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Short communication

Synthesis and evaluation of spectroscopic properties of newly synthesized push–pull 6-amino-8-styryl purines

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ABSTRACT

New 6-amino-8-styryl purines were synthesized using direct C–H bond functionalization. These push–pull compounds showed strong fluorescence, high quantum yields and a noteworthy fluorosolvatochromism. Deprotected purines **7a**–**c** are promising targets for incorporation into nucleic acids as they are still fluorescent in aqueous media.

1. Introduction

Over the past decade, fluorescence spectroscopy has gained considerable attention because of its wide range of applications. Notably, fluorescent molecules are known for their use in bioimaging, sensing, following chemical interactions of biomolecules or monitoring the delivery of therapeutics [1–3]. The photophysical properties of nucleosides have been studied lately as the purine scaffold exhibits promising fluorescent properties [4–10]. Indeed, nucleoside derivatives find applications in biological imaging of live cells [11,12], in DNA detection [13,14] or as fluorescent probes [15]. As such, developing new fluorescent purines fulfilling the standards of an ideal fluorophore and thus displaying high quantum yield combined with large Stokes shift is of great interest. Indeed, extending the π-conjugation usually results in a bathochromic shift of absorption and emission wavelengths and an increased quantum yield. Also, the fluorescent properties can be enhanced using push–pull structures which skeleton is composed of a conjugated π-electron system substituted by an electron withdrawing group and an electron donor one. In this context, we envisioned the synthesis of new push–pull purines bearing an amino substituent on the position 6 as the electron donor moiety and a π-conjugated styryl linker on the position 8, substituted with an electron withdrawing group in the para position of the phenyl ring. Although a styryl spacer has already been used in the examination of the fluorescent properties of caffeine or guanosine respectively bearing an oxo or a free amino group on position 6, the combination of both an *N*,*N*′-substituted amino group and a styryl group on the purine ring has never been reported.[11,15–17] Moreover, examination of the fluorescence properties of these compounds in water was not investigated.

Given that we have recently developed methods for the C–H bond direct functionalization of azoles [18,19], including purines [20], we applied these conditions for the synthesis of our molecules thereby optimizing the number of steps and providing an easy and rapid access to these potential fluorescent compounds. Herein, we disclose the synthesis and the investigation of photophysical properties of new push–pull 6-amino-8-styryl purines in organic solvents and water. The impact of the different substituents is discussed.

2. Experimental

2.1. General experimental methods

Commercially available reagents and solvents were used without further purification unless otherwise stated. Yields refer to

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isolated and purified products. Reactions were monitored by thin-layer chromatography carried out on silica gel plates (60F-254) visualized under UV light. Column chromatography was performed on silica gel 60, 40–63 μm. Chemical shifts of ^{1}H NMR and ^{13}C NMR were reported in ppm (δ units) and residual non deuterated solvent was used as internal reference. The following abbreviations were used to designate the multiplicities : s = singlet, d = doublet, t = triplet, q = quadruplet, bs = broad singlet, m = multiplet. Microwave irradiation was performed on CEM Explorer (CEM Corporation). Temperature measurement of the reaction mixture within the Discovery series was achieved by an IR sensor. The method was set with maximum power of 150 W, with maximum pressure of 17 bar and used without powermax. Reaction times refer to the hold time at the desired set temperature. Reaction cooling was performed by compressed air after the heating period was over. UV–vis experiments were monitored on a Cary Series UV–vis spectrophotometer (Agilent Technologies). Fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies) at room temperature. Measurements were performed with solutions of OD < 0.1 to avoid re-absorption of the emitted light, and data were corrected with a blank and from the variations of the detector with the emitted wavelength. Fluorescence quantum yield were measured according to Williams comparative method using quinine sulfate in 1 M H_2SO_4 as reference. [21] Absorption and fluorescence spectra were recorded for four solutions of increasing concentrations with an absorbance comprised between 0.01 and 0.1 to avoid re-absorption phenomenon. Electrospray ionization mass spectrometry (ESI-MS) was performed at the Institut Curie and HRMS were performed at the Small Molecule Mass Spectrometry platform of IMAGIF (ICSN, Gif-Sur-Yvette, France).

2.2. Chemical

*N*9-protected purines **1** and **4** were prepared according to the literature [22,23]. 8-styrylpurines **2a**–**b** and **5a**–**b** were synthetized through our formerly developed direct alkenylation method [20]. 6-aminopurines **3a**–**h** and **6a**–**c** were obtained following our previously described procedure under Buchwald-Hartwig-inspired palladium cross-coupling conditions [24]. Purines **7a**–**c** were deprotected under acidic conditions [25].

2.2.1. 9-benzyl-6-chloro-9H-purine (**1**)

White solid (51%) : ^{1}H NMR (300 MHz, $CDCl_3$) 8.80 (s, 1H), 8.10 (s, 1H), 7.37–7.33 (m, 5H), 5.46 (s, 2H). Spectroscopic data were in agreement with those reported in the literature [22].

2.2.2. (E)-9-benzyl-6-chloro-8-(4-(trifluoromethyl)styryl)-9H-purine (**2a**)

White solid (60%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.16 (d, J = 15 Hz, 1H), 7.67–7.59 (m, 4H), 7.40–7.33 (m, 3H), 7.23 (d, J = 7.9 Hz, 2H), 7.08 (d, J = 15.9 Hz, 1H), 5.62 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.3, 153.2, 151.8, 149.7, 139.3, 138.4, 135.1, 131.8, 131.7, 129.4, 128.8, 127.9, 127.0, 126.11, 126.06, 114.4, 46.4. MS (ES+) m/z (%) : 437.2 (80) $[M + Na]^+$. HRMS (ESI) calcd for $C_{21}H_{15}ClF_3N_4$ $[(M + H)^+]$ 415.0937, found 415.0945.

