


Cite this: *RSC Adv.*, 2024, 14, 37216

# Efficient convergent synthesis of 1,3-diazepinone nucleosides by ring-closing metathesis and direct glycosylation†

Adam K. Hedger,<sup>ab</sup> Jonathan Findell,<sup>ac</sup> Dinesh S. Barak,<sup>a</sup> Celia A. Schiffer,<sup>a</sup> Jonathan K. Watts<sup>\*b</sup> and Akbar Ali<sup>\*a</sup>

A new and highly efficient ring-closing metathesis-based strategy was developed for the synthesis of the cyclic urea 1,3-diazepinone, presenting significant improvement upon previous methods. Using a direct glycosylation approach, analogues of the potent cytidine deaminase (CDA) inhibitor diazepinone riboside were then synthesized including 2'-deoxyribo-, 2'-deoxy-2'-fluoroarabino-, and 2'-deoxy-2',2'-difluoro-diazepinone nucleosides, all with moderate to good yield and excellent anomeric selectivity. Crucially, in contrast to the previous multistep linear synthesis of 2'-deoxyribo- and 2'-deoxy-2'-fluoroarabino-diazepinone nucleosides, this is the first report of direct glycosylation to access these nucleosides. Overall, we report efficient convergent routes to multiple 2'-modified-diazepinone nucleosides for further evaluation as CDA and potential APOBEC3 inhibitors.

Received 12th October 2024  
Accepted 13th November 2024

DOI: 10.1039/d4ra07318e

rsc.li/rsc-advances

## Introduction

Cytidine deaminase enzyme (CDA) catalyzes the deamination of cytidine and deoxycytidine to uridine and deoxyuridine, respectively,<sup>1</sup> as a part of the pyrimidine salvage pathway.<sup>2</sup> CDA has been found to readily metabolize and deactivate pyrimidine-based anticancer and antiviral nucleoside drugs such as gemcitabine, azacytidine and decitabine, leading to resistance and poor clinical outcomes.<sup>2,3</sup> These anticancer nucleoside drugs show improved therapeutic efficacy when co-administered with a CDA inhibitor (*e.g.* cedazuridine), culminating in the FDA approval of decitabine/cedazuridine combination therapy. The APOBEC3 family of enzymes also catalyzes the deamination of cytosine (C) to uracil (U), but only in nucleic acids (DNA and RNA) rather than single nucleosides. The APOBEC3 proteins play an important role in innate immunity, but when misregulated cause somatic mutations to the human genome that lead to cancer evolution and drug resistance.<sup>4,5</sup> Recent biochemical and structural data has shown that nucleoside-based CDA inhibitors can also inhibit APOBEC3 enzymes when incorporated into short oligonucleotides.<sup>6–8</sup>

Therefore, there is significant interest in synthetically straightforward routes to modified nucleosides that can inhibit CDA and APOBEC3 enzymes.

Several riboside-based CDA inhibitors have been reported including zebularine, tetrahydrouridine, and 1,3-diazepinone riboside (Fig. 1). However, unlike the CDA enzyme, APOBEC3s do not bind single nucleosides, and prefer to bind DNA substrates rather than RNA. Therefore, CDA inhibitors must be incorporated into oligonucleotides as their 2'-deoxy counterparts to have good activity against APOBEC3 enzymes, as is the case for zebularine and 2'-deoxyzebularine (Fig. 1B). The diazepinone riboside is one of the most potent CDA inhibitors reported with a  $K_i$  of 25 nM.<sup>9</sup> The corresponding 2'-deoxy- and 2'-fluorinated-diazepinone nucleosides also potently inhibit CDA,<sup>10,11</sup> and we hypothesize that they could inhibit APOBEC3 enzymes once incorporated into oligonucleotides. Indeed, the 2'-fluorination of CDA inhibitor tetrahydrouridine has also been shown to increase stability while retaining good levels of activity, resulting in the FDA approved cedazuridine (Fig. 1C).<sup>12</sup>

The first reported synthesis of the 7-membered diazepinone nucleobase involved an undesirable and low yielding ring-closing reaction of (*Z*)-but-2-ene-1,4-diamine using carbonyl sulfide (COS) gas (Scheme 1).<sup>13</sup> A recent method reported by Kim *et al.*<sup>14</sup> avoids the use of COS gas, but still requires 5 steps with orthogonal protection and deprotection steps. This improved method utilized a ring-closing metathesis-based strategy originally developed by the Marquez laboratory for the synthesis of carbocyclic analogues of diazepinone riboside.<sup>15</sup> To access the diazepinone riboside, both reports employ a direct glycosylation strategy using mercury salts as catalysts, which are commonly used for non-aromatic cyclic-urea

