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Syntheses of 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives as novel anticancer nucleoside analogs

Marc A. Labroli [a], Michael P. Dwyer [b,*], Ruichao Shen [c], Janeta Popovici-Muller [c], Qinglin Pu [c], Judson Richard [c], Kristen Rosner [c], Kamil Paruch [a], Timothy J. Guzi [c]

[a] Merck Research Laboratories, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA
[b] Merck Research Laboratories, PO Box 2000, 126 E. Lincoln, Rahway, NJ 07065, USA
[c] Merck Research Laboratories, 33 Louis Pasteur Avenue, BMB-3, Boston, MA 02115, USA

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ABSTRACT

A novel class of 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives has been synthesized in order to identify anticancer nucleoside analogs. Several synthetic routes were devised and implemented which relied upon either S_N2 displacement or reductive amination to provide the desired derivatives.

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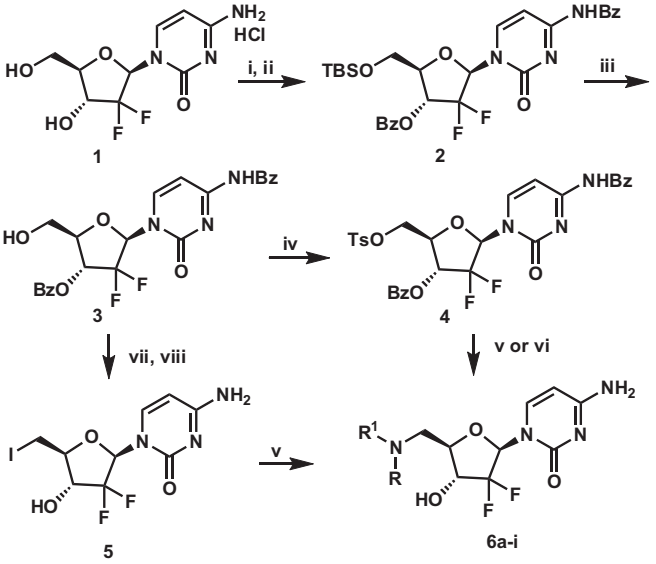
2′-Deoxy-2′,2′-difluorocytidine, the antimetabolite nucleoside known as gemcitabine (**1**), is approved for the treatment of pancreatic, breast, and non-small cell lung cancers either as a single agent or in combination with other chemotherapeutic agents.[1] Gemcitabine acts as a prodrug which is intracellularly phosphorylated to its active diphosphate and triphosphate intermediates.[2] Gemcitabine triphosphate competes with deoxycytidine triphosphate for incorporation into DNA which results in termination of DNA polymerization.[3] The diphosphate intermediate effectively inhibits ribonucleotide reductase (RRM1) which leads to the depletion of the deoxynucleotide pool and halt of DNA synthesis.[4] Since the discovery of gemcitabine by Hertel et al.,[5] numerous gemcitabine derivatives have been synthesized in search for new anticancer or antiviral agents. For example, the base-modified gemcitabine derivatives include adenosine, guanine, and uracil analogs.[6] The ribose-modified derivatives include 4′-azido analogs,[7] 4′-allene substituted analogs,[8] 3′-deoxy analogs,[9] and thio/aza/carbocyclic analogs.[10] However, to our knowledge, the 5′-amino derivatives of gemcitabine, a novel and potentially biologically interesting class of compounds, have not been actively investigated. As part of our program to identify novel and selective anticancer compounds, we initiated efforts to develop efficient syntheses of 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives in order to profile this novel series. Herein, we describe our chemistry efforts to prepare 5′-amino derivatives of gemcitabine.[11]



Scheme 1. Reagents and conditions: (i) TBSCl, imidazole, DMF, rt, 12 h, 92%; (ii) BzCl, DMAP, pyridine, rt, 12 h, 90%; (iii) TBAF, THF, 0 °C, 3.5 h then AcOH, 85%; (iv) TsCl, pyridine, Et_3N, 85%; (v) HNRR[1], DMF, 90–100 °C; (vi) NH_3, MeOH, 58%; HNRR[1], DMF; (vii) $MeP(OPh)_3I$, DMF, 60 °C, 84%; (viii) NH_3, MeOH, 92%.