2.2.3. (E)-4-(2-(9-benzyl-6-chloro-9H-purin-8-yl)vinyl) benzonitrile (**2b**)

Beige solid (56%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.13 (d, J = 15.9 Hz, 1H), 7.70–7.58 (m, 4H), 7.40–7.35 (m, 3H), 7.22 (d, J = 6.8 Hz, 2H), 7.09 (d, J = 15.8 Hz, 1H), 5.62 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.3, 152.8, 151.9, 149.9, 139.3, 138.6, 135.1, 132.8, 131.7, 129.5, 128.8, 128.1, 126.9, 118.5, 115.4, 113.1, 46.4. MS (ES+) m/z (%) : 394.3 (100) $[M + Na]^+$. HRMS (ESI) calcd for $C_{21}H_{15}ClN_5$ $[(M + H)^+]$ 372.1016, found 372.1009.

2.2.4. (E)-4-(2-(9-benzyl-6-((4-methoxybenzyl)amino)-9H-purin-8-yl)vinyl)benzonitrile (**3a**)

Yellow solid (67%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.71 (d, J = 15.9 Hz, 1H), 7.62–7.46 (m, 4H), 7.36–7.28 (m, 5H), 7.20 (d, J = 6.8 Hz, 2H), 7.01 (d, J = 15.8 Hz, 1H), 6.85 (d, J = 8.5 Hz, 2H), 6.35 (bs, 1H), 5.51 (s, 2H), 4.81 (bs, 2H), 3.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 154.2, 153.5, 147.3, 140.0, 136.0, 134.3, 132.7, 130.5, 129.2, 128.3, 127.6, 126.8, 119.9, 118.7, 116.6, 114.1, 112.1, 55.4, 45.8, 44.2. MS (ES+) m/z (%) : 473.3 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{29}H_{25}N_6O$ $[(M + H)^+]$ 473.2090, found 473.2094.

2.2.5. (E)-9-benzyl-N-(4-methoxybenzyl)-8-(4-(trifluoromethyl) styryl)-9H-purin-6-amine (**3b**)

Yellow solid (68%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.75 (d, J = 15.9 Hz, 1H), 7.61–7.50 (m, 4H), 7.37–7.29 (m, 5H), 7.22 (d, J = 6.5 Hz, 2H), 7.01 (d, J = 15.9 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 6.24 (bs, 1H), 5.52 (s, 2H), 4.83 (bs, 2H), 3.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 154.2, 153.4, 147.6, 139.1, 136.1, 134.9, 130.6, 129.3, 128.2, 127.3, 126.8, 125.8, 122.2, 119.9, 115.7, 114.1, 55.3, 45.7, 44.3. MS (ES+) m/z (%) : 516.5 (80) $[M + H]^+$. HRMS (ESI) calcd for $C_{29}H_{25}F_3N_5O$ $[(M + H)^+]$ 516.2011, found 516.1995.

2.2.6. (E)-4-(2-(9-benzyl-6-(pyrrolidin-1-yl)-9H-purin-8-yl)vinyl) benzonitrile (**3c**)

Yellow solid (99%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.73 (d, J = 15.8 Hz, 1H), 7.64–7.51 (m, 4H), 7.36–7.27 (m, 3H), 7.18 (d, J = 6.5 Hz, 2H), 7.03 (d, J = 15.8 Hz, 1H), 5.52 (s, 2H), 4.28 (bs, 2H), 3.82 (bs, 2H), 2.07 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.2, 152.8, 151.6, 146.0, 140.5, 136.3, 133.2, 132.6, 129.1, 128.1, 127.5, 126.7, 120.8, 118.8, 117.1, 111.8, 49.2 45.5, 30.2. MS (ES+) m/z (%) : 407.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{25}H_{23}N_6$ $[(M + H)^+]$ 407.1984, found 407.1978.

2.2.7. (E)-9-benzyl-6-(pyrrolidin-1-yl)-8-(4-(trifluoromethyl) styryl)-9H-purine (**3d**)

Yellow solid (84%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.76 (d, J = 15.8 Hz, 1H), 7.62–7.53 (m, 4H), 7.35–7.28 (m, 3H), 7.19 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 15.8 Hz, 1H), 5.52 (s, 2H), 4.29 (bs, 2H), 3.82 (bs, 2H), 2.07 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.1, 152.8, 151.6, 146.4, 139.6, 136.4, 133.9, 130.7, 130.2, 129.1, 128.1, 127.3, 126.8, 125.8, 120.7, 116.2, 49.1, 45.6, 30.2. MS (ES+) m/z (%) : 450.5 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{25}H_{23}F_3N_5$ $[(M + H)^+]$ 450.1906, found 450.1912.

