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Synthesis of 8-(1,2,3-triazol-1-yl)-7-deazapurine nucleosides by azide-alkyne click reactions and direct C-H bond functionalization

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Abstract

Treatment of toyocamycin or sangivamycin with 1,3-dibromo-5,5-dimethylhydantoin in MeOH (r.t./30 min) gave 8-bromotoyocamycin and 8-bromosangivamycin in good yields. Nucleophilic aromatic substitution of 8-bromotoyocamycin with sodium azide provided novel 8-azidotoyocamycin. Strain promoted click reactions of the latter with cyclooctynes resulted in the formation of the 1,2,3-triazole products. Iodine-mediated direct C8-H bond functionalization of tubercidin with benzotriazoles in the presence of *tert*-butyl hydroperoxide gave the corresponding 8-benzotriazolyltubercidin derivatives. The 8-(1,2,3-triazol-1-yl)-7-deazapurine derivatives showed moderate quantum yields and a large Stokes shifts of ~ 100 nm.

Graphical Abstract

Keywords

Azido Nucleosides; Click Chemistry; Deazapurine nucleosides; Direct C-H Functionalization; Fluorescence; Triazoles

Synthetic transformations, biological activities and physicochemical properties of 7-deazapurine derivatives (mainly tubercidin, toyocamycin, and sangivamycin antibiotics) have been extensively studied^{1–9} and are subject of the excellent reviews.^{10–12} In recent years 7-deazapurine nucleosides have been explored as substrates for 1,4-regioselective

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Supplementary Material

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copper-catalyzed azide/alkyne cycloaddition (CuAAC), which resulted in developing fluorogenic DNA probes.^{13–16} These studies have mainly concentrated on the application of 7-alkynyl derivatives of 7-deazapurines and 7-deazanucleosides for the preparation of the fluorescent 1,2,3-triazol-4-yl probes.^{4,17–18} Studies with 7-azido-7-deazapurine nucleosides as well as 8-azido- or 8-alkynyl-7-deazapurine nucleosides have received much less attention due to the lack of availability of the convenient synthetic protocols for their synthesis.^{17,19} This is despite the fact that azidonucleosides^{20–22} including 8-azido as well as 8-alkynyl purine²³ nucleosides have been used as convenient substrates for CuAAC and strain-promoted azide-alkyne cycloaddition (SPAAC).^{24–26} This approach is rapidly growing field with various applications, including *in vivo* imaging, of azido/alkyne-modified nucleoside/nucleotides^{26–27} or DNA/RNA fragment click adducts.^{28–33}

Herein, we report two independent methods for the synthesis of novel 8-triazol-1-yl derivatives of 7-deazapurine nucleosides. One protocol involves strain-promoted click chemistry between 8-azido-7-deazapurine nucleosides and cyclooctynes whereas the second approach explores direct C8-H bond functionalization of 7-deazapurine nucleosides and coupling with benzotriazoles.

The synthesis of the 8-azido-7-deazapurines (**3a–c**) was attempted from 8-halo-7-deaza substrates. Thus, bromination of 2',3',5'-tri-*O*-acetyltubercidin **1a** using recently reported protocols for the C-8 bromination of purine nucleosides,³⁴ employing 1,3-dibromo-5,5-dimethylhydantoin (DBH, 0.65 eq.) in CH₂Cl₂ at 0 °C for 40 min, provided 7-bromo **2a** (32%) in addition to 7,8-dibromo **2b** (10%, TLC; Scheme 1). Deacetylation of **2a** and **2b** with methanolic ammonia gave 7-bromotubercidin **2c** (80%) and 7,8-dibromotubercidin **2d** (57%). Treatment of tubercidin **1b** with *N*-bromosuccinimide (NBS, 2 eq.) in presence of KOAc in DMF yielded 8-bromotubercidin³⁵ **2e** (34%), whereas attempted bromination of **1b** with DBH in DMF gave a complex reaction mixture.

