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Synthesis of Polycyclic Hetero-Fused 7-Deazapurine Heterocycles and Nucleosides through C−H Dibenzothiophenation and Negishi Coupling

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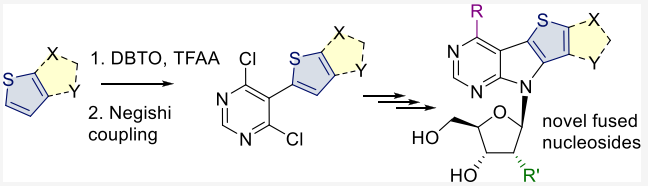
ABSTRACT: A new approach for synthesizing polycyclic heterofused 7-deazapurine heterocycles and the corresponding nucleosides was developed based on C−H functionalization of diverse (hetero)aromatics with dibenzothiophene-*S*-oxide followed by the Negishi cross-coupling with bis(4,6-dichloropyrimidin-5-yl)zinc. This cross-coupling afforded a series of (het)aryl-pyrimidines that were converted to fused deazapurine heterocycles through azidation and thermal cyclization. The fused heterocycles were glycosylated to the corresponding 2′-deoxy- and ribonucleosides, and a series of derivatives were prepared by nucleophilic substitutions at position 4. Four series of new polycyclic thieno-fused 7-deazapurine nucleosides were synthesized using this strategy. Most of the deoxyribonucleosides showed good cytotoxic activity, especially for the CCRF-CEM cell line. Phenyl- and thienyl-substituted thieno-fused 7-deazapurine nucleosides were fluorescent, and the former one was converted to 2′-deoxyribonucleoside triphosphate for enzymatic synthesis of labeled oligonucleotides.



INTRODUCTION

Modified nucleoside and nucleotide analogues are important cytostatic[1] and antiviral drugs.[2] Recent outbreaks of RNA-virus diseases, including the current pandemics caused by SARS-CoV-2 virus, started a renaissance[3] of this class of compounds because several modified nucleosides and their prodrugs (i.e., Remdesivir[4] or Molnupiravir[5]) are precursors of metabolites (usually nucleoside triphosphates) that inhibit RNA-dependent RNA polymerases[6] or cause mutations in viral replication.[7] Clearly there is a great need for novel types of nucleoside and nucleotide derivatives in the development of antiviral drugs against emerging viruses or anticancer agents against drug resistant tumors or leukemias.

Previously, we have discovered several classes of substituted 7-deazapurine ribonucleosides **1** with potent and selective cytotoxic effect[8] against cancer cell lines that act through incorporation to DNA causing DNA damage and apoptosis.[9] Other related derivatives exerted antiviral[10] or antiparasitic[11] effects. Recently, we designed and synthesized novel types of tri- and tetracyclic fused 7-deazapurine ribonucleosides and found some benzo-fused derivatives were potent antivirals against RNA viruses.[12] More importantly, the thieno-,[13] furo-,[14] pyrrolo-,[14] pyrazolo-,[15] and some pyrido-fused[16] deazapurine nucleosides **2a−e** showed strong cytostatic effects in submicromolar concentrations and their mechanism of action involved DNA damage and apoptosis. The corresponding tetracyclic naphtho-[17] and benzothieno-fused[18] nucleosides **3a,b** (Figure 1) were less or noncytotoxic but still showed

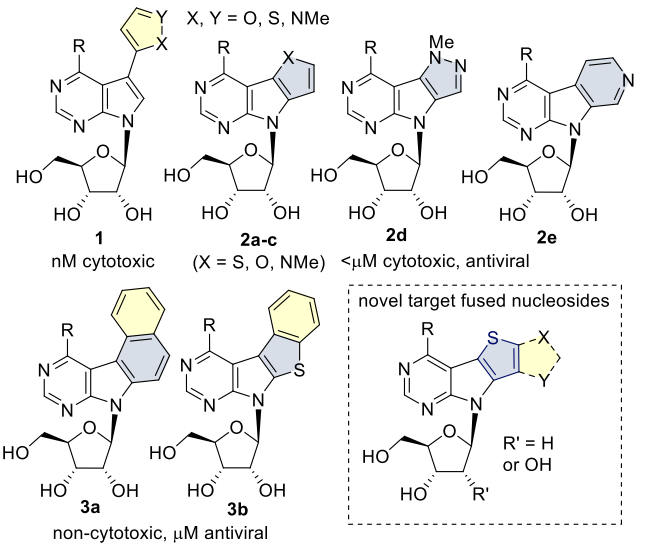


Figure 1. Structures of previously developed biologically active substituted and fused 7-deazapurine nucleosides and the design of target compounds (in dashed square).

