2'-Deoxy-2',2'-difluorothymidine analogues for radiolabeling with fluorine-18 and other biomedical applications

Andreas M. Doepner, Eric O. Aboagye, Anthony G.M. Barrett

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Graphical Abstract.

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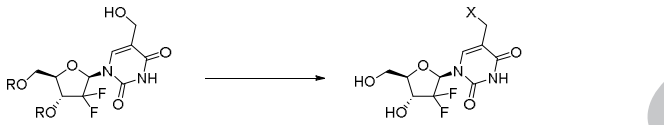

X= MeO, N_3, β-D-glucopyranosyl, dansyl-NH, 4-(2-fluoroethyl)-1,2,3-triazole, urea-bridged dimer

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2'-Deoxy-2',2'-difluorothymidine analogues for radiolabeling with fluorine-18 and other biomedical applications

Andreas M. Doepner[a], Eric O. Aboagye[b] and Anthony G. M. Barrett[a]*

[a] Department of Chemistry, Imperial College, London SW7 2AZ, England
[b] Department of Surgery and Cancer, Imperial College, Hammersmith Campus, London W12 0NN, England

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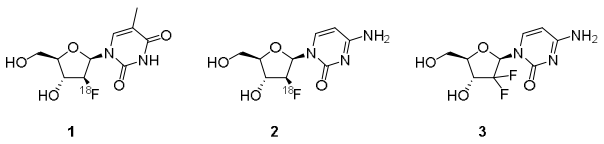
ABSTRACT

Novel 2'-deoxy-2',2'-difluorothymidine analogues with potential applications as antiviral, cytotoxic and cancer imaging agents have been synthesized. Introduction of the hydroxymethyl functionality at the 5-position of 2'-deoxy-2',2'-difluoruridine provided a key intermediate with a suitable synthetic handle for the generation of these nucleoside derivatives.

Positron emission tomography (PET) is a highly sensitive imaging modality for visualizing physiological processes occurring within the body through the use of molecular probes labelled with short-lived positron emitting radionuclides such as: ^{11}C, ^{18}F, ^{15}O and ^{13}N.[1] Rapidly proliferating tissues show a strong reliance on the DNA salvage pathway for nucleotide synthesis.[2] Targeting this pathway therefore permits PET based imaging of cancer. Radiolabelled nucleoside analogues are a class of PET probes highly suited to this task, which have demonstrated utility as agents for the visualization of tumors.[3] Many of these are thymidine based derivatives whose accumulation within cells correlates with the activity of the salvage enzyme thymidine kinase 1 (TK1) and hence the extent of cellular proliferation.[4] There are however some exceptions, for example, ^{11}C and ^{18}F-1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-thymine (FMAU) **1**, which also show retention in tumors and other proliferating tissues.[5] However, such nucleosides show preferential selectivity for the cell-cycle independent thymidine kinase 2 (TK2) enzyme over TK1 and are not regarded as proliferation markers.[6] Furthermore Radu and co-workers recently published details of the synthesis of a radiolabelled deoxycytidine derivative, ^{18}F-1-(2'-deoxy-2'-fluoroarabinofuranosyl)-cytosine (FAC) **2**, which is a substrate for deoxycytidine kinase (dCK).[7] This probe has shown potential for monitoring DNA damage response in cancer as well as immune activation.[8] Radu *et al* also highlighted the antineoplastic agent 2'-deoxy-2',2'-difluorocytidne (dFdc or Gemcitabine) **3** as a compound displaying similar selectivity for proliferating tissues to FAC.[7] Moreover, it has been hypothesized that the presence of the 2'-deoxy-2',2'-difluoro moiety can increase dCK activity towards nucleoside analogues.[9] However, radiolabelling of this compound was not investigated due to possible synthetic difficulties.[7]