Our initial route to the 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine analogs is outlined in Scheme 1. The synthesis began

* Corresponding author. Tel.: +1 (732) 594 1733.
E-mail address: michael.dwyer@merck.com (M.P. Dwyer).

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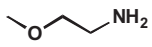
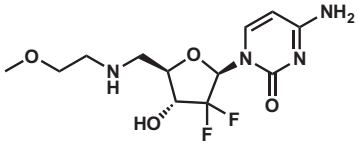
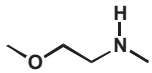
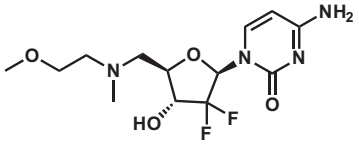
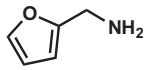
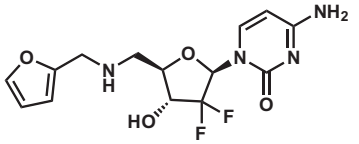
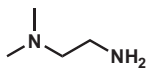
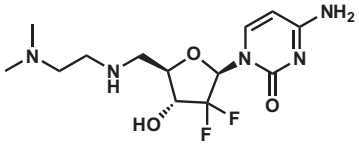
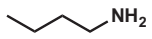
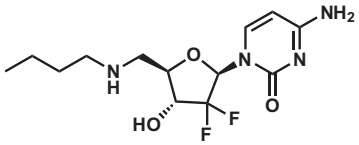
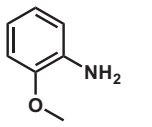
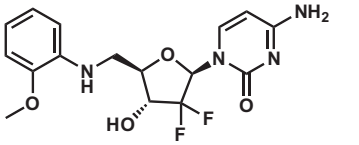
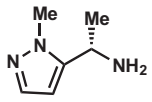
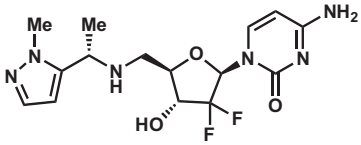
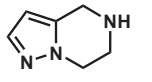
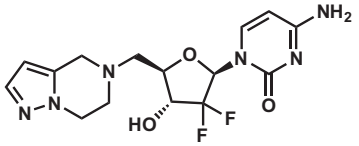
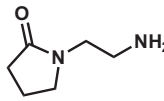
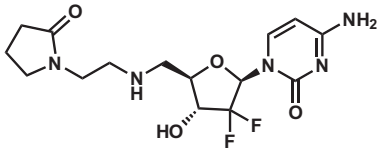
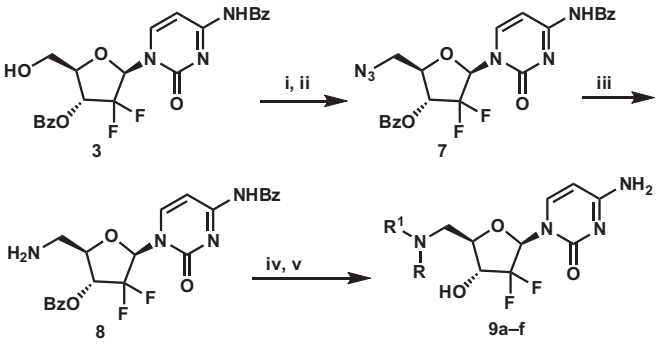
with protection of the 5′-hydroxy group of gemcitabine hydrochloride (**1**) as the *tert*-butyldimethylsilyl (TBDMS) ether followed by global benzoyl protection to afford compound **2**. Desilylation of the silyl ether was achieved with TBAF at 0 °C to provide alcohol **3**[12] which was converted to tosylate **4** under standard conditions. Treatment of intermediate **4** with excess amine coupling partner at 100 °C promoted both tosylate displacement and benzoyl group cleavage to afford products **6a**–**d**,**i** (Table 1). Alternatively, this protocol could be carried out in a step-wise fashion by first removing the benzoyl groups of **4** by treatment with 7 N ammonia in methanol to provide the penultimate product. Treatment of the resulting tosylate with excess amine provided the desired compounds **6e**,**f** (Table 1). While the displacement reaction with the amine coupling afforded modest yields of the desired products (Table 1), multiple purifications were required to completely remove the resultant TsOH from desired product. In order to