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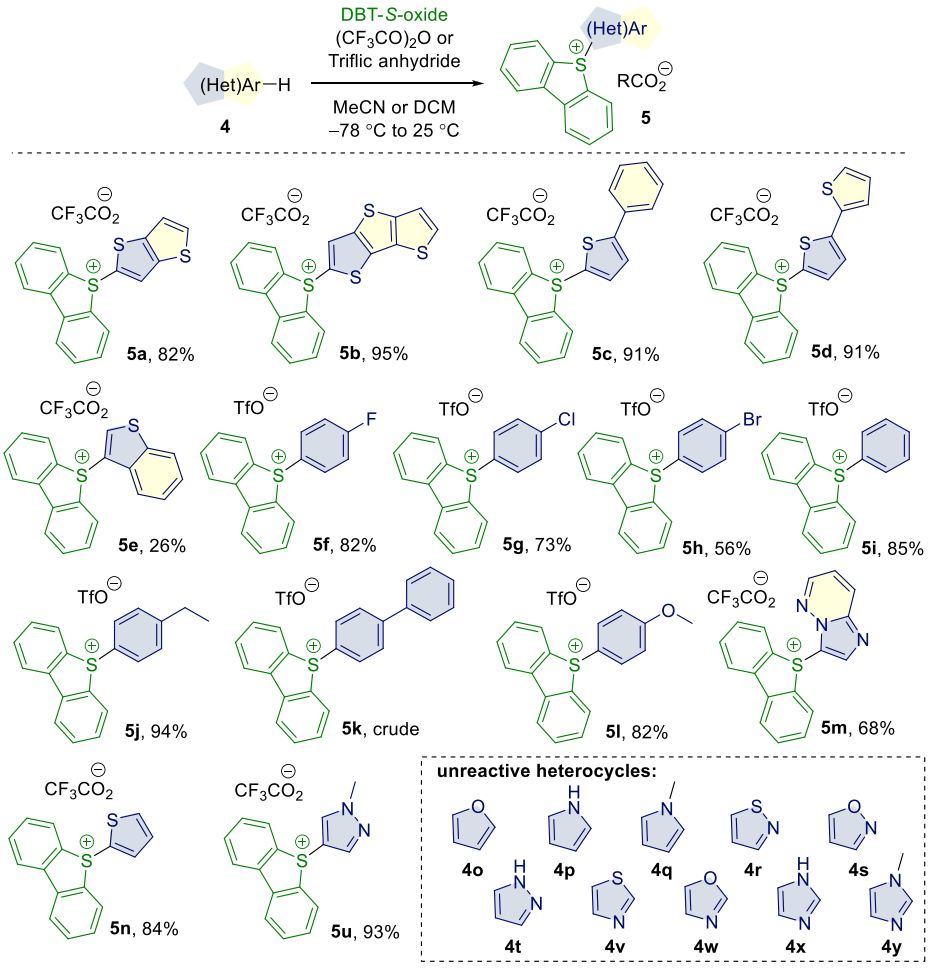
Scheme 1. Preparation of (Het)aryl Sulfonium Salts



antiviral effects suggesting that the increased bulkiness of the fused heteroaromatic system might lead to selectivity in antiviral versus cytotoxic activities. These tri- and tetracyclic fused nucleobases can be synthesized either by multistep heterocyclization approach[12,15,16] or through cross-coupling of *in situ* generated 4,6-dichloropyrimidine-5-zinc reagent with hetaryl halides,[18,13,14] but for some heterocycles, the corresponding halides are inaccessible, expensive, or unreactive. Therefore, there is a need for an alternative general approach that would enable synthesis of a wide range of novel fused deazapurine bases for further applications.

To overcome the above-mentioned synthetic problems, we turned our attention to recently developed C−H functionalization of (hetero)aromatics with sulfur heterocycles and further cross-coupling of the resulting sulfonium intermediates.[19,20] The Ritter group has reported seminal works[21] on metal-free C−H thianthrenylation followed by Negishi coupling with aliphatic alkylorganozinc compounds or aromatic nucleophilic substitutions with diverse nucleophiles. Dibenzothiophene (DBT) and dibenzothiophene-*S*-oxide (DBTO) were also successfully used[22] for C−H functionalization and further derivatizations. Diverse substituted benzenes showed good reactivity and selectivity, whereas only several examples of five-membered heterocycles (mostly substituted thiophenes) were reported[23] in this reaction sequence suggesting that their reactivity is problematic. Other groups have reported generation and Negishi coupling of alkenyl-[24] or alkylsulfonium[25] salts with arylzinc reagents, but no example of (het)aryl-(het)aryl cross-coupling and neither applications of this chemistry for the synthesis of biologically active compounds was reported so far. We report here a general approach to a portfolio of novel tri-, tetra-, and even pentacyclic fused 7-deazapurine bases through the C−H functionalization of heterocycles followed by the Negishi cross-coupling of the resulting dibenzothiophenium derivatives, their glycosylations to nucleosides, and the photophysical properties and biochemical and biological profiling of the corresponding nucleosides and nucleotides.

RESULTS AND DISCUSSION

Chemistry. As mentioned above, (het)aryl-fused 7-deazapurine heterocycles can be prepared through the following approaches: (1) buildup of the heterocycles from chloronitrobenzene through heterocyclization of 2-amino-1*H*-indole-3-carboxylate,[12,16,26] (2) transition metal-catalyzed intramolecular C−H arylation of arylamino-iodopyrimidine,[27] and (3) thermal or photochemical cyclization of 5-aryl-6-azidopyrimidines through nitrene formation.[13,14,18,28] However, the starting materials for each approach are not always easily obtained due to the regioselectivity of halogenation or nitrozation of the heterocycles. Therefore, we envisaged an advantageous use of the recently reported C−H functionalization of diverse heterocycles with DBTO followed by the

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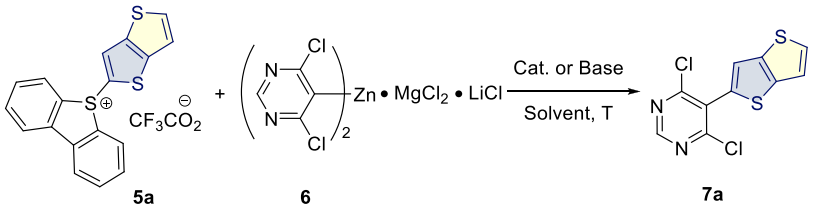
J. Am. Chem. Soc. 2022, 144, 19437−19446

