{13}C NMR (MeOD, 50 MHz) δ=42.0, 56.0, 63.0, 72.1, 75.0, 89.9, 90.7, 119.5, 125.1, 126.2, 126.4, 127.3, 129.4, 129.7, 130.0, 130.5, 130.7, 135.4, 137.6, 139.2, 139.5, 156.5, 176.1. MS (ESI, MeOH) m/z=457.9 $[M+Na]^+$, 473.8 $[M+K]^+$. HRMS (ESI) calcd for $C_{23}H_{22}N_3O_6$ $[M+H]^+$, 436.1508; found, 436.1509.

4.4.3. β-1-(4-(3-Hydroxy-chromone-2)-triazol-1-yl)-2-deoxy-D-ribofuranose **1b**

To a degassed solution of the above triazolyl-compound intermediate (0.3 mmol) in THF (10 mL) was added Pd/C (10% molar). Hydrogenolysis was then conducted in a high pressure autoclave (Parr apparatus, 3 bar) for 10 h. The catalyst was filtered and washed with THF. The filtrate was concentrated in vacuo and the obtained residue was purified by flash chromatography to afford **1b** as a white powder (34 mg, 90%). R_f=0.32 (8:2 CH_2Cl_2/MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz) δ=2.42–2.47 (m, 1H, $H_{2'}$), 2.73 (ddd, 1H, J=5.8 and 13.5 Hz, $H_{2'}$), 3.45 (dd, 1H, J=4.8 and 11.7 Hz, $H_{5'}$), 3.55 (dd, 1H, J=4.3 and 11.7 Hz, $H_{5'}$), 3.91 (dd, 1H, J=4.8 and 8.8 Hz, $H_{4'}$), 4.43–4.44 (m, 1H, $H_{3'}$), 4.87 (br s, 1H, $OH_{5'}$), 5.36 (br s, 1H, $OH_{3'}$), 6.52 (t, 1H, J=5.9 Hz, $H_{1'}$), 7.48 (ddd, 1H, J=1.0, 7.0 and 8.0 Hz), 7.73 (d, 1H, J=8.2 Hz), 7.81 (ddd, 1H, J=1.6, 7.0 and 8.6 Hz), 8.13 (dd, 1H, J=1.6 and 8.0 Hz), 8.81 (s, 1H, H-5), 10.00 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ=40.0, 61.5, 70.4, 88.4, 88.5, 118.4, 122.0, 124.7, 124.9, 125.3, 133.8, 137.9, 138.0, 140.3, 154.5, 172.1. MS (ESI, MeOH) m/z: 343.7 $[M-H]^-$, 368.0 $[M+Na]^+$. HRMS (ESI) calcd for $C_{16}H_{16}N_3O_6$ $[M+H]^+$, 346.1039; found, 346.1042. IR (KBr) ν: 3392, 1621 cm^{-1}.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.07.021.

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