The 8-bromination of toyocamycin **1c** with 0.75 equiv. of DBH in MeOH (30 min.) proceeded efficiently to give after crystallization **2f** (85%). Treatment of **1c** with NBS (1.1 eq.) in DMF at r.t. for 2.5 h also yielded **2f** (45%) after column chromatography. Replacement of DMF with MeOH gave **2f** (64%) after crystallization, although reaction took 4 h for completion. The 8-bromotoyocamycin (**2f**) has been previously prepared either by coupling of the corresponding 8-bromo-7-deazapurine with the ribose precursor³⁶ or by bromination of toyocamycin **1c** with Br₂/H₂O (61%).³⁷ Thus the DBH/MeOH combination is the most efficient for synthesizing **2f** in terms of yield, reaction time, and product isolation.

Treatment of sangivamycin **1d** with DBH (1.1 eq.) in MeOH (r.t., 30 min) yielded **2g** (63%), whereas bromination of **1d** with NBS (1.5 eq.) in DMF for 1.5 h gave **2g** in 40% yield in addition to unidentified byproducts. The synthesis of 8-bromosangivamycin **2g** is usually achieved by the hydrolysis of 8-bromotoyocamycin **2f** with a concentrated NH₄OH and 50% H₂O₂ solution.³⁶

Attempts to synthesize 8-azidotubercidin **3a** from 8-bromotubercidin **2e** [NaN₃ (1–5 eq)/DMF/rt–153 °C; NaN₃ (3 eq)/TsOH (3 eq)/EtOH/reflux) were unsuccessful. The higher

electron density of π -excessive pyrrole ring most probably prevented the nucleophilic substitution reaction. However, treatment of 8-bromotoyocamycin **2f** with NaN_3 in DMF at room temperature for 16 h produced 8-azidotoyocamycin **3b** in 46% isolated yield (70%, based on TLC and ^1H NMR of the crude reaction mixture). Although this azidation reaction was light- and heat-sensitive and required the reaction, purification, and characterization to be carried out in the dark, the isolated 8-azido product **3b** was stable for days when stored in refrigerator at 4 °C.^{23,38} The presence of the EWG on the 7-position of the pyrrole ring in toyocamycin, as compared to tubercidin, could be the reason for successful substitution of bromide by azide in **2f**. Treatment of 8-bromosangivamycin **2g** with NaN_3 in DMF (as well as in DMSO or MeOH) at r.t or 70 °C failed to produce the desired 8-azidosangivamycin **3c**.

SPAAC reaction of 8-azidotoyocamycin **3b** with symmetrically fused cyclopropyl cyclooctyne **4** (OCT) in an aqueous solution of acetonitrile (ACN) at ambient temperature (4 h) gave the 8-(1,2,3-triazol-1-yl) product **5** as mixture of two isomers (60%, Scheme 2). Analogous reaction (4 h) of **3b** with a strain modulated dibenzylcyclooctyne **6** (DBCO) produced triazole **7** as mixture of isomers (47%) after RP-HPLC purification. The resulting triazolyl products have "light-up" (inherited) fluorescent properties (Table 1), due to increased conjugation in the 7-deazapurine ring, and can therefore be used for fluorescent imaging in cancer cells, as recently reported with 8-triazolyl purine and 5-triazolyl pyrimidine nucleosides.^{26–27} CuAAC reaction of 8-azido **3b** with phenylacetylene in aqueous MeOH (4 days/r.t.) gave 8-(4-phenyl-1*H*-1,2,3-triazol-1-yl)toyocamycin **9** (65%) after silica column purification.