2.2.8. (E)-4-(2-(9-benzyl-6-((2-methoxyethyl)amino)-9H-purin-8-yl)vinyl)benzonitrile (**3e**)

Yellow solid (95%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.78 (d, J = 15.9 Hz, 1H), 7.66–7.51 (m, 4H), 7.36–7.29 (m, 3H), 7.20 (d, J = 6.8 Hz, 2H), 7.03 (d, J = 15.9 Hz, 1H), 6.15 (bs, 1H), 5.52 (s, 2H), 3.91 (bs, 2H), 3.67 (*t*, J = 5.1 Hz, 2H), 3.43 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.4, 153.3, 150.6, 147.3, 140.1, 136.1, 134.2, 132.6, 129.2, 128.2, 127.6, 126.8, 120.2, 118.7, 116.7, 112.1, 71.3, 58.9, 45.7, 29.8. MS (ES+) m/z (%) : 411.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{24}H_{23}N_6O$ $[(M + H)^+]$ 411.1933, found 411.1933.

2.2.9. (E)-9-benzyl-N-(2-methoxyethyl)-8-(4-(trifluoromethyl) styryl)-9H-purin-6-amine (**3f**)

Yellow solid (62%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.80 (d, J = 15.9 Hz, 1H), 7.62–7.53 (m, 4H), 7.36–7.28 (m, 3H), 7.20 (d, J = 7.0 Hz, 2H), 7.01 (d, J = 15.9 Hz, 1H), 6.24 (bs, 1H), 5.52 (s, 2H), 3.92 (bs, 2H), 3.67 (*t*, J = 4.9 Hz, 2H), 3.42 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.4, 153.2, 147.6, 139.2, 136.1, 134.9, 130.9, 130.5, 129.2,

128.3, 127.4, 125.9, 122.2, 120.1, 115.7, 71.3, 58.9, 45.8, 29.8. MS (ES+) m/z (%) : 454.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{24}H_{23}F_3N_5O$ $[(M + H)^+]$ 455.1855, found 455.1846.

*2.2.10. (E)-4-(2-(7-benzyl-4-(benzyl(methyl)amino)-7H-pyrrolo [2,3-d]pyrimidin-6-yl)vinyl)benzonitrile (**3g**)*

Yellow oil (78%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.70 (d, J = 16.0 Hz, 1H), 7.48–7.63 (m, 4H), 7.37–7.28 (m, 8H), 7.21 (d, J = 6.7 Hz, 2H), 7.02 (d, J = 15.8 Hz, 1H), 5.54 (s, 2H), 3.50 (s, 2H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.5, 152.9, 152.3, 145.7, 140.4, 138.0, 136.3, 133.8, 132.7, 129.2, 128.7, 128.2, 127.9, 127.6, 126.8, 120.5, 118.8, 116.9, 111.9, 45.7, 43.6, 30.3. MS (ES+) m/z (%) : 457.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{29}H_{25}N_6$ $[(M + H)^+]$ 457.2141, found 457.2144.

*2.2.11. (E)-N,7-dibenzyl-N-methyl-6-(4-(trifluoromethyl)styryl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**3h**)*

Yellow oil (88%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.74 (d, J = 15.9 Hz, 1H), 7.60–7.50 (m, 4H), 7.37–7.28 (m, 8H), 7.23 (d, J = 6.4 Hz, 2H), 7.01 (d, J = 15.9 Hz, 1H), 5.54 (s, 2H), 3.51 (s, 2H), 1.66 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.4, 152.6, 152.1, 145.9, 139.3, 137.9, 136.2, 134.2, 130.6, 130.2, 129.0, 128.6, 128.0, 127.8, 127.3, 127.2, 126.7, 125.7, 122.1, 120.2, 115.8, 45.5, 43.4, 30.1. MS (ES+) m/z (%) : 500.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{29}H_{25}F_3N_5$ $[(M + H)^+]$ 500.2062, found 500.2059.

*2.2.12. 6-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (**4**)*

Pale yellow solid (89%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.35 (s, 1H), 5.80 (dd, J = 10.2, 2.5 Hz, 1H), 4.23–4.18 (m, 1H), 3.80 (td, J = 11.4, 3.1 Hz, 1H), 2.20–2.00 (m, 3H), 1.85–1.67 (m, 3H). Spectroscopic data were in agreement with those reported in the literature [23].

*2.2.13. (E)-6-chloro-9-(tetrahydro-2H-pyran-2-yl)-8-(4-(trifluoromethyl)styryl)-9H-purine (**5a**)*

Yellow solid (36%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.15 (d, J = 16.6 Hz, 1H), 7.75–7.68 (m, 4H), 7.60 (d, J = 16.0 Hz, 1H), 6.04 (d, J = 11.8, 1H), 4.34 (d, J = 11.0 Hz, 1H), 3.82 (*t*, J = 11.5 Hz, 1H), 2.30–1.78 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.1, 152.2, 151.4, 149.7, 138.9, 138.4, 131.4, 127.9, 126.1, 126.0, 122.2, 117.1, 83.2, 69.6, 31.9, 25.3, 23.2. MS (ES+) m/z (%) : 431.2 (100) $[M + Na]^+$. HRMS (ESI) calcd for $C_{19}H_{17}ClF_3N_4O$ $[(M + H)^+]$ 409.1043, found 409.1046.

