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Synthesis of a Protected *keto*-Lysidine Analogue via Improved Preparation of *Arabino*-isoCytosine Nucleosides

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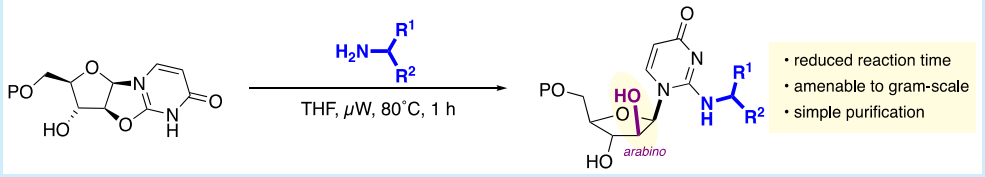
Supporting Information



ABSTRACT: Anhydrouridines react with aliphatic amines to give *N*-alkyl isocytosines, but reported procedures often demand very long reaction times and can be low yielding, with narrow scope. A modified procedure for such reactions has been developed, using microwave irradiation, significantly reducing reaction time and allowing facile access to a diverse range of novel nucleosides on the gram scale. The method has been used to prepare a precursor to a novel analogue of lysidine, a naturally occurring iminonucleoside found in ^{t}RNA.

Noncoded ("unnatural") nucleoside analogues are privileged chemical motifs, possessing a diverse range of biological and clinical properties. Most nucleoside drug candidates contain modified heterocyclic components; however, modification of the native ribose core can also confer significant biological activity, and 2′-modified nucleosides are found at the heart of marketed antisense oligonucleotide[1] medications, including mipomersen (Kynamro, used to treat homozygous familial hypercholesterolemia) and nusinersen (Spinraza, for spinal muscular atrophy). 2′-Modified mononucleosides also exhibit biological potency, and *arabino*-configured nucleosides Cytarabine (ara-C **1**, acute myeloid and lymphocytic leukemias, and lymphomas; Figure 1), Clevudine (**2**, hepatitis B), and Fludarabine (**3**, chronic lymphocytic leukemia, non-Hodgkin lymphoma) are used in the clinic, leading to great interest in methods for synthesis of this class of compound.[2,3] In addition to therapeutic significance, modified nucleosides are also of great utility as structural probes and as chemical start points for functional synthetic polynucleotides. *Arabino*-configured antisense systems have been prepared and shown to possess interesting properties,[4] and aminated *arabino*-isocytosines **4** are also biologically active nucleoside analogues, possessing anticancer activity,[5] and as precursors to polynucleotides able to form duplexes with isoG-containing sequences.[6] As part of a research program directed toward synthesis and testing of novel catalytic polynucleotides possessing both modified base and ribose motifs, compounds rarely reported in the literature, we sought a synthetic entry to *arabino*-configured isocytosines **4**.

In addition, for similar purposes, we also sought access to arabino-configured analogues of the naturally occurring ^{t}RNA nucleoside Lysidine **5**, as potential inhibitors of lysidine synthetase.[7]

We envisaged entry to *arabino*-isocytosines, including the previously unreported lysidine analogue **6**, by means of ring opening of 2,3′-anhydrouridines **7** by aliphatic amines (Figure 2): thus, reaction of a suitably protected lysine **8** would directly give the protected lysidine analogue. However, we found existing methods capricious and low yielding and so sought an alternative entry to these noncoded nucleoside analogues; we report here a modified procedure which ameliorates these problems, delivering the target nucleosides on the gram scale and representing a significant advance over the existing methodology.

The ring opening of anhydrouridine by ammonia was first reported by Todd et al.;[8] the method was later refined[9] and broadened in scope, to include higher amines.[10] In general, the ring opening of anhydrouridine with alkylamines to give ara-isocytosines is a slow process,[11] with reactions typically taking several days to reach completion (e.g., ring opening of anhydrouridine by cyclohexylamine takes 32 days to reach completion[10]). Moreover, the isolation of aminated products is inefficient, with

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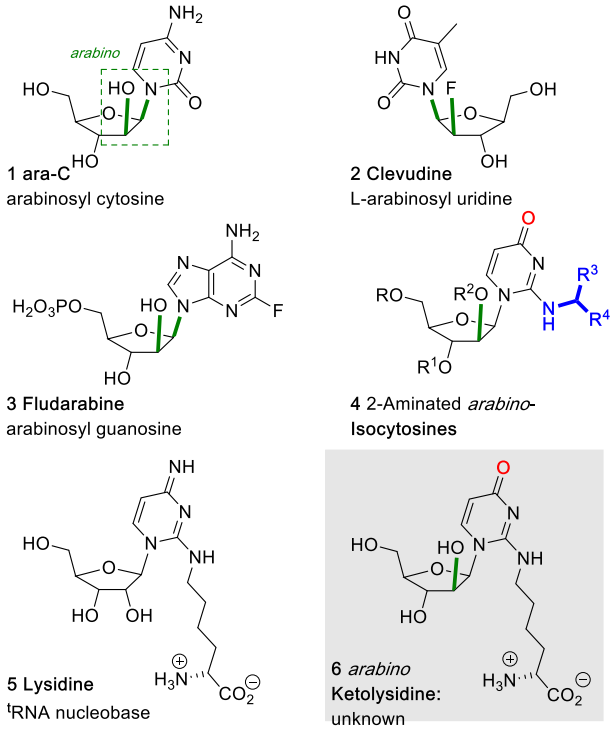