Our attempts to prepare 8-alkynyl-7-deazapurine derivatives, which could serve as substrates for the syntheses of the underdeveloped 8-(1,2,3-triazol-4-yl) adducts *via* click reactions with organoazides were unsuccessful. Thus, treatment of 8-bromotubercidin (**2e**), 8-bromotoyocamycin (**2f**), 8-bromo-2',3',5'-tri-*O*-acetyltoyocamycin,³⁹ or 8-iodotoyocamycin [prepared by treatment of the silylated toyocamycin with LDA (5 equiv.) and iodine (I_2 , 1.5 equiv.) in THF (16%)] with trimethylsilylacetylene (TMSA) in the presence of TEA and $(\text{PPh}_3)_2\text{PdCl}_2/\text{CuI}$ in anhydrous DMF showed mainly unchanged starting material under various reaction conditions. These results are in sharp contrast to 7-halo-7-deazapurines that undergo smooth Sonagashira cross-coupling providing 7-alkynyl analogues, which after further modifications provide derivatives with interesting photophysical and biological properties.^{4,17}

Since syntheses of 8-azidotubercidin **3a** for the click reactions were unsuccessful, the possibility to prepare 8-triazolyl adducts of tubercidin **1b**, using a direct C8-H activation of the 7-deazapurine ring, was explored next. Direct C-H functionalization of purines and purine nucleosides has recently gained much attention.^{19,40–43} The examples include regioselective Pd-catalyzed direct C8-H arylation of the 6-phenyl-7-deazapurines with aryl halides to provide 8-arylated products albeit in low to moderate yields.⁴⁴ A regioselective direct C-H amination of the 7-deazapurines to give access to the 8-amino-,⁴⁵ or 7-amino-7-deazapurine analogues has been also reported.⁴⁶ We found that treatment of tubercidin **1b** with benzotriazole (2 equiv.) in the presence of I_2 (0.4 equiv.) and *tert*-butylhydroperoxide (TBHP; 5–6 M/decane, 2 equiv.)⁴⁷ in anhydrous DMF at 35 °C for 96 h showed ~30% conversion (TLC) to 8-(benzotriazol-1-yl)tubercidin **10** which was isolated in 18% yield

after column chromatography and HPLC (Scheme 3). Increasing the amount of iodine from catalytic to stoichiometric (3 equiv.) afforded **10** in 35% yield after 16 h. ^1H NMR data showed that the 7.60 ppm doublet for H7 in **1b** collapsed to a singlet at 7.04 ppm in **10**.

The C8 regioselectivity for the oxidative cross-coupling product **10** was established by a X-ray crystal structure determination (Figure 1).⁴⁸ It is noteworthy that the 7-deazapurine ring and the benzotriazole ring are twisted to each other with an angle of 67.9° between the planes, which does not favor intermolecular π - π interactions. The glycosyl torsion angle C4-N9-C1'-O4' is 59.8° (*syn* conformation) and the furanose pseudorotation angle⁴⁹ is 162.9° (^2E conformation). The C3'-C4'-C5'-O5' torsion angle is 54.1° and is in *g*+/*g**g* range.

Treatment of **1b** with 5-methylbenzotriazole [I_2 (0.2 equiv.)/TBHP/DMF/rt/5 days] produced a 3:2 mixture of 5-methyl- and 6-methylbenzotriazol-1-yl adducts **11a/11b** (6%). Reaction with stoichiometric amount of iodine (3 equiv.) resulted in the efficient conversion to **11a/11b** (48%; ~80% based on TLC after 15 min.), showing generality of the iodine-mediated direct activation of tubercidin ring at C8. Analogous treatment of **1b** with 5-chlorobenzotriazole (3 equiv.) produced 5/6-chlorobenzotriazol-1-yl adducts **12a/12b** (1:1, 21%; ~60% conversion on TLC). Because 7-deazapurine is structurally similar to indole, the coupling of **1b** with benzotriazole might have occurred *via* initial iodination of the pyrrole ring of 7-deazapurine followed by trapping of the resulting iodonium, or 7-iodo iminium intermediates, with nucleophilic triazole. Subsequent elimination of HI, as proposed for the iodine-catalyzed direct activation of indoles with azoles mediated by TBHP,⁴⁷ should yield products **10–12**. Subjection of the sangivamycin **1c** and toyocamycin **1b** to direct C8-H functionalization with benzotriazole (r.t. to 80°C /48 h) gave unchanged substrate or resulted in rather complex reaction mixture. These results reiterate the literature report that the iodine catalyzed C-H activation of indoles with azoles works rather sluggishly with electron deficient indole substrates.⁴⁷