Table 1
Preparation of **6a–i** by S_N2 substitution

Entry	Amines	Product	Yield (%)
6a			20[a]
6b			54[a]
6c			32[a]
6d			19[a]
6e			84[a,c]
6f			13[a]
6g			53[b]
6h			55[b]
6i			53[a]

[a] Yield for one step.
[b] Combined yield for two steps.
[c] Rxn heated at 40 °C.

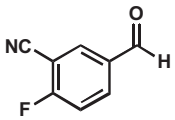
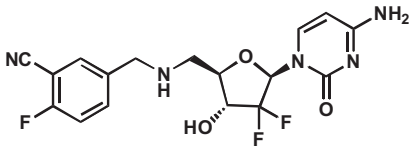
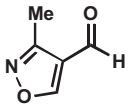
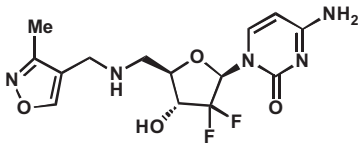
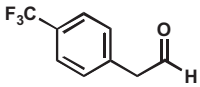
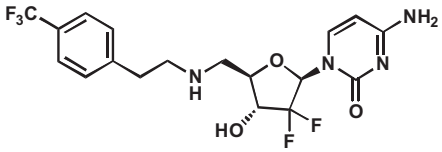
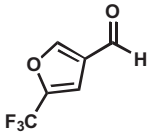
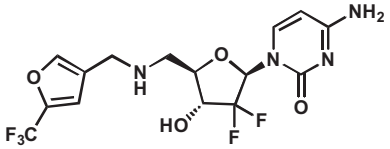
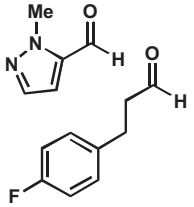
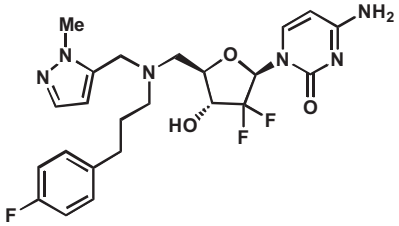
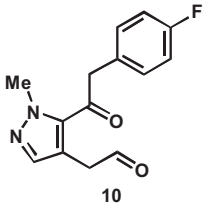
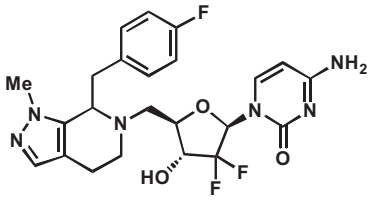


Scheme 2. Reagents and conditions: (i) MsCl, Et_3N, pyridine, 89%; (ii) NaN_3, DMF, 70 °C, 98%; (iii) Me_3P, MeCN/H_2O, 85%; (iv) RCHO, $NaBH_3CN$, MeOH, AcOH, 35–75%; (v) 7 M NH_3/MeOH, 45–85%.