Figure 1. *arabino*-Nucleosides: privileged chemical motifs

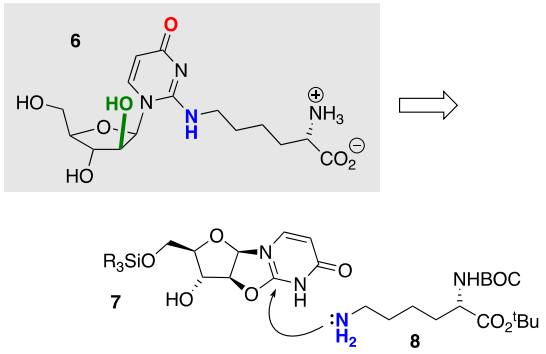
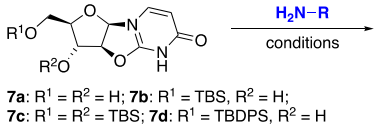
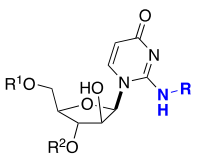


Figure 2. *Arabino*-ketolysidine synthesis: strategy.

Table 1. Nucleoside Amination by Ring Opening of Anhydrouridine: Traditional vs Microwave Conditions



7a: $R^1 = R^2 = H$; **7b**: R^1 = TBS, R^2 = H; **7c**: $R^1 = R^2$ = TBS; **7d**: R^1 = TBDPS, R^2 = H



$R^1 = R^2 = H$
4aa: R = Me; **4ab**: R = ^{n}Bu

R^1 = TBS, R^2 = H;
4ba: R = Me; **4bb**: R = ^{n}Bu

$R^1 = R^2$ = TBS,;
4ca: R = Me; **4cb**: R = ^{n}Bu

R^1 = TBDPS, R^2 = H
4db: R = ^{n}Bu

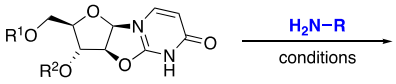
7	R	conditions	reaction time/h	product	yield/%
7a	Me	DMF, 7 equiv of amine, rt[a10]	N/A	**4aa**	N/A
7a	^{n}Bu	DMF, 7 equiv amine, rt[a]	72	**4ab**	71[b]
7b[12]	Me	THF 10 equiv of amine, μW, 80 °C	1	**4ba**	89

Table 1. continued

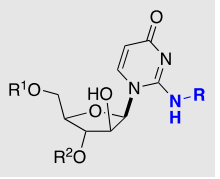
7	R	conditions	reaction time/h	product	yield/%
7c[12]	Me	THF 10 equiv of amine, μW, 80 °C	1	**4ca**	65
7b	^{n}Bu	THF 10 equiv of amine, μW, 120 °C	1	**4bb**	mixture
7c	^{n}Bu	THF 10 equiv of amine, μW, 80 °C	0.5	**4cb**	55
7c	^{n}Bu	THF 10 equiv of amine, μW, 120 °C	0.5	**4cb**	0
7c	^{n}Bu	THF 10 equiv of amine, $ZnCl_2$, μW, 120 °C	0.5	**4cb**	0
7d[13]	^{n}Bu	THF 10 equiv of amine, μW, 80 °C	1	**4db**	68

[a]Ref 10. [b]Yield of corresponding triacetate.