The photophysical properties of 8-(1,2,3-triazol-1-yl)-7-deazapurine adducts **5**, **7**, **9** and **10–12** were determined in MeOH. All triazol derivatives have similar absorption and excitation spectra with an absorption maximum between 276 nm and 295 nm and comparable extinction coefficient values (Table 1 and Figure S2). Moderate quantum yields and a large Stokes shifts of ~ 100 nm observed here are analogous to the fluorescence properties of aliphatic and benzylic derivatives of 1-(purin-8-yl)triazoles.²³ The emission maximum of triazol **5** (380 nm) is blue shifted compared to other derivatives (412–480 nm), likely due to the absence of an aromatic substituent (Figure 2). Emission spectrum of chloro substituted benzotriazole **12** is red-shifted compared to benzotriazoles **10** and **11**, suggesting a resonance conjugation between the aromatic ring and Cl electrons.

Emission properties of the triazole **10** were also investigated in aqueous solutions. Compound **10** is fluorescent at neutral and basic pH (7.0 to 12.0) and becomes non-fluorescent at acidic pH. Interestingly, compounds **10** and **11** seem to be highly sensitive to UV light, as an increase in emission intensity (8.1 and 2.4 fold) upon irradiation with 280 nm light was observed (Figure S3). Triazole **10** has an average lifetime about 3.8 ns with two well-defined components 1 ns (10%) and 4.1 ns (90%).

In summary, bromination of 7-deazapurine nucleosides with DBH/MeOH gave 8-bromotocamycin and 8-bromosangivamycin in high yields. Treatment of the 8-bromotocamycin with sodium azide yielded 8-azidotocamycin. Strain promoted click chemistry of 8-azidotocamycin with cyclooctynes provided the corresponding 8-triazol-1-yl products. Iodine-mediated direct C-H arylation of tubercidin with benzotriazoles gave the corresponding 8-(benzotriazol-1-yl)tubercidin products. The 8-(1,2,3-triazol-1-yl)-7-deazapurine derivatives showed moderate quantum yields and a large Stokes shifts of ~ 100 nm.

Supplementary Material

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Highlights

- Efficient bromination of 7-deazapurine nucleosides with DBH in MeOH
- Click reactions of 8-azidotoyocamycin with alkynes gave fluorescent triazoles
- Direct C8-H bond functionalization of tubercidin with benzotriazoles

Figure 1.

X-ray crystal structure of **10** showing atom labeling. Solvent molecules and H-atoms are omitted for clarity.

Figure 2.
Emission spectra for 8-(1,2,3-triazol-1-yl)-7-deazapurine nucleosides.

Scheme 1.

Synthesis of 8-bromo- and 8-azido-7-deazapurine nucleosides.

Scheme 2.

Click reactions between 8-azidotoyocamycin and cyclooctynes **4**, **6** and phenylacetylene **8**.

Scheme 3.

Iodine-mediated oxidative cross-coupling of tubercidin with benzotriazole.

Table 1

Photophysical data for 8-(1,2,3-triazol-1-yl)-7-deazapurine nucleosides.

	5	7	9	10	11^a	12^a
ϵ_{max} (M ⁻¹ cm ⁻¹)	12200	12600	16000	16700	17100	17700
λ_{max} (abs) ^b (nm)	287	290	295	278	278	276
λ_{max} (exc) ^b (nm)	295	299	301	321	322	343
λ_{max} (emi) ^b (nm)	380	434	412	416	419	480
Stokes Shift (nm)	85	135	111	95	97	137
ϕ_F^b (%)	1.4	0.5	0.4	0.2	0.7	0.8

^a As mixture of 5- and 6-isomers.

^b In MeOH