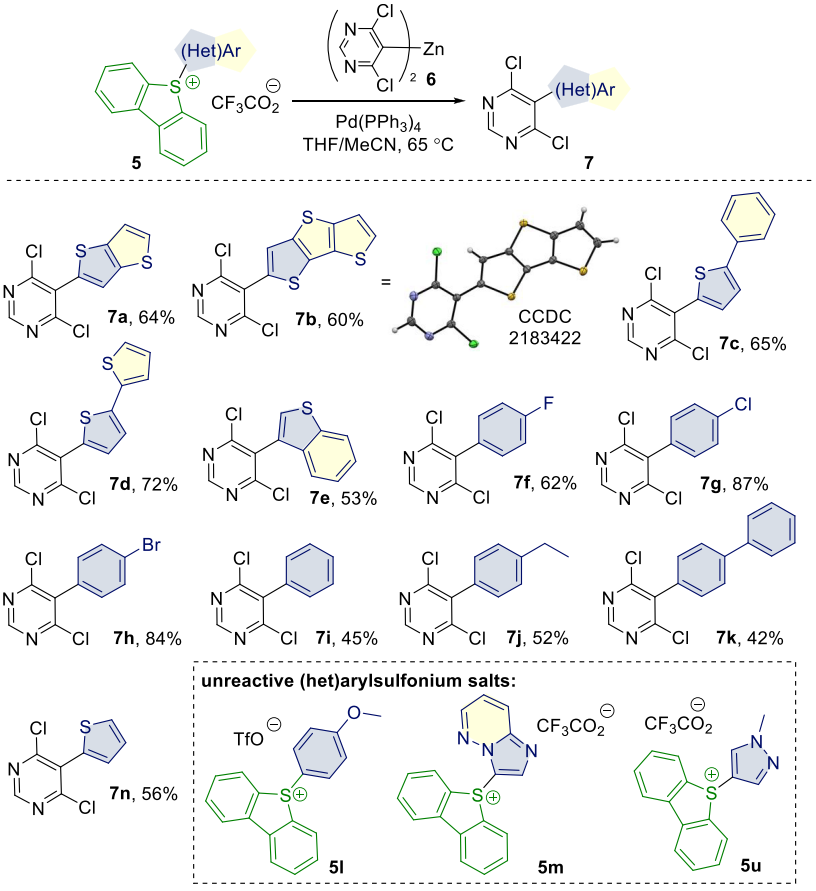
Table 1. Optimization of Negishi Cross-Coupling with 6[a]



Entry	Cat. or Base	6 (equiv)	Solvent	T/°C	Time	Yield or Result
1	$Pd(PPh_3)_2Cl_2$	0.5	THF	65	overnight	no product
2	$Pd(PPh_3)_4$	0.5	THF	65	overnight	mixture[b]
3	$Pd(PPh_3)_4$	0.5	THF	65	40 h	42%
4	$Pd(PPh_3)_4$	1	THF	65	40 h	60%
5	$Pd(PPh_3)_4$	1	THF/MeCN	65	40 h	64%

[a]Reaction conditions: Sulfonium salt (0.23−0.45 mmol, 1.0 equiv), catalyst (0.05−0.08 equiv), zincated pyrimidine (0.5−1 equiv) and the solvent (THF or MeCN; c = 0.2 M) were stirred at 65 °C for 12−48 h. [b]Inseparable mixture containing product **7a** (TLC).

Scheme 2. Investigation of the Substrate Scope of the Negishi Cross-Coupling of (Het)arylsulfonium Salts 5a−5u with Dichloropyrimidine-Zinc 6



Negishi coupling of sulfonium salts with zincated dichloropyrimidine.

First, we tested the C−H functionalization of a wide range of substituted benzenes and substituted and fused thiophenes, as well as imidazo[1,2-*b*]pyridazine and a set of other five-membered heterocycles with dibenzothiophene-*S*-oxide (that was reported[23] to perform better on thiophenes compared to thianthrene). The reactions were performed in the presence of trifluoroacetic or triflic anhydride according to literature protocols (Scheme 1).[22,23] In the case of all thiophenes, benzenes, imidazopyridazine, and *N*-methylpyrazole, we obtained the desired (het)arylsulfonium salts in good yields. Only in the case of benzothiophene, the yield was lower and in the case of biphenyl the dibenzothiophenium salt was inseparable and was used directly for the Negishi coupling. On the other hand, the other five-membered heterocycles **4o−4y** including furan and diverse azoles did not give any detectable sulfonium products. In all these cases, we also tried C−H thianthrenylation[19,21] that also did not provide the desired products. It seems that this C−H functionalization has a severe limitation for most five-membered heterocycles beyond thiophene.