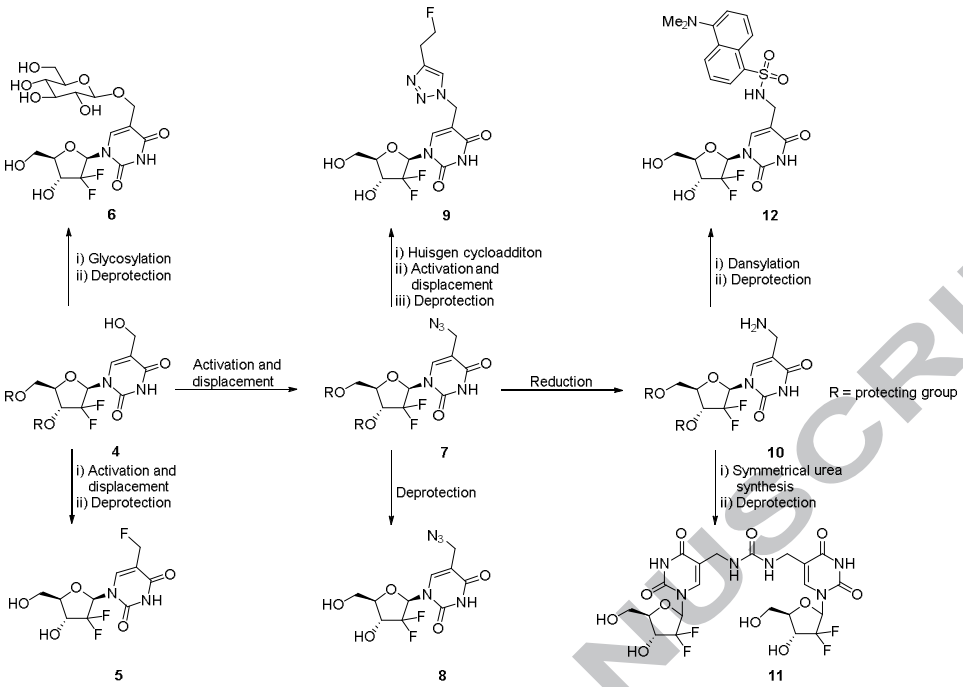


1 **2** **3**

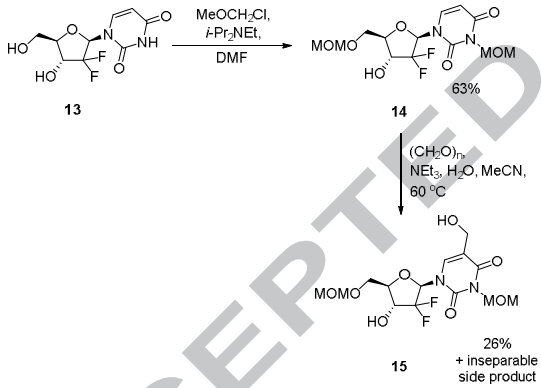
Figure 1. ^{18}F-FMAU (**1**), ^{18}F-FAC (**2**) and Gemcitabine (**3**)

Inspired by these developments and as part of our ongoing efforts into the development of new molecular probes for cancer imaging, we sought to establish a synthetic route for the generation of thymidine based nucleoside analogues incorporating the 2'-deoxy-2',2'-difluoro moiety suitable for radiolabelling with ^{18}F or other radionuclides. Their selectivity could be assessed for TK1, TK2 and dCK, and their utility as PET probes for proliferation or DNA damage response monitoring evaluated. Our chemistry strategy focused on the synthesis of a key uridine intermediate, **4**, hydroxymethylated at the 5-position (Scheme 1). Activation and S_N2 substitution of this alcohol should produce fluoride **5** and azide **7**. The azide could then be further functionalized to analogue **9,** based on the modified nucleoside N^3-((1-(2-[^{18}F]fluoroethyl)-1H-[1,2,3]-triazol-4-yl)methyl)thymidine (FOT), previously developed within our group.[10] Furthermore, owing to continued interested in base modified Gemcitabine derivatives as potential antiviral and cytotoxic agents,[11] we saw the opportunity for the synthesis of a number of other nucleoside analogues including: a 5-azidomethyl derivative **8**,[12] a covalently linked nucleoside dimer **11**,[13] a fluorescent nucleoside **12**[14] and a glycosylated nucleoside **6**[15] (Scheme 1). Herein, we report the successful syntheses of most of these analogues.

Corresponding author. Tel.: +44 020 7594 5766; fax: +44 020 7594 5805.
E-mail address: agm.barrett@imperial.ac.uk (A.G.M. Barrett).