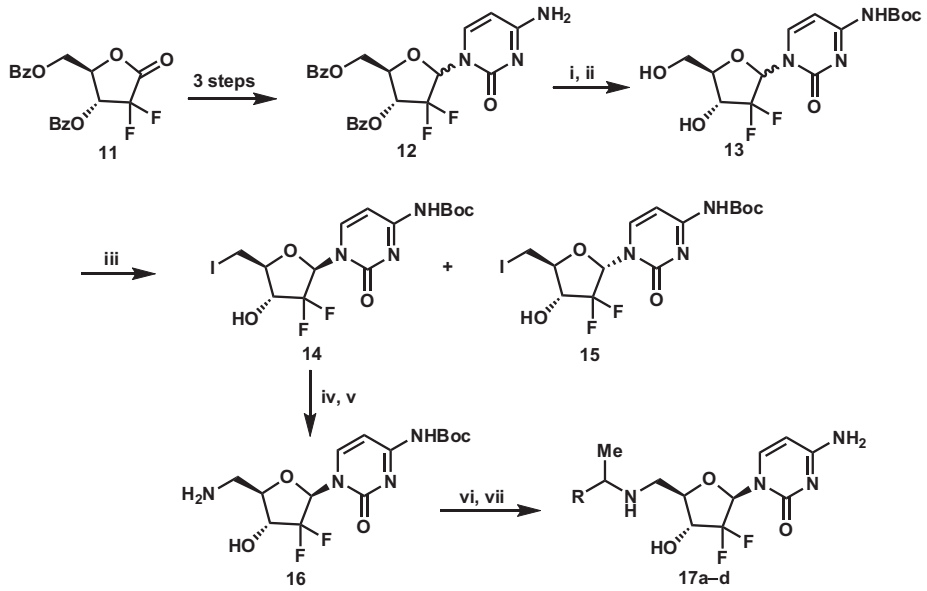
circumvent this issue, alcohol **3** was converted to the iodide intermediate **5** using methyltriphenoxyphosphonium iodide followed by benzoyl deprotection. Treatment of iodide **5** with 3 equiv of amines at 90 °C provided 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine **6g**,**h** in respectable yields (Table 1). The use of iodide **5** allowed us to scale back the number of equivalents of amine for the displacement reaction while making the purification of the final analogs much easier.

While the S_N2 strategy depicted in Scheme 1 represented a concise preparation of 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives, we decided to pursue other alternatives due to the poor to modest yields for the final compounds. Toward this end, a reductive amination approach was explored not only to improve the yield of the final 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine analogs but to broaden the scope of coupling partners. As shown in Scheme 2, alcohol **3** was converted to the mesylate followed by treatment with NaN_3 to afford the corresponding azide **7**. Treatment of azide **7** with trimethylphosphine provided amine intermediate **8** which was used directly without silica gel purification to prevent benzoyl transfer to the 5′-amino group. Treatment of **8** with various aldehydes under reductive amination conditions ($NaBH_3CN$) afforded the N-alkylated products which were treated with 7 M NH_3 in MeOH to afford 5′-substituted amino analogs

Table 2
Reductive amination protocol/deprotection from **8** to afford **9a–f** (Scheme 2)

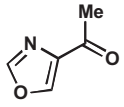
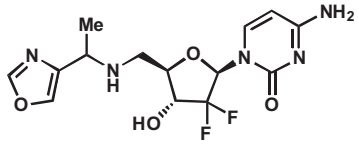
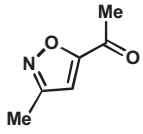
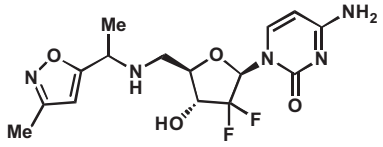
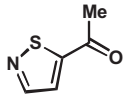
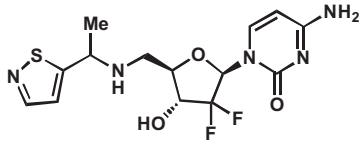
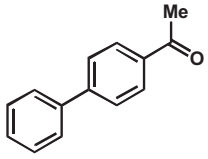
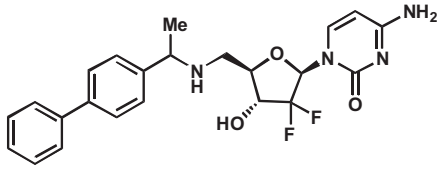
Entry	Aldehydes	Product	Yield (%) of step iv[a]/step v
8a			72/82
b			66/70
c			68/45
d			69/84
e			59[b]/50
f	 10		35/85

[a] Amine **8** was used as the crude product of reduction of azide **7**.
[b] Prepared by one-pot stepwise reductive amination.



