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Synthesis of Responsive Fluorescent Nucleobases 7-(Benzofuran-2-yl)-7-deazahypoxanthine and 7-(Benzofuran-2-yl)-7-deazaguanine Using Cross-coupling Reaction

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In order to develop fluorescent guanine analogs having substitutions at the 7-positions, 7-(benzofuran-2-yl)-7-deaza-hypoxanthine (**1a**) and 7-(benzofuran-2-yl)-7-deazaguanine (**1b**), were synthesized from 7-deaza-7-iodohypoxanthine and 7-deaza-7-iodo-2-*N*-pivaloylguanine via a Suzuki–Miyaura cross-coupling, respectively. Compound **1b** showed strong fluorescence, with higher fluorescent quantum yields in less polar solvents; meanwhile, **1a** showed higher activity in more polar solvents. It is expected that the guanine analogs can be incorporated into nucleosides to develop new fluorescent oligonucleotides.

In the past several decades, various fluorescent nucleobase analogs have been developed to aid the study of the structure and activity of biomolecules such as nucleotides, oligonucleotides, and proteins.[1–3] Although there are many reports on fluorescent pyrimidine analogs,[4–9] very few fluorescent purines have been developed, with guanine analogs being particularly lacking.[10–17] Prior research has found some success in arylating the 8-position of guanine,[14–16] which has yielded analogs with high fluorescent activity; however, these compounds significantly decrease the stability of DNA and RNA duplexes because the added substituent forces the sugar–guanine conformation from *anti* to *syn*. Moreover, Sproviero and co-workers reported that the inclusion of an aryl substituent at the 8-position of deoxyguanosine increases susceptibility to acid-catalyzed depurination.[18]

An alternate approach to synthesizing fluorescent guanine analogs is through 7-substitution of 7-deazaguanines; several reports exist that detail the photophysical properties of these compounds.[12,17] Saito and co-workers reported that 7-naphthylethynylated 8-aza-7-deaza-2′-deoxyguanosine (^{na}G) showed high fluorescence in aprotic solvents; importantly, oligodeoxynucleotides containing ^{na}G formed DNA duplexes that were as stable as those containing unmodified deoxyguanosine.[17] This improved stability is a marked advantage over the 8-arylated analogs.

Herein, we report the synthesis and photophysical properties of fluorescent 7-(benzofuran-2-yl)-7-deazaguanine analogs **1a** and **1b** (Figure 1). We chose benzofuran as a substituent given a recent report that the fluorescent intensity of 5-(benzofuran-2-yl)uracil changes depending on the solvent used and the microenvironments,[19] properties which we hoped would also manifest themselves in guanine analogs. As a first trial to synthesize **1a** and **1b**, we chose not to include any substituents at the 9-position because there are very few reports on the synthesis of 7-arylated purine analogs that are unmodified at this position.[20] However, we believe that the photophysical properties of nucleosides or single-strand oligonucleotides containing

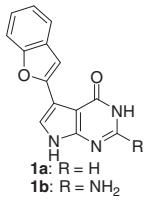
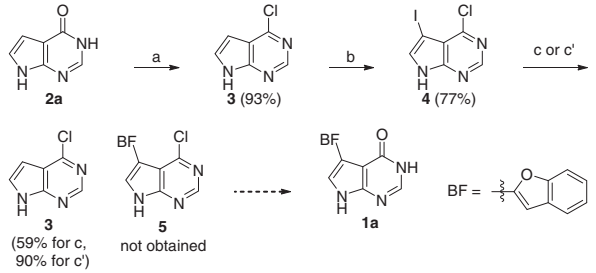


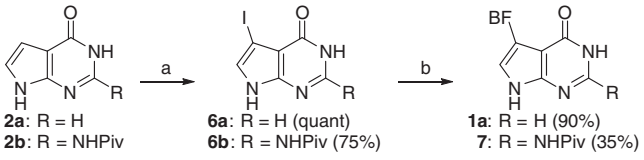
Figure 1. Structures of **1a** and **1b**.