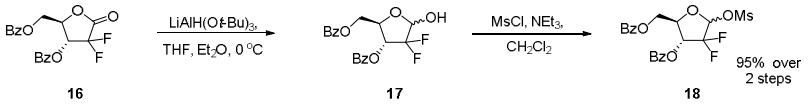
Scheme 1. Nucleoside targets of interest



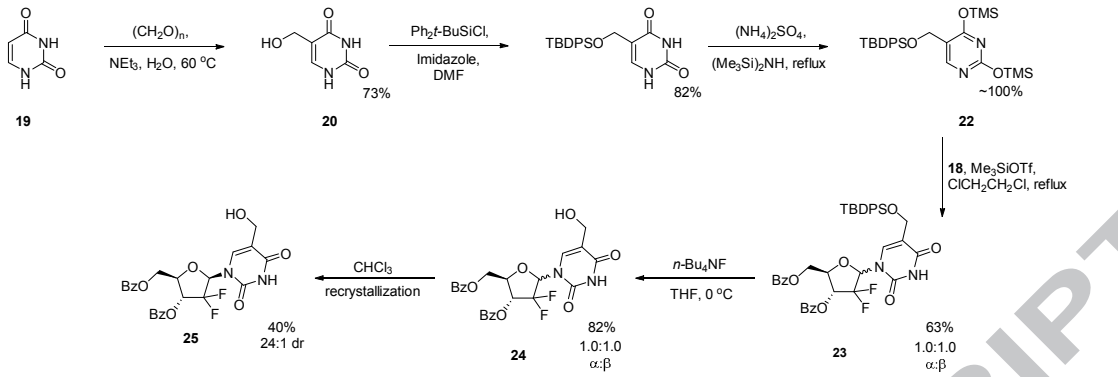
Scheme 2. Synthesis of hydroxymethylated nucleoside **15**

Protection of 2'-deoxy-2',2'-difluoruridine (**13**)[16] as its methoxymethyl ether (MOM) **14** followed by hydroxymethylation using *para*-formaldehyde was carried out in an initial attempt at producing a suitably functionalized derivative of **4**.[17, 18] Yields for the hydroxymethylation were poor and the nucleoside, **15**, could not be isolated as a pure compound, a result corroborated by Chung *et al* on similar substrates (Scheme 2).[19] Since multi-gram quantities of the intermediate **4** were required, an alternative approach was investigated.

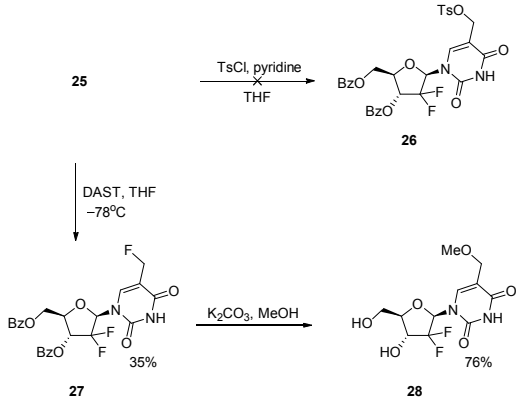
Zhang *et al.* described the synthesis of nucleoside analogues modified in the 5-position by pre-functionalization of the free nucleobase followed by Vorbrüggen glycosylation with a deoxyribose partner.[20] Encouraged by this report, synthesis of the required coupling partners for benzoyl protected **4** was carried out. Mesylates **18** were synthesized in high yield in two steps from commercially available ribonolactone **16** (Scheme 3).[16] Hydroxymethylation of uracil proceeded in 73% yield to give base **20**[18] which was subsequently protected with *tert*-butyldiphenylsilyl chloride (TBDPSCl).[19] This was further silylated using hexamethyldisilazane and directly coupled with **18** in the Vorbrüggen reaction to give protected nucleoside **23** as a mixture of anomers in 63% yield (Scheme 4). At this stage, separation of the anomers proved to be difficult. Only partial separation was achieved by column chromatography and re-crystallisation proved not to be possible. Based on the crystallisation of similar nucleosides in the literature, it was hoped that removing the bulky TBDPS group would render the nucleoside more susceptible to recrystallization.[21] Deprotection with tetra-*n*-butylammonium fluoride[22] proceeded smoothly and recrystallization gave the pure β-anomer **25** from chloroform, thereby allowing separation of the anomers whose identity was ascertained by NOESY experiments. Using this route, large quantities of nucleoside **25** could be made rapidly and efficiently, thus permitting examination of the synthesis of the desired analogues **5**, **6**, **8**, **9**, **11** and **12** .