Scheme 3. Reagents and conditions: (i) Boc_2O, DMAP, THF, 75%; (ii) $Et_3N/MeOH/H_2O$, rt, 12 h, 92%; (iii) $MeP(OPh)_3I$, DMF, rt, 1 h, 31% (**14**), 45% (**15**); (iv) NaN_3, DMF, 40 °C, quant.; (v) Me_3P, $MeCN/H_2O$, 73%; (vi) RCOMe, $Ti(i\text{-}OPr)_4$, then $NaBH_3CN$, 51–68%; (vii) TFA, CH_2Cl_2, 71–98%.

Table 3
Reductive amination/deprotection protocol from **16** to afford **17a–d** (Scheme 3)

Entry	Ketones	Product	Yield (%) of step vi[a]/step vii
17a			68/71
17b			60/95
17c			50/71
17d			51/98

9a–**f** shown in Table 2. The reductive amination/deprotection protocol worked well for both aromatic and aliphatic aldehydes (entries **9a**–**d**) with respectable yields observed for both steps (Table 2). Additionally, this protocol was adapted to a stepwise format by employing two distinct aldehydes to afford **9e** or by employing a dicarbonyl substrate **10** to afford bicyclic derivative **9f**.

While the reductive amination approach utilizing amine **8** proceeded well with aldehydes, this protocol yielded very low yields of α-substituted amine products when ketones were used as coupling partners. Owing to the reduced electrophilicity and increased steric hindrance of the ketone coupling partners, it was rationalized that a strong Lewis acid may be required to promote the initial imine formation for these substrates. It was rationalized that one might need an alternative 5′-amino intermediate with a suitable protecting group for harsher imine formation conditions due to potentially labile 3′-benzoate of **8**. Additionally, it was desirable to develop a flexible route which could allow for rapid base modification (other than cytosine) while at the same time circumventing the need to use expensive gemcitabine as a starting material. Based on the above considerations, a second reductive amination synthetic route was developed to prepare

5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives derived from ketone coupling partners.

The synthesis started with commercially available 2-deoxyl-2,2-difluoro-D-*erythro*-pentafuranous-1-ulose-3,5-dibenzoate (**11**) (Scheme 3). Following the protocol described by Chou et al.,[13] lactone **11** was converted to nucleoside **12** in three steps as a mixture of both anomers (β:α = 1:1.5). The 4-amino group of the cytosine was protected as the Boc derivative followed by hydrolysis of the 3′,5′-dibenzoate under mild basic conditions to provide diol **13**. Based on the selective iodination protocol of thymidine reported by Verheyden and Moffatt,[14] treatment of diol **13** with methyltriphenoxyphosphonium iodide afforded only the 5′-iodo nucleoside anomers **14** and **15**. Presumably, the selectivity of this transformation is driven by the reduced nucleophilicity of the 3′-hydroxyl caused by the geminal di-fluorine atoms which yields only the 5′-iodo products. Fortunately, the 5′-iodo anomers **14** and **15** could be separated by regular silica gel chromatography. Treatment of β-anomer **14** with sodium azide followed by reduction furnished the 5′-amino intermediate **16**. Treatment of amine **16** with aromatic ketones in the presence of Ti(*i*-OPr)$_4$[15] followed by treatment with $NaBH_3CN$ and Boc deprotection leads to α-methylated 5′-amino compounds **17a–d** as a mixture of diastereomers in moderate to good yields (Scheme 3). The specific yields for the reductive amination step as well as deprotection are detailed in Table 3.

In summary, we have described three unique synthetic routes for the syntheses of a novel class of 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives. The first route relied upon a S_N2 displacement of either a 5′-tosylate or 5′-iodide intermediate using excess amine as coupling partners. To circumvent the modest yields and challenging purifications of final products using the first route, a second route was developed which relied upon a reductive amination of a 5′amino derivative with aldehydes to afford a wide variety of 5′-N-alkylated derivatives. Finally, the final route relies upon a reductive amination step with ketones but also offers the ability to change the base with fewer manipulation of protecting groups than the previous routes. While the 5′-amino intermediates **8** and **16** were critical components for the reductive amination protocols described, one could envision the utility of these materials for preparation of such non-basic analogs such as amides, sulfonamides, and ureas. The biological activity associated with this novel class of 5′-amino-2′,5′-dideoxy-2′,2′-difluorocytidine derivatives will be reported in due course.[16]

References and notes

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