Scheme 1. Reagents and conditions: (a) $POCl_3$, reflux, 2 h; (b) *N*-iodosuccinimide, DMF, r.t., 7.5 h; (c) benzofuran-2-boronic acid pinacol ester, 3 mol % $Pd(OAc)_2$, 8 mol % trisodium triphenylphosphine-3,3′,3″-trisulfonate, Na_2CO_3, H_2O–MeCN (2:1, v/v), 100 °C, 3.5 h, or (c′) potassium (benzofuran-2-yl)trifluoroborate, 3 mol % [$PdCl_2$(dppf)], CH_2Cl_2, Et_3N, EtOH, reflux, 19 h. dppf: 1,1′-bis(diphenylphosphino)ferrocene.

7-arylated purine analogs can be reasonably predicted by studying those of the 9-unsubstituted purine analogs. For example, the UV–vis absorption and emission spectra of 2-aminopurine, the corresponding nucleoside, and single-strand oligonucleotides are similar.[21,22] In addition, Kovaliov and co-workers showed that there was only a slight difference in the UV–vis absorption and emission spectra of fluorescent *p*-(trifluoromethyl)phenylimidazolocytosine and the corresponding nucleoside.[23] As we described in this letter, we found that in some reactions the 9-unsubstituted purines showed reactivities different from those of 9-substituted derivatives. Therefore, keeping this position unmodified makes our newly established synthetic protocol useful not only for the development of fluorescent nucleobases but also for other purine analogs such as bioactive compounds.

Our first attempted synthetic route for the synthesis of **1a** is outlined in Scheme 1. This route began by treating **2a** with phosphorus oxychloride to afford chlorinated compound **3** in 93% yield; this followed a previous established procedure.[24]



Scheme 2. Reagents and conditions: (a) *N*,*O*-bis(trimethylsilyl)acetamide, DMF, 40 °C, 4 h, followed by *N*-iodosuccinimide, r.t., 14 h; (b) benzofuran-2-boronic acid, 6 mol % $Pd(OAc)_2$, 12 mol % triphenylphosphine-3,3′,3″-trisulfonic acid trisodium, Na_2CO_3, H_2O–MeCN–DMF (2:1:2, v/v/v), 45 °C, 8 h (**1a**) or 3 h (**7**). Piv: pivaloyl.

Subsequent iodination at the 7-position with *N*-iodosuccinimide afforded **4** in 77% yield. We then attempted a Suzuki–Miyaura cross-coupling of **4** to introduce the benzofuran-2-yl group (BF) at the 7-position in order to obtain **5**. However, the reaction conditions (c in Scheme 1) unexpectedly resulted in deiodination, giving **3** in 59% yield instead of the desired **5**. Furthermore, the use of alternate conditions (c′ in Scheme 1)[25] produced **3** in even higher yield, at 90%. There are many examples of palladium-catalyzed coupling reactions of 6-chloro-7-deaza-7-iodopurine analogs with deoxyribose or alkyl substituents at the 9-position, suggesting that these substituents are required to prevent deiodination.[26–28]

Given the lack of success of Scheme 1, we then attempted an alternate synthesis, as outlined in Scheme 2. First, **2a** was quantitatively iodinated to yield **6a**, following the Barnett's procedure.[29] The subsequent Suzuki–Miyaura cross-coupling of **6a** and benzofuran-2-boronic acid yielded the desired **1a** in 90% yield. Similarly, compound **7** was prepared from **2b**;[30] iodination of **2b** afforded **6b**[29] in 75% yield, while the following cross-coupling of **6b** and benzofuran-2-boronic acid yielded **7** in 35% yield. Recently, Nauš and co-workers reported the synthesis of 7-(benzofuran-2-yl)-7-deazaruanosine via Suzuki–Miyaura coupling between benzofuran-2-boronic acid and 7-deaza-7-iodo-6-*O*-methylguanosine.[31] Our result, shown in Scheme 2, revealed that a similar coupling reaction proceeded without protection of the 6-O position.