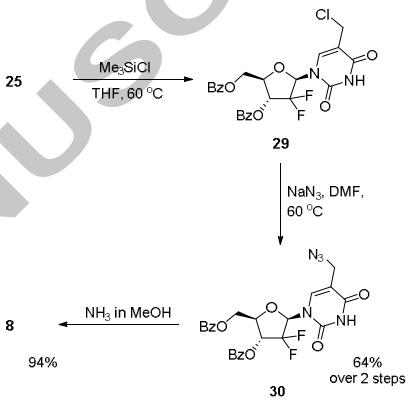
Scheme 3. Synthesis of ribofuranosyl methanesulfonates **18**



Scheme 4. Synthesis of hydroxymethylated nucleoside **25**



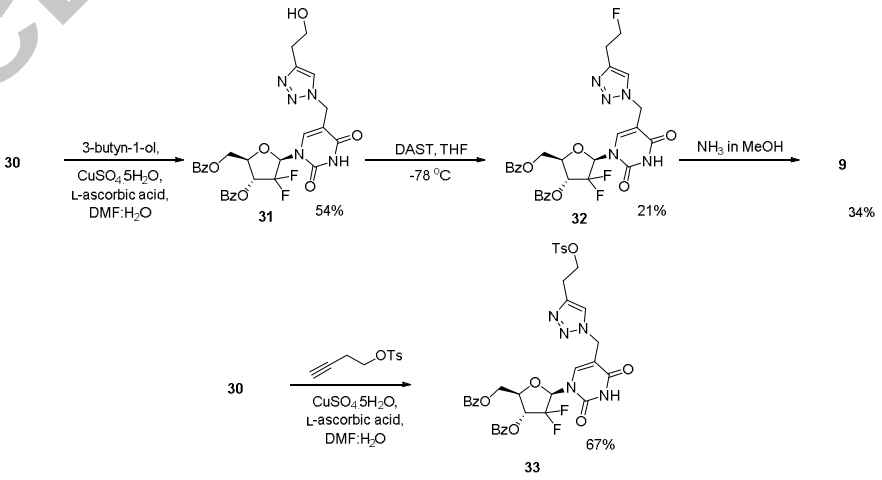
Scheme 5. Attempted synthesis of fluoro-nucleoside **5**



Scheme 6. Synthesis of azidomethyl-nucleoside **8**

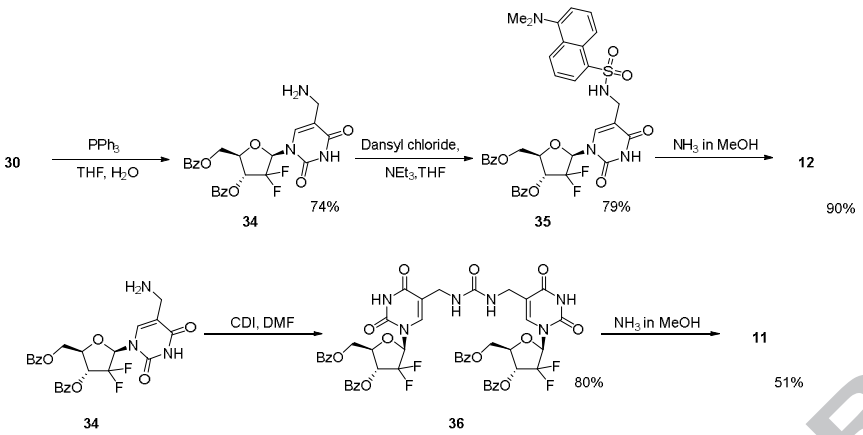
Initial efforts towards the synthesis of nucleoside **5**, by 4-toluenesulfonylation of alcohol **25** followed by displacement with fluoride, were unsuccessful, resulting in decomposition (Scheme 5). In contrast, the use of diethylaminosulfur trifluoride (DAST), which has been used to incorporate ^{18}F,[23] was successful.[24] Unexpectedly, deprotection of the benzoyl esters of diester **27** using potassium carbonate in methanol also resulted in displacement of the fluoride to form methyl ether **28** (Scheme 5).