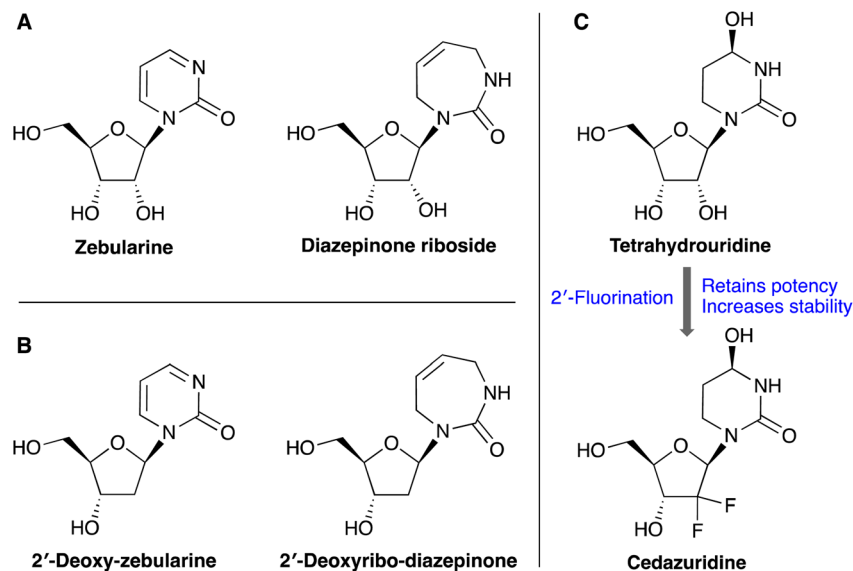
<sup>a</sup>Department of Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, Worcester, Massachusetts, 01605, USA. E-mail: Akbar.Ali@umassmed.edu; Tel: +1 508 856 8873

<sup>b</sup>RNA Therapeutics Institute, University of Massachusetts Chan Medical School, Worcester, Massachusetts, 01605, USA. E-mail: Jonathan.Watts@umassmed.edu; Tel: +1 774 455 3784

<sup>c</sup>Department of Chemistry, School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4ra07318e>





**Fig. 1** Example of chemically modified cytidine analogues: (A) ribose-based nucleoside inhibitors of cytidine deaminase (CDA). (B) Corresponding 2'-deoxy analogues which inhibit APOBEC3 enzymes when incorporated into oligonucleotides. (C) 2'-Fluorination has successfully been used to increase the stability of CDA inhibitors.

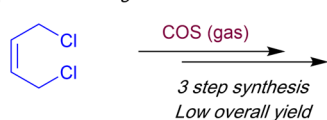
nucleobases.<sup>9,14,16</sup> However, for 2'-modified diazepinone nucleosides, including the recently reported 2'-deoxy-, and 2'-fluoroarabino diazepinone nucleosides, a linear synthetic strategy of building the nucleobase on the sugar itself is used (Scheme 1).<sup>11,17</sup> Starting from the sugar, these multistep linear routes are less efficient as the glycosylation and diazepinone ring-building is carried out early in the synthesis, with the key ring-closing

metathesis (RCM) steps resulting in only ~40% yield. Furthermore, this approach would likely require re-optimization of many steps for each new target nucleoside.

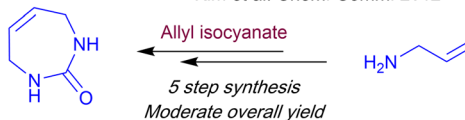
Here we report an efficient convergent route to the 1,3-diazepinone cyclic urea nucleobase and corresponding 2'-modified nucleosides. Using our approach, the 7-membered diazepinone ring can be synthesized in 3 steps utilizing RCM with 70%