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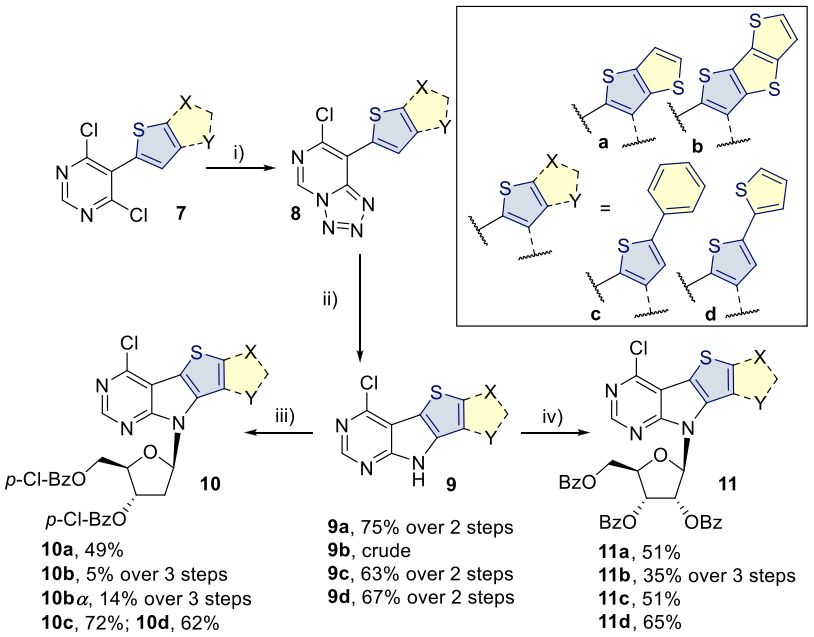
J. Am. Chem. Soc. 2022, 144, 19437−19446

Scheme 3[a]



[a]Reagents and conditions: (i) NaN_3 (1 equiv), LiCl (1 equiv), THF, r.t., 40 h; (ii) 1,4-dibromobenzene, 180 °C, 35 min; (iii) *for* ***10a***, ***10c***: 1. KOH (2.6 equiv), TDA-1 (1.5 equiv), Hoffer's chlorosugar (1.5 equiv), MeCN, r.t., 50 min; *for* ***10b***: 1. BSA (1 equiv), MeCN, 60 °C, 35 min. 2. 1-chloro-3,5-di-(4-chlorobenzoyl)-2-deoxy-α-D-ribose (2 equiv), TMSOTf (2.5 equiv), MeCN, 60 °C, 30 min. (iv) 1. BSA (1 equiv), MeCN, 60 °C, 30 min. 2. 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2 equiv), TMSOTf (2.5 equiv), MeCN, 60 °C, 25−60 min.

With the portfolio of (het)arylsulfonium salts in hand, we then tested the feasibility of the Negishi cross-coupling reaction. Previously, only two examples of Negishi coupling of a substituted phenyl-tetrafluorothianthrenium salt with alkylzinc halides was reported.[21] Initially, we tested the model reaction of thienothiophene-derived dibenzothiophenium salt **5a** with *in situ* generated zincated dichloropyrimidine **6**[13] under different conditions. Reaction with a 0.5 equiv of **6** in the presence of $Pd(PPh_3)_2Cl_2$ did not proceed, whereas the same reaction in the presence of $Pd(PPh_3)_4$ overnight led to a complex inseparable mixture containing the desired product **7a** (TLC-MS). However, simple prolonging of the reaction time to 40 h afforded the desired product **7a** with 42% isolated yields. The yield was improved to 60% when using 1 equiv of organozinc **6** (that offers 2 equiv of the arylorganometallic moiety for transmetalation[29]). Moreover, using the THF/MeCN mixture as the solvent further slightly improved the yield to 64%, since the thienothiophene-derived dibenzothiophenium salt **5a** has better solubility in MeCN. Therefore, the conditions outlined in Table 1, entry 5 were chosen as the optimized conditions for subsequent investigations.

With the optimized reaction conditions, we then examined the substrate scope of this new Negishi coupling (Scheme 2). All the dibenzothiophenium salts derived from thiophene-based heterocycles (thienothiophene, dithienothiophene, phenylthiophene, bithiophene, benzothiophene, and thiophene) **5a**−**5e** and **5n** reacted very well to produce the desired products **7a**−**e** and **7n** in moderate to good yields (53%−72%). Similarly, most of the sulfonium salts derived from substituted benzenes **5f**−**5k** were amenable to this Negishi coupling reaction with zincated dichloropyrimidine **6** to form the corresponding products **7f**−**k** in acceptable yields (42%−87%) with excellent site selectivity. On the other hand, no reaction was observed with strongly electron-rich methoxybenzene- and imidazopyridazine-derived sulfonium salts **5l** and **5m**.

Since the site selectivity of iodination of some (hetero)-aromatics, such as thienothiophene and benzothiophene, is poor, the traditional Negishi coupling using (het)aryl iodides can be quite problematic. Therefore, the regioselective C−H functionalization followed by the Negishi coupling of sulfonium salts and aryl-zinc reagents can be an excellent complementary strategy for the synthesis of complex heterocyclic biaryls.

Based on the biological activity of benzo-, thieno-, benzothieno-, and naphtho-fused deazapurine nucleosides, we designed novel tetracyclic thienothieno- and pentacyclic thienothienothieno-fused deazapurines **9a** and **9b**, as well as phenyl- and thienyl-substituted thieno-fused 7-deazapurines **9c** and **9d** as key intermediates in the synthesis of the corresponding nucleosides and their synthesis started from the new thienyl-pyrimidines **7a**−**d** (Scheme 3).

First, the azidation of dichloropyrimidines **7a**−**d** with sodium azide in THF was performed to get tetrazolopyrimidines **8a**−**d**, which have poor solubility in organic solvents and were used directly for the next step without chromatographic purification. The NMR spectra in DMSO showed that crude compounds **8a**−**d** exist mainly as the form of tetrazolopyrimidines. Based on our previous experience with the synthesis of heteroaryl-fused 7-deazapurine nucleobases, the thermal condition was applied for the heterocyclization of tetrazolopyrimidines to nucleobases. For compounds **8a**, **8c**, and **8d**, the robust thermal cyclization at 180 °C successively gave us the desired fused deazapurine products **9a**, **9c**, and **9d** in 75%, 63%, and 67% yield over 2 steps, respectively. In the case of nucleobase **9b**, the poor solubility did not allow chromatographic purification; hence, the crude compound **9b** was used directly for the next glycosylation step.