Table 2. Microwave-Mediated Anhydrouridine Amination: Yield Comparison



7a: $R^1 = R^2 = H$; **7d**: R^1 = TBDPS, R^2 = H



$R^1 = R^2 = H$
4ab: R = ^{n}Bu; **4ac**: R = Allyl
4ad: R = Bn; **4ae**: R = ^{c}Hex

R^1 = TBDPS, R^2 = H
4db: R = ^{n}Bu; **4dc**: R = Allyl
4dd: R = Bn; **4de**: R = ^{c}Hex

7	R	conditions	reaction time/h	product	yield/%
7a	^{n}Bu	DMF, 7 equiv of amine, rt[10]	36	**4ab**	71[a]
7d	**^{n}Bu**	**THF 10 equiv of amine, μW, 80 °C**	**1**	**4db**	**68**
7a	allyl	DMF, 7 equiv of amine, rt[10]	48	**4ac**	77[a]
7d	**allyl**	**THF 10 equiv of amine, μW, 80 °C**	**1**	**4dc**	**66**
7a	benzyl	DMF, 7 equiv of amine, rt[10]	180	**4ad**	90[a]
7d	**benzyl**	**THF 10 equiv of amine, μW, 80 °C**	**1**	**4dd**	**56**
7a	^{c}Hex	DMF, 7 equiv of amine, rt[10]	384	**4ae**	72[a]
7d	**^{c}Hex**	**THF 10 equiv of amine, μW, 80 °C**	**1**	**4de**	**51**

[a]Yield of corresponding triacetate.

postreaction derivatization of the unprotected product necessary to allow reasonable yields to be achieved.[8] Prior protection of the 5′-hydroxyl group directly delivers tractable products but does not noticeably improve the rate of reaction. The use of microwave irradiation to accelerate reactions, in particular those performed in polar solvents, is a contemporary paradigm, and we envisaged that this would be a valuable method to accelerate the ring opening of anhydronucleosides by amines. After a screen of reaction conditions, we were, therefore, gratified to observe that anhydrouridines **7b–d** reacted with methylamine and ^{n}butylamine in THF at 80 °C at 300 W to give the aminated products, *arabino*-isocytosines **4ba**, **4ca**, **4cb**, and **4db**, in good yields, after only 1 h (Table 1). The reaction with methylamine had not been reported in the literature, presumably due to the volatility of the amine component.

Although the ring-opened products proved stable to chromatography, showing little tendency to return to the anhydro

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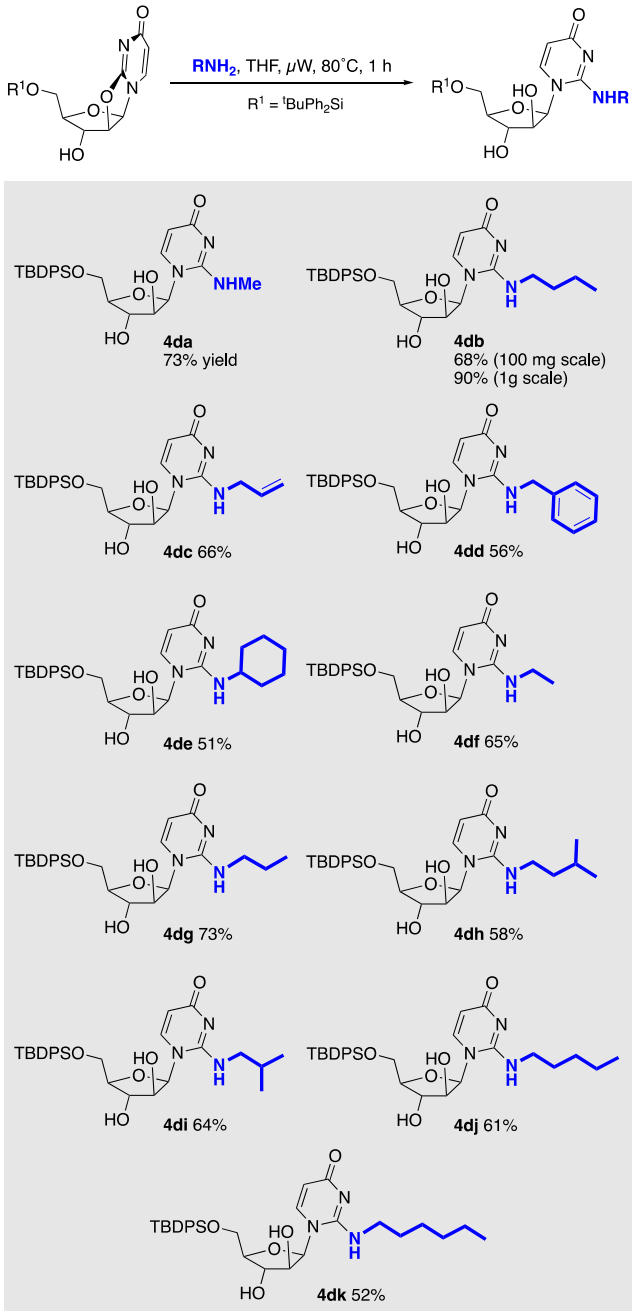
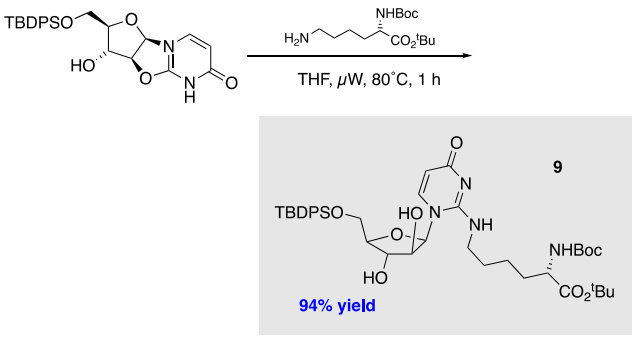


Figure 3. Amination of 5′-*O*-TBDPS-2,2′-anhydrouridine: scope.