*2.2.14. (E)-4-(2-(6-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)vinyl)benzonitrile (**5b**)*

Yellow solid (38%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.12 (d, J = 16.0 Hz, 1H), 7.72 (m, 4H), 7.62 (d, J = 16.0 Hz, 1H), 6.04 (dd, J = 11.0, 2.6 Hz, 1H), 4.34 (dd, J = 12.0, 3.6 Hz, 1H), 3.83 (td, J = 11.5, 3.8 Hz, 1H), 2.32–2.09 (m, 2H), 2.00–1.75 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.7, 152.2, 151.5, 149.9, 139.8, 137.8, 132.8, 131.4, 128.2, 118.6, 118.1, 112.9, 83.3, 69.6, 32.0, 25.3, 23.2. MS (ES+) m/z (%) : 282.1 (100) $[M\text{-THP}]^+$, 366.2 (60) $[M + H]^+$. HRMS (ESI) calcd for $C_{19}H_{17}ClN_5O$ $[(M + H)^+]$ 366.1122, found 366.1137.

*2.2.15. (E)-4-(2-(6-((4-methoxybenzyl)amino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)vinyl)benzonitrile (**6a**)*

Yellow solid (82%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.76–7.55 (m, 6H), 7.30 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 6.19 (bs, 1H), 5.96 (dd, J = 11.0, 2.4 Hz, 1H), 4.80 (bs, 2H), 4.30 (d, J = 12.9 Hz, 1H), 3.84–3.79 (m, 4H), 2.24–2.04 (m, 2H), 1.96–1.68 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 154.2, 153.2, 147.1, 140.5, 133.6, 132.7, 130.5, 129.3, 127.6, 119.5, 118.8, 114.1, 111.9, 82.7, 69.5, 55.4, 43.5, 32.3, 25.4, 23.2. MS (ES+) m/z (%) : 467.3 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{27}H_{27}N_6O_2$ $[(M + H)^+]$ 467.2195, found 467.2206.

*2.2.16. (E)-6-(pyrrolidin-1-yl)-9-(tetrahydro-2H-pyran-2-yl)-8-(4(trifluoromethyl)styryl)-9H-purine (**6b**)*

Yellow solid (68%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.79 (d, J = 16.0 Hz, 1H), 7.70–7.63 (m, 4H), 7.58 (d, J = 16.0 Hz, 1H), 5.97 (d, J = 11.1 Hz, 1H), 4.32–4.28 (m, 3H), 3.84–3.77 (m, 3H), 2.18–2.05 (m, 6H), 1.92–1.64 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6, 150.4, 146.0, 139.9, 133.1, 129.9, 127.2, 125.8, 120.1, 118.9, 82.4, 69.4, 32.1, 30.2, 25.3, 23.2. MS (ES+) m/z (%) : 444.3 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{23}H_{25}F_3N_5O$ $[(M + H)^+]$ 444.2011, found 444.2022.

*2.2.17. (E)-N-(4-methoxybenzyl)-9-(tetrahydro-2H-pyran-2-yl)-8-(4-(trifluoromethyl)styryl)-9H-purin-6-amine (**6c**)*

Yellow solid (84%) : ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.76 (d, J = 16.1 Hz, 1H), 7.65 (s, 4H), 7.55 (d, J = 16.1 Hz, 1H), 7.32 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 6.14 (bs, 1H), 5.95 (dd, J = 11.1, 2.4 Hz, 1H), 4.80 (bs, 2H), 4.31 (d, J = 9.9 Hz, 1H), 3.84–3.79 (m, 4H), 2.23–2.07 (m, 2H), 1.95–1.64 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2, 154.2, 153.0, 147.5, 139.6, 134.2, 130.8, 130.5, 130.4, 129.4, 127.4, 125.9, 122.3, 119.5, 118.5, 114.2, 82.7, 69.5, 55.4, 43.6, 32.2, 25.4, 23.3. MS (ES+) m/z (%) : 510.3 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{27}H_{27}F_3N_5O_2$ $[(M + H)^+]$ 510.2117, found 510.2123.

*2.2.18. (E)-4-(2-(6-((4-methoxybenzyl)amino)-9H-purin-8-yl) vinyl)benzonitrile (**7a**)*

Yellow solid (99%) : ^{1}H NMR (300 MHz, DMSO-d6) δ 8.20 (bs, 2H), 7.88–7.80 (m, 4H), 7.64 (d, J = 16.7 Hz, 1H), 7.35–7.29 (m, 3H), 6.86 (d, J = 8.2 Hz, 2H), 4.65 (bs, 2H), 3.70 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 158.1, 152.7, 147.3, 140.2, 132.8, 132.2, 131.9, 128.6, 127.6, 120.9, 118.8, 113.6, 110.6, 55.0, 29.0. MS (ES+) m/z (%) : 383.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{29}H_{19}N_6O$ $[(M + H)^+]$ 383.1620, found 383.1625.

*2.2.19. (E)-6-(pyrrolidin-1-yl)-8-(4-(trifluoromethyl)styryl)-9H-purine (**7b**)*

Yellow solid (93%) : ^{1}H NMR (300 MHz, DMF-d7) δ 13.18 (bs, 1H), 8.21 (s, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.83–7.75 (m, 3H), 7.43 (d, J = 16.6 Hz, 1H), 4.21 (bs, 2H), 3.70 (bs, 2H), 2.02 (bs, 4H). ^{13}C NMR (75 MHz, DMF-d7) δ 152.9, 152.6, 152.32, 152.29, 152.2, 147.0, 140.4, 131.9, 127.6, 125.9, 121.2, 120.8, 22.5, 13.7. MS (ES+) m/z (%) : 360.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{18}H_{17}F_3N_5$ $[(M + H)^+]$ 360.1436, found 360.1441.