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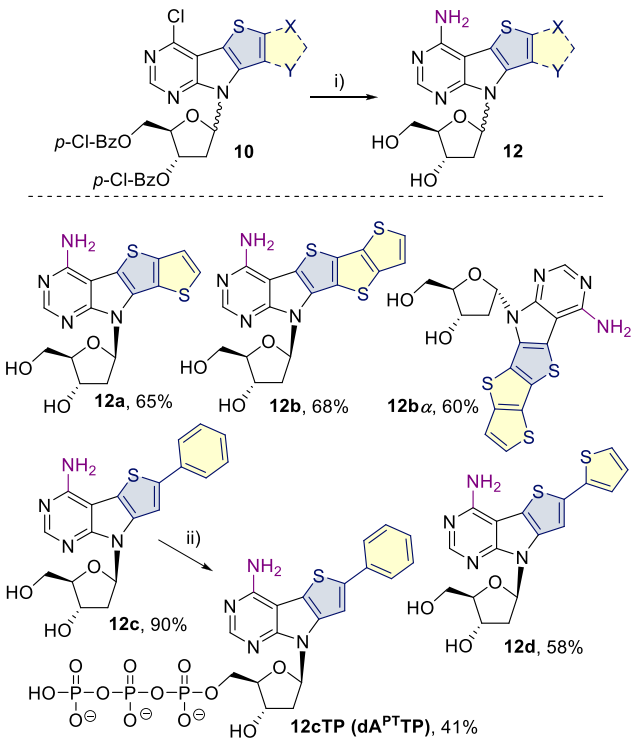
The glycosylation of nucleobases **9a,c,d** with the 1-chloro-3,5-bis-*O*-(4-chlorobenzoyl)-2-deoxy-α-D-ribofuranose (Hoffer's chlorosugar) under basic conditions gave the desired protected deoxynucleosides **10** in moderate to good yields (**10a** 49%, **10c** 72%, **10d** 62%) and with exclusive stereoselectivity to form β-anomers. The less soluble crude heterocycle **9b** was first treated with BSA at 60 °C and then TMSOTf and chlorosugar, which resulted in the formation of the desired β-anomeric nucleoside **10b** in a low yield of 5% over 3 steps and its α-anomer **10bα** in 14% yield over 3 steps. Next we performed the glycosylation of nucleobases **9** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose to prepare the corresponding ribonucleosides. We used a modified procedure of Vorbrüggen glycosylation as in our previous works.[13,14,18] A MeCN solution of nucleobase **9a−d** and BSA was heated to 60 °C for 30 min. After addition of TMSOTf and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, the mixture was stirred at the same temperature for 25−60 min. The protected ribonucleosides **11a**, **11c**, **11d** were obtained in good yields of 51%−65%, and also the ribonucleoside **11b** was synthesized from crude **9b** in an acceptable yield of 35% (over 3 steps) as the pure β-anomer. All nucleosides **11a−11d** were obtained as pure β-anomers.

The protected chloro derivatives of deoxyribonucleosides **10a−d** and **10bα** were converted to the corresponding analogues of 2′-deoxyadenosine (Scheme 4). Their nucleophilic substitution reaction with aqueous ammonia at 120 °C in dioxane substituted the chlorine at position 4 with the amino group with concomitant deprotection of the sugar part to form the desired fused 2′-deoxy-7-deazaadenosine derivatives **12a−d** and **12bα** in good yields (58%−90%).

Scheme 4[a]



[a]Reagents and conditions: (i) NH_3 (aq.), dioxane, 120 °C, 24 h; (ii) 1. $POCl_3$ (1.5 equiv), $PO(OMe)_3$, 0 °C, 3 h; 2. $(NHBu_3)_2H_2P_2O_7$ (5 equiv), Bu_3N, DMF, 0 °C, 2 h; 3. TEAB.

Triphosphorylation of **12c** by the standard procedure[30] furnished the desired phenylthieno-fused 7-deazapurine 2′-deoxynucleoside triphosphate **12cTP** (**dA^{PT}TP**) in 41% yield after HPLC purification (Scheme 4).

In the ribonucleoside series, we designed 4-amino-, 4-methoxy-, and 4-methyl derivatives in each type of fused deazapurine nucleoside, as these were the most active in the related tricyclic fused nucleosides in our previous works. Starting from the protected 4-chloro nucleosides **11a−d**, the amino group was introduced to position 4 using the same procedure and conditions as those for the 2′-deoxynucleosides with aqueous ammonia in dioxane to give the desired products **13a−d** (fused analogues of adenosine) in 41−74% yields. Reactions of intermediates **11a−d** with sodium methoxide displaced the chloro group with methoxy at position 4 and simultaneously cleaved the benzoyl protecting groups to give a series of 4-methoxy nucleosides **14a−d** in moderate to good yields. Due to the poor solubility of the intermediates **11**, the mixture of 1,4-dioxane and methanol and refluxing at 65 °C were used to accelerate the reaction and increase the yield. The introduction of a methyl group to position 4 was achieved through cross-coupling reactions of **11a−d** with trimethylaluminum and $Pd(PPh_3)_4$ in THF to obtain a set of protected 4-methyl nucleosides **15a−d** (62−80% yields) that were deprotected by MeONa in MeOH/dixane to obtain free nucleosides **16a−d** in 65−86% yield. All the target free nucleosides **13a−d**, **14a−d**, and **16a−d** were obtained in sufficient amounts and purity for further biological profiling (Scheme 5).