Scheme 1. Synthesis of Novel Ketolysidine Derivative 9



starting material, the products could more conveniently be isolated by precipitation from the crude reaction medium.

Thus, the microwave-mediated procedure reduced the reaction time by 97%; when compared to the reported procedure[10] using other amines, the increase in the rate of reaction was even more striking (Table 2). The use of THF rather than a high-boiling polar solvent (such as DMF[10]) also represents a significant simplification in the process.

The process can be applied to a range of aliphatic amines (Figure 3), delivering *arabino*-2-(alkyl)amino isocytosines in good yields. The simplicity of the method enables gram-scale reactions to be carried out in short order, and the ability to isolate pure products directly from the reactions enables rapid and convenient access to novel nucleosides.

Armed with a new and efficient entry into 2-(alkyl)amino isocytosine nucleosides, we turned our attention to our original target molecule, ketocytosine analogue **6**, and we were delighted to observe that reaction of lysine derivative **8** with anhydrouridine **7d** delivered the key product **9** (a protected precursor to **6**) in excellent yield (Scheme 1).

In summary, we have reported an improved method for amination of 2,2′-anhydrouridine, enabling a practical and high yielding entry to a range of 2-aminated nucleosides. We are currently engaged in the use of this technology to deliver novel analogues of bioactive nucleosides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b00086.

Experimental details and associated spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Khvorova, A.; Watts, J. K. *Nat. Biotechnol.* **2017**, *35*, 238−248.

(2) For recent synthetic activity in the area, see: Dostie, S.; Prevóst, M.; Guindon, Y. *J. Org. Chem.* **2012**, *77*, 7176−7186.

(3) Ewald, B.; Sampath, D.; Plunkett, W. *Oncogene* **2008**, *27*, 6522−6537.

(4) (a) Resmini, M.; Pfleiderer, W. *Helv. Chim. Acta* **1993**, *76*, 158. (b) Damha, M. J.; Wilds, C. J.; Noronha, A.; Brukner, I.; Borkow, G.; Arion, D.; Parniak, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 12976−12977. (c) Li, F.; Sarkhel, S.; Wilds, C. J.; Wawrzak, Z.; Prakash, T. P.; Manoharan, M.; Egli, M. *Biochemistry* **2006**, *45*, 4141−4152.

(5) Krizmanić, I.; Višnjevac, A.; Luić, M.; Glavaš-Obrovac, L.; Žinić, M.; Žinić, B. *Tetrahedron* **2003**, *59*, 4047−4057.

(6) Ozaki, H.; Nakajima, K.; Tatsui, K.; Izumi, C.; Kuwahara, M.; Sawai, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2441−2443.

(7) (a) Suzuki, T.; Miyauchi, K. *FEBS Lett.* **2010**, *584*, 272−277. (b) Shapiro, A. B.; Plant, H.; Walsh, J.; Sylvester, M.; Hu, J.; Gao, N.;

DOI: 10.1021/acs.orglett.9b00086
Org. Lett. XXXX, XXX, XXX−XXX

Livchak, S.; Tentarelli, S.; Thresher, J. *J. Biomol. Screening* **2014**, *19*, 1137−1146.
(8) Brown, D. M.; Parihar, D. B.; Todd, A. R.; Varadarjan, S. *J. Chem. Soc.* **1958**, *0*, 3028−3035.
(9) Doerr, I. L.; Fox, J. J. *J. Org. Chem.* **1967**, *32*, 1462−1471.
(10) Delia, T. J.; Berańek, J. *J. Carbohydr., Nucleosides Nucleotides* **1977**, *4*, 349−362.
(11) See, for instance: Ozaki, H.; Nakajima, K.; Tatsui, K.; Izumi, C.; Kuwahara, M.; Sawai, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2441−2443.
(12) Ferreri, C.; Costantino, C.; Romeo, R.; Chatgilialoglu, C. *Tetrahedron Lett.* **1999**, *40*, 1197−1200.
(13) Sebesta, D. P.; Rourke, S. S. O.; Martinez, R. L.; Piekcn, W. A.; McGee, D. P. C. *Tetrahedron* **1996**, *52*, 14385−14402.

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