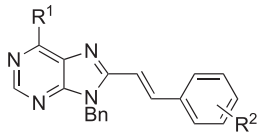
*2.2.20. (E)-N-(4-methoxybenzyl)-8-(4-(trifluoromethyl)styryl)-9H-purin-6-amine (**7c**)*

Yellow solid (87%) : ^{1}H NMR (300 MHz, DMF-d7) δ 8.23 (s, 1H), 7.92 (d, J = 8.1 Hz, 1H), 7.81–7.74 (m, 3H), 7.58–7.51 (m, 2H), 7.43–7.35 (m, 3H), 6.91–6.85 (m, 3H), 4.80 (bs, 2H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, DMF-d7) δ 13C NMR (75 MHz, DMF-d^7) δ 158.8, 153.1, 152.1, 147.7, 140.2, 132.5, 129.4, 128.9, 127.7, 126.4, 125.9, 125.8, 124.9, 122.8, 120.6, 113.7, 54.9, 22.5. MS (ES+) m/z (%) : 426.4 (100) $[M + H]^+$. HRMS (ESI) calcd for $C_{22}H_{19}F_3N_5O$ $[(M + H)^+]$ 426.1542, found 426.1534.

3. Results and discussion

During our previous work on developing new synthetic methodologies in the area of direct C–H bond functionalization, new 6-amino- or 6-thio-8-styrylpurines were synthesized through a key C-8 direct alkenylation step (Fig. 1) [20].

First, the optical properties of these newly synthesized compounds were studied (results not shown). Among those 19 compounds, eight were fluorescent in dichloromethane with a quantum yield higher than 0.32. From a structural point of view, they bear either electron withdrawing group on the para position of the styryl group or an amino substituent on the 6 position of the



R^1= SPh, R^2 = EWG, EDG
or R^1= NHR, R^2 = H

Fig. 1. Structure of 8-styrylpurines previously synthetized.

purine ring. Since we were interested in generating new push–pull chromophores with enhanced fluorescent properties, a small library of new purines derivatives combining an amino substituent at position 6 and an electron withdrawing group on the para position of the styryl group was designed. Hence, new molecules **3a**–**h** were synthesized, in a straightforward way, through direct alkenylation and Buchwald–Hartwig reactions using conditions both developed in our laboratory [20,24,26].

3.1. Synthesis

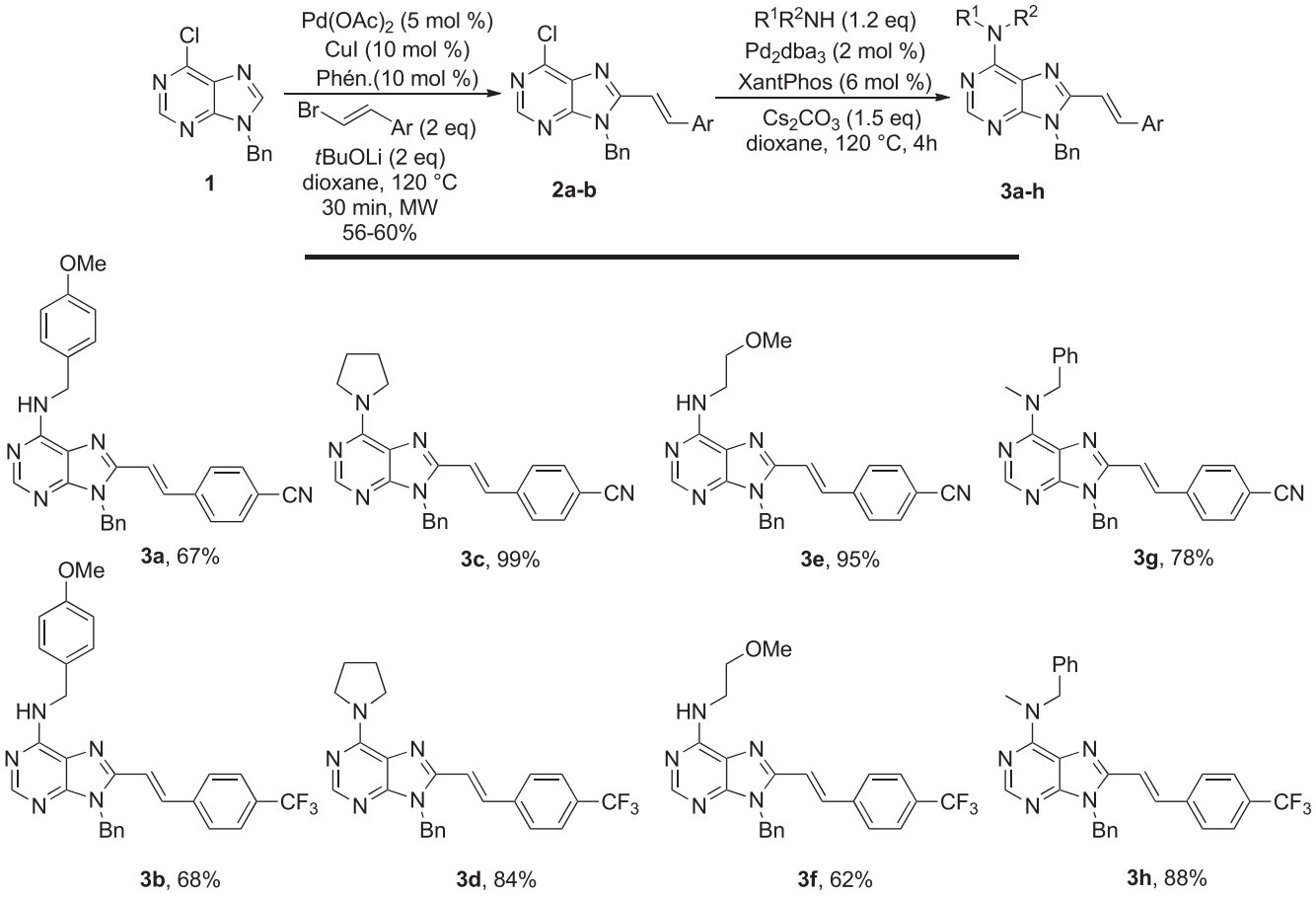
The synthetic route of 8-vinylpurines is outlined in Schemes 1 and 2.