Fluorescence Properties of Polycyclic Fused Deazapurine Nucleosides. Previous studies reported that benzo-[31,32] and naphtho-fused[17,33] 7-deazapurine nucleosides showed useful fluorescence properties and were used for construction of fluorescent DNA probes. Therefore, we have investigated the photophysical properties of the new polycyclic fused 7-deazapurine nucleosides. Table 2 shows the results of measurement of the UV−vis and fluorescence of the fused 2′-deoxyadenosine analogues **12a−12d** in three different solvents (Table 2). All of the four amino derivatives exerted fluorescence with emission maxima at 359−424 nm. Among these amino derivatives, the phenyl-thieno-fused 7-deazapurine nucleoside **12c** showed the strongest fluorescence with high quantum yields. Interestingly, it exerted the highest fluorescence quantum yield of 48% in water. The thienyl-thieno-fused 7-deazapurine nucleoside **12d** also exhibited relatively strong fluorescence with a 16−25% quantum yield. The fluorescence emission maxima and quantum yields of **12c** and **12d** did not significantly change in different solvents. Surprisingly, the extended tetra- and pentacyclic thienothieno- and thienothienothieno-fused nucleosides **12a** and **12b** showed only very weak fluorescence.

Enzymatic Synthesis and Photophysical Properties of Modified Oligonucleotide. Since phenyl-thieno-fused 7-deazapurine nucleoside **12c** showed strong fluorescence in water, we decided to study enzymatic incorporation of the modified nucleotide and investigate the photophysical properties of the resulting oligo-2′-deoxyribonucleotides. The enzymatic synthesis was performed using **dA^{PT}TP** (**12cTP**) as a substrate in primer extension (PEX) in the presence of KOD XL DNA polymerase with a 19-nt template Temp1A encoding for incorporation of one **dA^{PT}**-modified nucleotide into the extended primer (for sequences of oligonucleotides, see Supporting Information (SI), Table S1). A FAM-labeled

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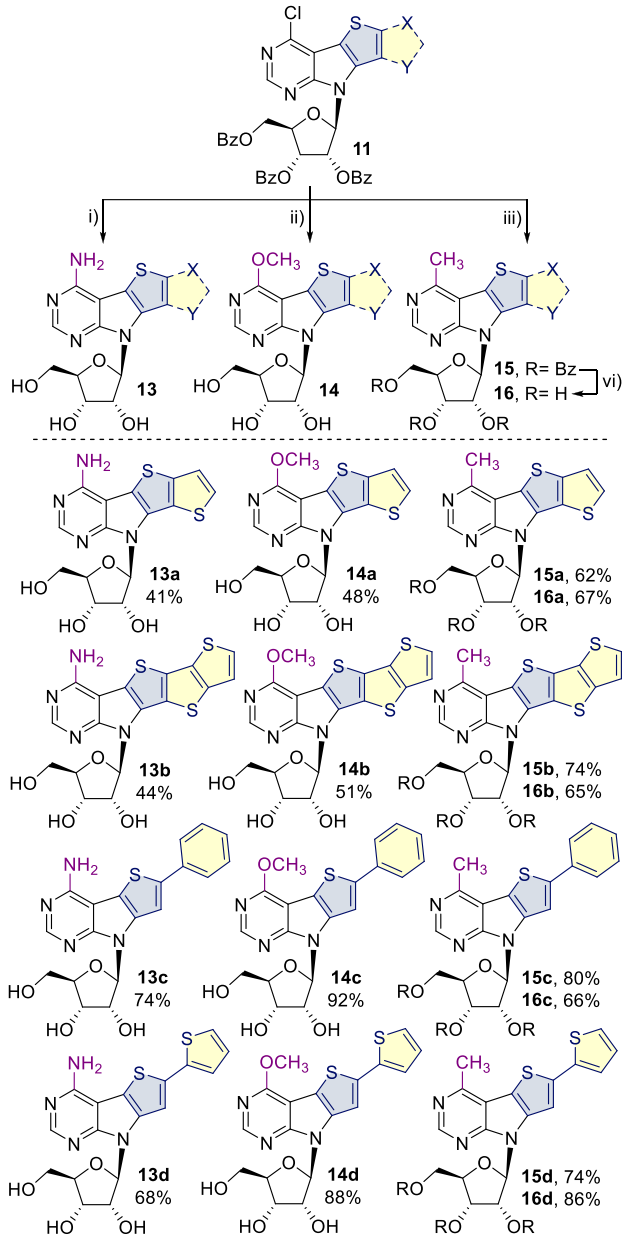
J. Am. Chem. Soc. 2022, 144, 19437−19446

Scheme 5[a]



[a]Reagents and conditions: (i) NH_3 (aq.), dioxane, 120 °C, 24 h; (ii) MeONa, MeOH/dioxane, 65 °C, overnight; (iii) Me_3Al (2.2 equiv), $Pd(PPh_3)_4$ (0.15 equiv), THF, 65 °C, overnight; (vi) MeONa (3 equiv), MeOH/dioxane, 60 °C, overnight.