We next examined pivaloyl deprotection of **7** to **1b** following Taylor's method.[30] However, deprotection using aqueous sodium hydroxide was unsuccessful probably because of the low solubility of **7**. The addition of pyridine to improve substrate solubility also failed to effect deprotection, while the use of sodium methoxide resulted in a complex mixture. Conversely, aqueous ammonia afforded effective deprotection, yielding the desired **1b** in 63% yield (Table 1, Entry 1). Interestingly, sodium methanethiolate also effectively afforded **1b** in 43% yield (Table 1, Entry 2). These results suggest that less basic nucleophiles are required for this deprotection. It is likely that more basic reagents abstract protons at the 9-, 1-, or 2-positions and increase electron density on the 7-deazapurine ring, slowing C–N bond cleavage. The highly electron-rich species generated by this deprotonation might also be susceptible to oxidation by air.

Next, we studied the photophysical properties of **1a** and **1b** in various solvents, focusing specifically on methanol, acetonitrile, and ethyl acetate; the results are shown in Table 2 and Figures S1 and S2. The absorption maxima (Abs_{max}) for **1a** varied minimally, ranging from 312 to 313 nm for all solvents,

Table 1. Deprotection of pivaloyl-protected **7** to **1b**

Entry	Reagent	Solvent	Temp. /°C	Time /h	Yield /%
1	NH_3 (115 equiv)	pyridine-28% aq. NH_3 (1:1, v/v)	65	7.5	63
2	NaSMe (5.0 equiv)	MeOH	50	18	43

Table 2. Photophysical properties of **1a** and **1b**

Solvent	**1a**			**1b**		
	Abs_{max} /nm	Em_{max} /nm	Φ_F	Abs_{max} /nm	Em_{max} /nm	Φ_F
MeOH	312	417	0.03	318	374	0.02
MeCN	312	397	0.003	316	364	0.30
EtOAc	313	n.d.[a]	n.d.[a]	317	359	0.29

[a]n.d.: not detected.

while the absorption maxima of **1b** behaved similarly, ranging from 316 to 318 nm. However, the fluorescence quantum yield (Φ_F) values showed a dependence not only on the solvent but also on the presence or absence of the amino group at the 2-position. Compound **1a** showed the highest Φ_F in methanol, with a value of 0.03; acetonitrile and ethyl acetate afforded Φ_F values of 0.003 and lower than the detection limit (not determined), respectively. On the other hand, compound **1b** showed its lowest Φ_F value of 0.02 in methanol, while larger values were observed in acetonitrile and ethyl acetate. The maximum fluorescence wavelength (Em_{max}) values showed this dependency as well. **1a** and **1b** showed a blue shift from 417 to 397 nm and 374 to 364 nm, respectively, when the solvent was changed from methanol to acetonitrile. These effects observed in **1a** and **1b** are likely the result of increased stabilization of excited states as the polarity increases.

In conclusion, we synthesized two kinds of 7-substituted fluorescent purine analogs, **1a** and **1b**, and proved that 7-deaza-7-iodohypoxanthine (**6a**) and 7-deaza-7-iodo-2-*N*-pivaloylguanine (**6b**) were suitable intermediates for these compounds, respectively. By studying their photophysical properties, we revealed that the structural difference between these compounds influences the solvent dependency of their fluorescence activity. The overall importance of the solvent in influencing Φ_F was very noticeable, with a large difference from 0.03 to n.d. for **1a** and a 15-fold difference from 0.02 to 0.29 for **1b** as the solvent was changed between methanol and ethyl acetate. These significant effects suggest that the oligonucleotides containing these bases may change their fluorescence intensity upon binding to complementary strands or nucleic acid-binding proteins. For example, upon binding to complementary strands or nucleic acid-binding proteins, the polarity around the fluorescent nucleobase in the oligonucleotides decreases owing to interactions between the surrounding hydrophobic nucleobases and/or amino acid residues. Such alteration of the microenvironment could change the photophysical properties of the fluorescent nucleobase.[32] Recently, Hocek and co-workers reported the synthesis, potentially cytostatic, antimicrobial, and anti-HCV activities of 7-heteroaryl-7-deazapurine nucleosides,

including 7-(benzofuran-2-yl)-7-deazaguanosine.[31,33,34] Therefore, 7-(benzofuran-2-yl)-7-deazapurine skeletons may also be viable fluorescent probes in studies regarding antimicrobial or anti-virus activities. We are now studying the synthesis of 2′-deoxynucleosides and oligodeoxynucleotides containing **1a** and **1b** as base analogs; results are forthcoming.

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Supporting Information is available electronically on J-STAGE.

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