Molecules **3a**–**h** were synthesized in two steps from purine **1**. Purines **2a**–**b** were obtained by direct alkenylation of purine **1** in moderate yields, with (*E*)-styryl bromides under microwave irradiation using a Pd/Cu co-catalyst system, reaction developed in our laboratory. [20] (*E*)-bromostyrenes, bearing an electron withdrawing substituent in the para position of the phenyl ring, were prepared in a two-step literature procedure starting from the commercially available aldehydes [27,28]. Then, a range of amines were introduced at position 6 of 8-styrylpurines through a Buchwald–Hartwig cross-coupling reaction, which conditions are similar to those that we previously used with 8-iodopurines [24].

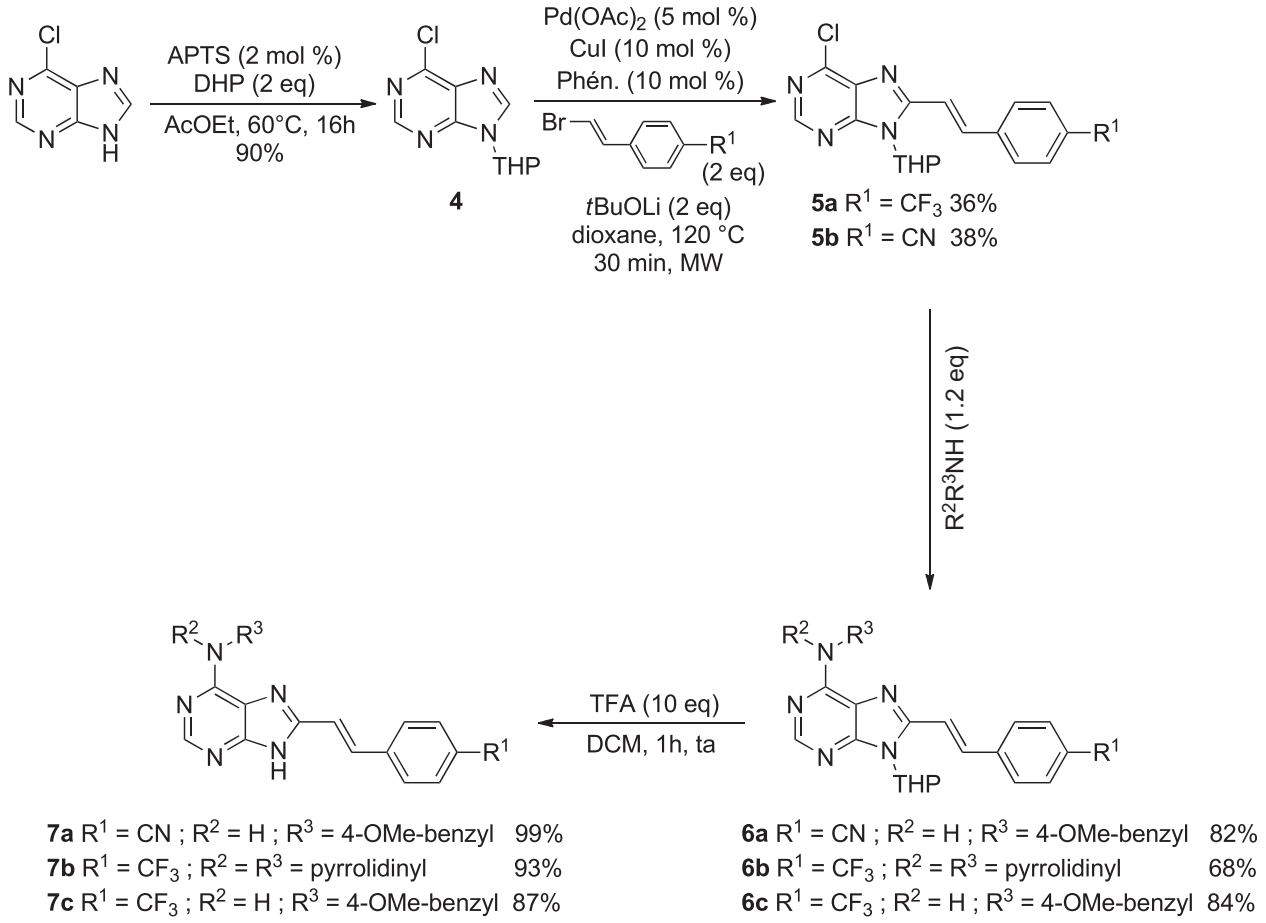
Assuming that we would create fluorescent DNA bases, it was necessary that 8-styrylpurines had no substituent at position 9, so as to make them more polar and then to increase their solubility in water. In order to synthetize *N*9–H free purines, this position had to be deprotected in the last step. However, it would have been difficult to remove the benzyl group without reducing the double bond. Thus, we decided to synthetize analogs with a tetrahydropyranyl protecting group, that could be easily introduced and removed under acidic conditions, compatible with the ethylenic double bond. Among the 9-benzylpurines **3a**–**h** synthetized, three molecules exhibiting the most interesting fluorescent properties were chosen for further study and resynthesized with a free NH at position 9. Thus, compounds **7a**–**c** were obtained in 4 steps from the commercially available 6-chloropurine. After THP protection on the *N*9 position of the purine ring, purine **4** underwent direct alkenylation reaction, followed by amination in the Buchwald–Hartwig conditions to afford molecules **6a**–**c**, which were efficiently deprotected using trifluoroacetic acid.