primer ($Prim^{FAM}$, 15-nt) was used in the PEX to visualize the extension on denaturing polyacrylamide gel electrophoresis (PAGE). Figure 2A confirms that the PEX reaction using **$dA^{PT}TP$** was successful giving the full-length product **19ON_1A^{PT}** which was also characterized by MALDI-TOF mass analysis (found: 6107.4 Da, calculated: 6105.9 Da, Figure S6 in SI). When the PEX reaction was performed with longer template Temp4A encoding for four modifications, the full-length product **ON_4A** (**31ON_4A^{PT}**) was also obtained, which was proved by both denaturing PAGE (Figure 2B) and MALDI-TOF mass (see SI, section 6), but it was accompanied by shorter products of incomplete primer extension. To gain more insight, we performed kinetic experiments of single nucleotide extension with **$dA^{PT}TP$** in comparison with dATP and 7-deaza-dATP (**$dA^{H}TP$**) (Figure S4 in SI) that show that indeed the incorporation of the very bulky tricyclic nucleotide was significantly slower, in particular when it was positioned against the 5′-terminal nucleotide in the template. Nevertheless, the fact that the polymerase was able to incorporate even such a bulky nucleotide is remarkable.

Table 2. UV Absorption Spectra and Fluorescence Properties of Nucleosides 12a–12d[a]

		absorption	emission	
Compd	solvent	λ_{abs} [nm] (ε [10^3 M^{-1} cm^{-1}])	λ_{em} [nm]	Φ_f
12a	MeOH	323 (21.1), 338 (19.8)	359	0.02
	Dioxane	325 (21.7), 340 (20.2)	367	0.01
	H_2O	322 (18.2), 337 (16.4)	367	0.04
12b	MeOH	341 (29.6), 358 (28.1)	385	0.03
	Dioxane	343(29.9), 360 (28.0)	392	0.02
	H_2O	341 (21.6), 357 (17.1)	386	0.04
12c	MeOH	344 (23.3)	398	0.40
	Dioxane	348 (23.0)	405	0.32
	H_2O	342 (19.8)	406	0.48
12d	MeOH	268 (12.4), 357 (22.9)	417	0.16
	Dioxane	268 (12.3), 362 (22.3)	424	0.19
	H_2O	268 (10.3), 356 (21.3)	419	0.25

[a]Fluorescence quantum yields were measured by using anthracene in EtOH (Φ_f = 0.27) as a reference (excitation wavelength is 320 nm).

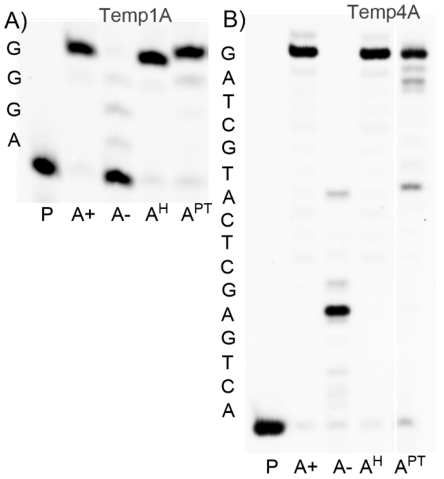


Figure 2. PEX with **12cTP** (**$dA^{PT}TP$**) and KOD XL DNA polymerase with templates encoding for incorporation of one (A) or four (B) modified nucleotide(s). A) template Temp1A, (P): FAM-labeled primer; (A+): dATP, dGTP; (A−): dGTP; (A^H): **$dA^{H}TP$**, dGTP; (A^{PT}): **$dA^{PT}TP$**, dGTP. B) template $Temp4A^{bio}$ (for original uncut gel, see Figure S2 in SI); (P): FAM-labeled primer; (A+): dATP, dGTP, dCTP, dTTP; (A−): dGTP, dCTP, dTTP; (A^H): **$dA^{H}TP$**, dGTP, dCTP, dTTP; (A^{PT}): **$dA^{PT}TP$**, dGTP, dCTP, dTTP.

In order to study the fluorescence properties of **dA^{PT}**-modified oligonucleotides (ONs), we first measured the fluorescence emission and quantum yields of the **19ON_1A^{PT}**. Fluorescence properties of the resulting ONs and duplexes were studied with an excitation wavelength of 320 nm. **ON_1A** in medium salt buffer showed fluorescence with emission maxima at 406 nm and with a quantum yield of 10.2%. Hybridization with matched or mismatched ONs did not show any significant difference either in emission maxima or in quantum yields (see SI for details). Therefore, we believe that this stable fluorescence property makes **dA^{PT}** promising for DNA labeling and quantification, but it is not an

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J. Am. Chem. Soc. 2022, 144, 19437−19446

Table 3. Cytotoxic Activities of Nucleosides

Compd	MTS, IC_{50} [μM]							
	BJ	MRC-5	CCRF-CEM	K562	A549	HCT116	HCT116 $p53^{-/-}$	U2OS
12a	>50	50	6.6	20.9	32.7	18.8	16.2	20.4
12b	18.1	23.8	0.48	1.9	14.2	1.91	1.46	2.8
12bα	>50	>50	0.73	3.7	>50	3.2	2.3	4.7
12c	50	50	0.74	2.2	12.6	2.5	2.7	2.1
12d	50	49.9	0.77	3.2	27.2	2.9	2.8	2.5
13a	48.1	43.8	14.2	20.4	27.2	17.2	19.1	16.6
13b	50	>50	1.4	4.2	8.8	2.3	2.5	4.5
13c	>50	>50	3.3	13.6	>50	14.8	14.3	10.6
13d	>50	>50	3.2	20.7	>50	12.5	9.6	13.5
14a	>50	>50	>50	>50	>50	>50	>50	>50
14b	>50	>50	>50	>50	>50	>50	>50	>50
14c	>50	>50	>50	>50	>50	>50	>50	>50
14d	>50	>50	>50	>50	>50	>50	>50	>50
16a	>50	>50	15.5	48.5	>50	35.2	46.2	41.7
16b	>50	>50	1.8	3.5	>50	5.5	n.d.	44.5
16c	>50	>50	2.0	>50	>50	>50	>50	>50
16d	>50	>50	2.0	12.3	>50	25.9	14.9	21.5
tubercidin	0.73	0.74	0.017	0.36	0.52	0.08	0.15	0.089
2′-deoxytubercidin	>50	>50	>50	>50	>50	>50	>50	>50