**a) Reported strategies for the synthesis of 1,3-diazepinone**

Marquez *et al.* *J. Org. Chem.* 1980



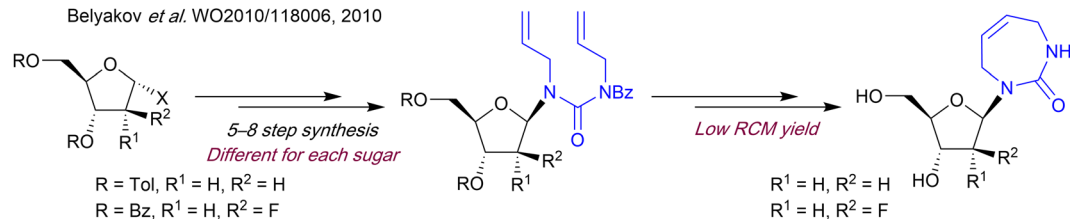
Kim *et al.* *Chem. Comm.* 2012



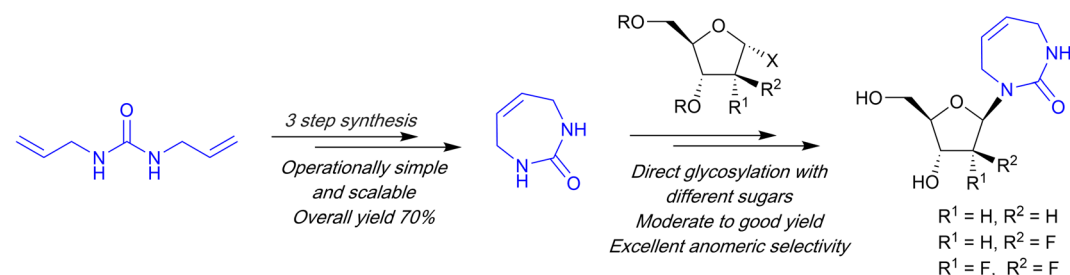
**b) Reported strategies for the synthesis of 2'-modified diazepinone nucleosides**

Kurup *et al.* *Org. Biomol. Chem.* 2023

Belyakov *et al.* WO2010/118006, 2010



**c) This work: Synthesis of 2'-modified diazepinone nucleosides by direct glycosylation**



**Scheme 1** Strategies for the synthesis of 1,3-diazepinone and 2'-deoxyribo and 2'-fluorinated diazepinone nucleosides.

overall yield. Next, we optimized a direct glycosylation approach to install the diazepinone onto various 2'-substituted sugars to provide easy access to diazepinone nucleosides in a convergent manner. In our hands the 2'-deoxy-diazepinone nucleoside shows limited stability, as reported for other non-aromatic nucleosides.<sup>18</sup> We expanded our direct glycosylation approach to access the corresponding 2'-fluoroarabino nucleoside, as well as the 2'-deoxy-2',2'-difluoro nucleoside. Crucially, 2'-fluorination improves the stability of the 2'-deoxy-nucleosides, suggesting promise for further evaluation as CDA and APOBEC3 inhibitors.

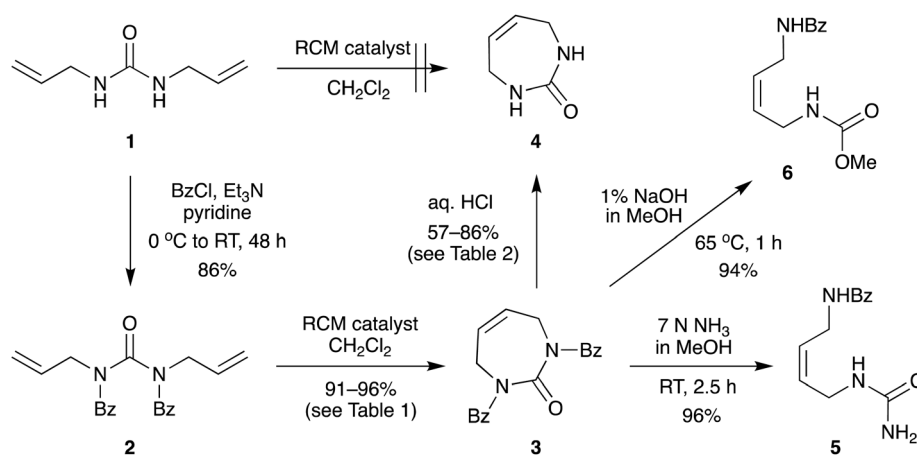
## Results and discussion

The direct glycosylation approach first involved developing an efficient route to the 7-membered diazepinone nucleobase **4**, which we envisaged could then be glycosylated directly to 2'-

modified sugars of interest. The direct RCM of unprotected diallylurea derivatives (*e.g.*, **1** to **4**, Scheme 2) does not work due to unfavorable orientation of the allyl groups,<sup>15,19</sup> as we also found. We hypothesized that a general strategy of bis-*N*-protection, RCM, and then deprotection would provide an efficient route to the target diazepinone **4** (Scheme 2), and that benzoyl protection would be a good candidate for this strategy.