Further attempts using ammonia in methanol and ethanol in potassium carbonate generated the corresponding amine and ethyl ether respectively. It was proposed that the enaminyl nature of the uracil system was causing activation of the primary fluoride in nucleoside **27** thereby increasing its lability and facilitating nucleophilic displacement under mild conditions to give the observed products.[25] Such an activation would also explain the instability of 4-toluenesulfonate **26**.

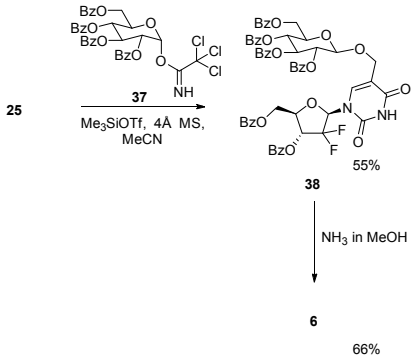


Scheme 7. Synthesis of FOT analogue **9**

Corresponding author. Tel.: +44 020 7594 5766; fax: +44 020 7594 5805.
E-mail address: agm.barrett@imperial.ac.uk (A.G.M. Barrett).



Scheme 8. Synthesis of dansylated **12** and bivalent **11** nucleoside analogues



Scheme 9. Synthesis of glycosylated nucleoside **6**

At this point it was evident that a different protecting strategy was required; however it was decided to continue the synthesis of the remaining analogues.

The azidomethyl-nucleoside **8** was easily synthesized in three steps starting from alcohol **25** (Scheme 6). Alcohol **25** was converted to the chloride, which was then substituted with sodium azide, as described by Seio *et al*, [26] to give azide **30** in a 64% yield over two steps. Subsequent debenzoylation of diester **30** gave the desired azidomethyl-nucleoside **8**.

With azide **30** readily available, the synthesis of the FOT analogue **9** was carried out (Scheme 7). Using a standard copper catalyzed azide-alkyne cycloaddition reaction with azide **30** and 3-butyn-1-ol, triazole **31** was generated in 54% yield.[10] Subsequent DAST mediated substitution of alcohol **31** gave fluoride **32**, which was deprotected under basic conditions to give nucleoside **9**. Having completed the synthesis of **9**, efforts were made to develop a route more amenable to radiochemistry. ^{18}F-DAST may be synthesized; however the specific activity of this reagent is comparatively low therefore the use of an ^{18}F fluoride metal salt is preferable.[23] As such the Huisgen cycloaddition reaction was repeated using but-3-ynyl 4-toluenesulfonate[27] to give sulfonate **33**, which is better suited to such fluoride displacement reactions.

Staudinger reduction[28] of azide **30** gave the corresponding amine **34**, which was converted into the urea **11** and sulfonamide **12** (Scheme 8). Reaction of amine **34** with dansyl chloride[29] proceeded smoothly to furnish **35**, which was deprotected to provide sulfonamide **12** in 90% yield. The symmetrical disubstituted urea **11** was synthesized by allowing two equivalents of amine **34** to react with a single equivalent of carbonyl diimidazole to generate the pseudo-di-nucleoside. Subsequent debenzoylation gave the di-nucleoside urea **11**.

The final target was the glycosylated nucleoside **6**. De Kort *et al*.[30] reported the synthesis of a similar analogue by reaction of a hydroxymethylated nucleoside using a modified D-glucose derivative. The glycosyl donor **37**, was synthesized from D-glucose according to the protocols of Egusa[31] and Mühlhausen.[32] Schmidt glycosylation of nucleoside **25** with trichloroacetimidate **37** was carried out using trimethylsilyl trifluoromethanesulfonate as the promoter and gave the fully-protected, glycosylated nucleoside **38** in good yields (Scheme 9).[30] Deprotection using ammonia in methanol gave the nucleoside **6**.

In conclusion, by adapting the methods of Zhang *et al*,[20] a reliable method for the generation of multiple gram quantities of hydroxymethylated nucleoside **25** was developed. Through the synthesis of this intermediate, the inefficient hydroxymethylation of nucleosides, which proceeds in low yields, was avoided. From the key intermediate **25** we were able to synthesize an array of diverse nucleoside analogues, which should be of potential use as imaging agents in PET and for other biomedical applications.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version.

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Corresponding author. Tel.: +44 020 7594 5766; fax: +44 020 7594 5805.
E-mail address: agm.barrett@imperial.ac.uk (A.G.M. Barrett).