3.2. UV–vis and photoluminescence data

The optical properties of purines **3a**–**h** and **7a**–**c** measured in dichloromethane at 25 °C using UV/visible and photoluminescence



Scheme 1. Synthetic route to molecules **3a**–**h**.



Scheme 2. Synthetic pathway to compounds **7a**–**c**.

spectroscopy are presented in Table 1. All compounds show absorption wavelengths in the UV region (349–390 nm) and emission wavelengths in the visible region (437–490 nm). In consequence, large Stokes shifts were observed, which suggests significant differences (vibrational, electronic, geometric) between the Franck-Condon state and the excited state. Furthermore, all compounds have moderate absorption coefficient allowing them to be sufficiently bright for detection. Also, high quantum yields were obtained for all compounds. The photophysical properties were moderately modified depending on the substituents. If we compare the two substituents used in the para position of the phenyl ring, we can notice that nitrile displays a larger bathochromic shift whereas trifluoromethyl group leads to higher Stokes shifts and quantum yields. Also, it should be noted that quantum yields are slightly better when amine in position 6 is substituted by pyrrolidinyl or 4-methoxybenzyl groups. Regarding 9-benzylpurines, compounds **3a,b,d** showed particularly higher quantum yields compared to other purine derivatives. Therefore, we were interested in generating analogs bearing no protecting group on the *N*9 position in order to examine the influence of such substituent. *N*9–H free purines **7a**–**c** still exhibited strong fluorescence in dichloromethane with quantum yields ranging from 0.71 to 0.93.

Table 1
Spectroscopic data of compounds **3a**–**h** and **7a**–**c**.

Compound[a]	λ_{abs}, nm (ε, L^{-1} cm^{-1})	λ_{em}, nm	φ_F[b]	Stokes shift, cm^{-1}
3a	369 (18,900)	480	0.75	6267
3b	354 (15,100)	456	0.81	6319
3c	390 (19,800)	490	0.62	5233
3d	373 (16,700)	472	0.79	5623
3e	369 (15,300)	475	0.63	6048
3f	354 (16,900)	454	0.65	6222
3g	385 (24,100)	486	0.63	5398
3h	369 (18,100)	464	0.63	5549
7a	362 (19,300)	437	0.93	4741
7b	359 (14,100)	443	0.71	5285
7c	349 (15,300)	437	0.74	5770

[a] All spectra were recorded in CH_2Cl_2 solutions at room temperature with *c* from 10^{-6} to 10^{-5} M.
[b] Fluorescence quantum yield (±10%) were determined relative to quinine sulfate in 1 M H_2SO_4 ($\varphi_F = 0.54$).

3.3. Fluorosolvatochromism

The spectroscopic properties of compounds **7a**–**c** were evaluated in different solvents and are summarized in Table 2. The aim of this study was to investigate the effect of solvent polarity on the photophysical properties of the fluorophores. We noted that the emission band was red-shifted while increasing solvent polarity, evaluated by solvent orientation polarizability [29]. On the contrary, the absorption band was not importantly shifted. This solvatochromic behavior, well established with donor-acceptor fluorophores, is due to an interaction charge transfer in the lowest excited state [30–32]. As an example, the emission spectra and color changes under UV irradiation of compound **7a** are shown in Fig. 2. Compounds **7a**–**c** showed high fluorescence combined with high quantum yields in organic solvents of different polarity

Table 2
UV/vis and photoluminescence data of compounds **7a**–**c** in organic solvents and water.

Compound[a]	Solvent	λ_{abs}, nm (ε, L^{-1} cm^{-1})	$\lambda_{ém}$, nm	φ_F[b]	Stokes shift, cm^{-1}
7a	Toluene	370 (20,900)	449	>0.99	4755
	DCM	362 (19,300)	437	0.93	4741
	MeOH	357 (20,800)	481	0.80	7221
	DMSO	371 (19,500)	496	0.81	6793
	H_2O	337 (6400)	491	0.02	9307
7b	Toluene	370 (15,700)	446	0.93	6150
	DCM	359 (14,100)	443	0.71	5285
	MeOH	353 (16,800)	457	0.80	6447
	DMSO	368 (15,600)	481	>0.99	6384
	H_2O	354 (11,800)	469	0.07	6927
7c	Toluene	358 (15,900)	437	>0.99	5050
	DCM	349 (15,300)	437	0.74	5770
	MeOH	346 (17,400)	444	0.90	6379
	DMSO	360 (15,300)	478	0.95	6857
	H_2O	353 (12,500)	463	0.03	6730

[a] All spectra were recorded at room temperature with c from 10^{-6} to 10^{-5} M.
[b] Fluorescence quantum yield (±10%) were determined relative to quinine sulfate in 1 M H_2SO_4 ($\varphi_F = 0.54$).