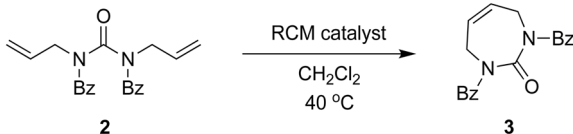
Therefore, diallylurea **1** was first bis-benzoyl (Bz) protected using excess BzCl in pyridine to form **2**. Next, RCM gave the Bz-protected diazepinone **3** in high yield, using as little as 1% RCM catalyst in anhydrous CH<sub>2</sub>Cl<sub>2</sub>. Finally, **3** was deprotected under strongly acidic conditions and recrystallized from MeOH to give the free diazepinone **4**, which was used in subsequent glycosylation reactions.

We explored optimization of the RCM step using multiple catalysts, catalyst loadings, and repeat reaction cycles in the same vessel (Table 1). We observed that the RCM reaction with



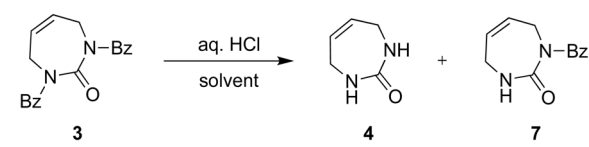
Scheme 2 Improved 3-step synthesis of 1,3-diazepinone by ring-closing metathesis.

Table 1 Optimization of ring-closing metathesis reaction conditions

						
Entry	Catalyst	Catalyst loading (mol%)	Reaction cycles <sup>a</sup>	Substrate conc. (mM)	Time	Yield <sup>b</sup> (%)
1	Grubbs 1st gen	10	1	6	2 h	90
2	Grubbs 2nd gen	10	1	6	1 h	91
3	Zhan-1b	10	1	6	1 h	93
4	Grubbs 2nd gen	5	1	6	1 h	91
5	Grubbs 2nd gen	2	1	6	1 h	96
6	Hoveyda-Grubbs 2nd gen	2	1	6	1 h	95
7	Zhan-1b	2	1	6	1 h	96
8	Zhan-1b	1	1	6	1 h	95
9	Zhan-1b	1	2	5	2 × 1 h	94
10	Zhan-1b	1	5	5	5 × 1 h	96

<sup>a</sup> Repeat cycles of substrate and catalyst addition in same vessel. <sup>b</sup> Isolated yield.



Table 2 Optimization of bis-*N*-benzoyl deprotection conditions<sup>a</sup>


Entry	[HCl]/solvent	Time (h)	Temp. (°C)	Product/yield (%)
1	6 N/THF	24	RT <sup>a</sup>	(3 major) + 7 <sup>b</sup>
2	6 N/1,4-dioxane	24	RT	(3 major) + 7 <sup>b</sup>
3	6 N/1,4-dioxane	48	RT	3 + 4 + (7 major) <sup>b</sup>
4	6 N/1,4-dioxane	24	30	3 + (7 major) <sup>b</sup>
5	6 N/1,4-dioxane	48	30	3 + 4 + (7 major) <sup>b</sup>
6	6 N/THF	24	50	4: 58%
7	6 N/1,4-dioxane	24	50	4: 57%
8	8 N/1,4-dioxane	24	RT	3 + 4 + (7 major) <sup>b</sup>
9	8 N/1,4-dioxane	48	RT	3 + 4 + (7 major) <sup>b</sup>
10	8 N/1,4-dioxane	48	45	4: 86%

<sup>a</sup> RT = 23 °C. <sup>b</sup> Monitored by TLC, products not isolated.

the Grubbs first-generation catalyst was slower compared to reactions with second-generation catalysts, although yields were similar. All reactions were high yielding, and we managed to systematically reduce catalyst loading. Optimal RCM conditions (Table 1, entry 10) used just 1 mol% catalyst, and allowed for a substantial reduction in solvent, by performing 5 repeat reaction cycles in the same flask.