environment-sensitive label for studying changes of secondary structures or hybridization.[34]

Biological Activity Profiling. Nucleosides **12a−12d**, **13a−13d**, **14a−14d**, and **16a−16d** were also tested for *in vitro* cytotoxic activity on the panel of leukemic cell lines (CCRF-CEM − acute lymphoblastic leukemia, K562 − myelogenous leukemia,)[11] and solid tumor cells (A549 − human lung adenocarcinoma, HCT116 and HCT116p53$^{-/-}$ − colon cancer cells with/without p53 gene, U2OS − human osteosarcoma)[35] using a 3-day MTS (3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) assay.[36] Nonmalignant fibroblast cell lines MRC-5 and BJ were used in the MTS assay to assess the cancer cell selectivity. Table 3 summarizes the cytotoxic activities of compounds in the MTS assay in comparison with known parent compounds: strongly and nonselectively cytotoxic tubercidin (7-deazaadenosine)[37] and 2′-deoxytubercidin (2′-deoxy-7-deazaadenosine).[38]

Ribonucleoside **13b** showed low micromolar activities against most of cancer cell lines but not nonmalignant fibroblasts in the MTS assay. Ribonucleosides **13a**, **13c**, **13d**, **16b**, and **16d** showed only moderate effects at micromolar concentrations with little selectivity to cancer cells. The remaining ribonucleosides are inactive against cancer cells. On the other hand, most of the deoxyribonucleosides **12b−12d** and the α-anomer **12bα** displayed good antitumor activities in micromolar or submicromolar (for CCRF-CEM cell line) concentrations. Compounds **12bα**, **12c**, and **12d** show good selectivity toward cancer cell lines and are nontoxic to fibroblasts as compared to toxic tubercidin. These rather surprising results are interesting because in the previous examples of tubercidin,[37] 7-substituted[8] or fused deazapurine nucleosides,[13,14] the ribonucleosides were always more active whereas 2′-deoxytubercidin[38] was inactive. This suggests that the mode of action of this class of nucleosides will probably be different from the previously reported tubercidin or tricyclic thieno-, furo-, and pyrrolo-fused 7-deazapurine ribonucleosides that become phosphorylated and incorporated to DNA causing DNA damage and apoptosis. The mechanism of action of these new polycyclic 2′-deoxyribonucleosides will need a separate study in the future.

All the title nucleosides were also screened for their antiviral activity against herpes simplex, influenza, human immunodeficiency virus (HIV), dengue, and SARS-CoV-2 viruses using previously published protocols.[10,16] None of the final nucleosides showed any significant activity at 25 μM concentration.

CONCLUSION

In this paper, we developed a new approach for the synthesis of fused 7-deazapurine heterocycles and nucleosides. The synthesis of the key polycyclic fused heterocycles relied on C−H functionalization of (hetero)aromatics with DBTO followed by the Negishi cross-coupling of the resulting sulfonium salts with bis(4,6-dichloropyrimidin-5-yl)zinc (**6**) giving a series of 5-(het)aryl-4,6-dichloropyrimidines **7** in good yields and excellent regioselectivity. It is the first example of the Negishi cross-coupling of (het)arylsulfonium salts with hetarylzinc reagent, and this cross-coupling has a very promising potential in synthesis of other complex heterocyclic biaryls. Selected examples of biaryls **7** were then azidated and cyclized to form novel substituted or extended thieno-fused 7-deazapurine heterocycles. These extended nucleobase analogues, which would be hardly accessible by previously known synthetic approaches, were then glycosylated to form 2′-deoxy- or ribonucleosides, and a series of derivatives were prepared by nucleophilic substitutions at position 4. Most of the modified ribonucleosides were inactive or moderately active against a panel of cancer cell lines in the cytotoxic MTS assay, whereas deoxyribonucleosides **12b−12d** displayed high anticancer activities, especially for T-lymphoblastic leukemia cells CCRF-CEM. On the other hand, the extended fused nucleosides did not show any significant antiviral activity. Fluorescent properties of the polycyclic deoxyribonucleosides were also studied, and we also prepared a triphosphate of 2′-deoxyribonucleoside **12c** (**12cTP**, as analogue of dATP) and successfully used it for enzymatic synthesis of fluorescently labeled DNA, which showed stable fluorescence in different sequences and was promising for DNA labeling. These

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J. Am. Chem. Soc. 2022, 144, 19437−19446

polycyclic fused nucleosides and nucleotides also might have potential in the construction of new types of expanded nucleic acids.[39]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.2c07517.

Absorption and emission spectra of modified nucleosides and DNA, sequences of oligonucleotides, full experimental section with synthetic procedures and characterization of all compounds, biochemical methods and procedures, and copies of NMR spectra. (PDF)

Accession Codes

CCDC 2183422 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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