(toluene, dichloromethane, methanol, DMSO). It should be noted that when a protic solvent such as methanol was used, the fluorescence was not quenched.

One of the main characteristics of an ideal fluorophore is its ability to be soluble in water. In fact, compounds **7a**–**c** were soluble in water, obeying the Beer–Lambert law up to a concentration of 30 μM. Interestingly, these molecules were still fluorescent in aqueous media. They showed low quantum yields but these could be sufficient enough to allow fluorescence detection. Indeed, Teulade-Fichou et al. used triphenylamine and DAPI, which show quantum yields of 0.021 and 0.019 respectively in aqueous media, for imaging DNA in cells [33]. Moreover, the purines **7a**–**c** absorbed in the UV region (337–360 nm) and emitted from 478 to 491 nm (Fig. 3). Thus, large Stokes shifts were observed (6730–9307 cm^{-1}), which could facilitate the detection and as such prevent from reabsorption phenomenon [34]. Under UV irradiation, the color

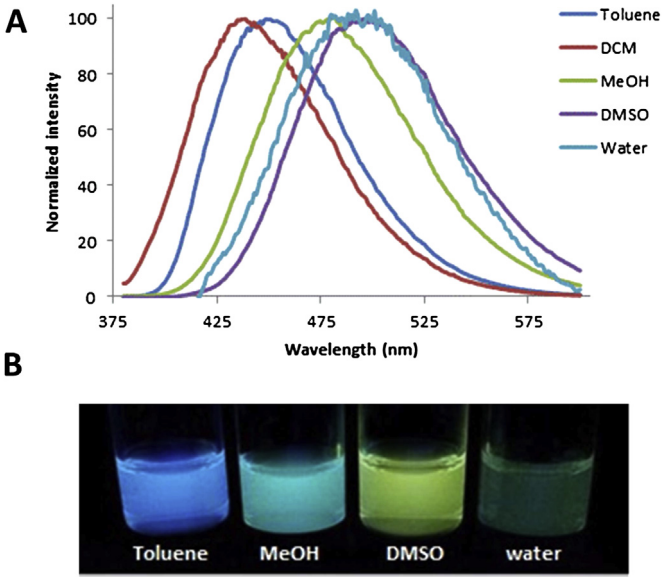


Fig. 2. A) Normalized emission spectra compound **7a** in various solvents with $c = 10^{-5}$ M. B) Color changes of solutions of **7a** with $c = 10^{-5}$ M for all solvents (except water $c = 10^{-3}$ M). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

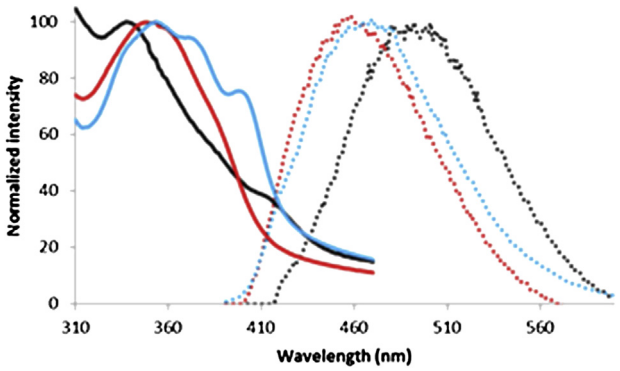


Fig. 3. Normalized UV–vis (solid lines) and emission spectra (broken lines) in water of compounds **7a** (black), **7b** (blue) and **7c** (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

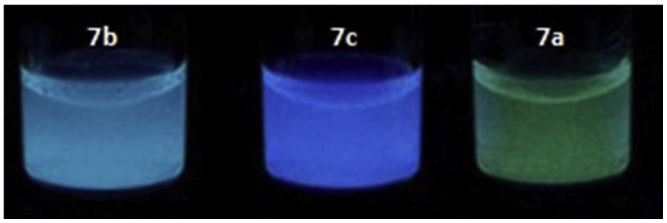


Fig. 4. Compounds **7a**–**c** in water under UV irradiation.

differences between molecules **7a**–**c** were pointed out as they evolved from purple to green (Fig. 4).

Finally, three products **7a**–**c** are promising as their characteristics are similar to DAPI, widely used in fluorescence microscopy [35]. Actually, DAPI has a low quantum yield of 0.019 (0.34 in DNA); and shows an absorption wavelength at 341 nm and emits at 496 nm. These features are quite close to our compounds, and more particularly **7a**, highlighting their potential application as good fluorophores.

4. Conclusion

In summary, new push–pull 6-amino-8-styryl purines have been successfully synthetized and characterized in a straightforward way *via* C–H bond direct functionalization and Buchwald–Hartwig cross-coupling. We showed that these molecules exhibit strong fluorescence and large Stokes shifts in organic solvents and among them, three compounds (**7a**–**c**) are fluorescent in water. This preliminary work could be useful for the synthesis of new nucleoside analogs through introduction of a ribose moiety on the *N*9 position of these 8-styrylpurines. Therefore, the spectroscopic properties of these nucleoside analogs could be investigated in order to determine their potential utility as DNA or RNA probes upon incorporation into oligonucleotides [36–38].

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