Surprisingly, deprotection of the *N*-Bz groups to provide the free nucleobase **4** proved to be quite challenging. Attempted deprotection under basic conditions provided only ring opened products (Scheme 2). For example, treatment of bis-*N*-Bz-diazepinone **3** with 7 N NH<sub>3</sub> in MeOH cleanly gave the ring-opened urea derivative **5** in high yield. Similarly, reaction with 1% NaOH in MeOH also provided the ring-opened product, in this case the carbamate derivative **6**, in 94% yield. Both these conditions have previously been reported to deprotect a *N*-Bz group on the diazepinone system, albeit with orthogonal substitution of the second urea nitrogen.<sup>15,17</sup>

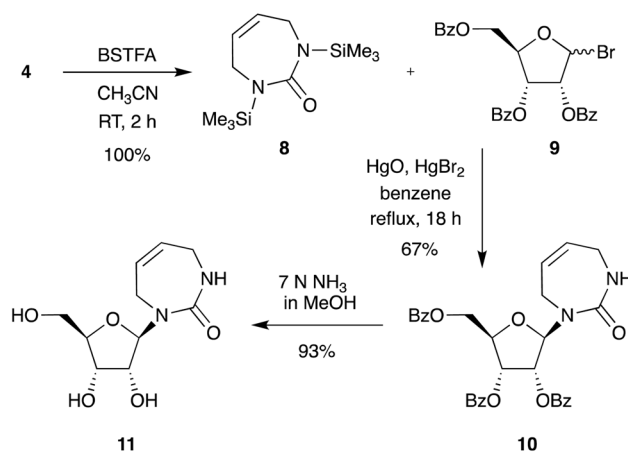
Next, we explored acidic conditions to remove the bis-Bz protection (Table 2). Reaction of **3** with aqueous 6 N HCl in THF or 1,4-dioxane at room temperature (RT) for 24 hours was unsuccessful, giving only small amounts of mono deprotected **7** (Table 2, entries 1 and 2). Extended time, or warming to 30 °C in 6 N HCl in 1,4-dioxane, gave a mixture of **4** and **7**, and unreacted **3** (Table 2, entries 3–5). Increasing the reaction temperature further to 50 °C, in either solvent gave predominantly the desired product **4** in good yield (~60%, Table 2, entries 6 and 7). These encouraging results, and the fact that the diazepinone ring remained intact, prompted us to further optimize the acid-catalyzed deprotection reaction. Next, we tried 8 N HCl in 1,4-dioxane at RT for 24 and 48 hours and observed **7** as the major product (Table 2, entries 8 and 9). Increasing the temperature to 45 °C for 48 hours gave our optimal conditions, producing fully deprotected **4** in 86% yield (Table 2, entry 10).

Overall, we present a highly efficient 3-step synthesis (70% overall yield) of the 7-membered diazepinone nucleobase. This improved method avoids the use of COS gas,<sup>9</sup> and requires fewer steps and significantly less RCM catalyst and solvent than the previous report.<sup>14</sup>

With efficient access to the free diazepinone nucleobase established, we next chose to explore synthesis routes to multiple diazepinone nucleosides. First, we repeated the synthesis of diazepinone riboside following the direct glycosylation conditions developed by Marquez *et al.* (Scheme 3).<sup>9</sup> Silylated diazepinone **8** was coupled to bromosugar **9** *via* mercury-catalyzed glycosylation in refluxing benzene, providing exclusively the β-anomer **10** in good yield (67%). The nucleoside **10** was deprotected using methanolic ammonia, and lyophilized to give the diazepinone riboside **11**. As with previous reports, we found the type and quality of mercury salts can affect the *N*-/*O*-linked glycoside ratio and must be carefully controlled.<sup>16</sup>

Next, we tried to obtain the corresponding 2'-deoxy-diazepinone nucleoside. As there is limited literature precedent for the direct glycosylation of non-aromatic nucleobases to 2'-deoxy sugars, and anticipating poor anomeric selectivity, we first opted to pursue 2'-deoxygenation of the diazepinone riboside (Scheme S1†). The Barton–McCombie deoxygenation has found widespread utility in carbohydrate synthesis, accessing novel ribonucleosides through exclusive β-anomer glycosylation selectivity, then removing the 2'-hydroxyl group to provide their 2'-deoxy counterparts.<sup>20–23</sup> To attempt 2'-deoxygenation we performed a series of protection/deprotection steps starting from protected riboside **10**, followed by deoxygenation under Barton–McCombie conditions to successfully give the fully protected target 2'-deoxy-diazepinone nucleoside **30**. However, deprotection proved troublesome and multiple conditions gave a mixture of products (Scheme S1† and associated text). These problems, along with the long synthetic scheme prompted us to explore an alternative strategy.

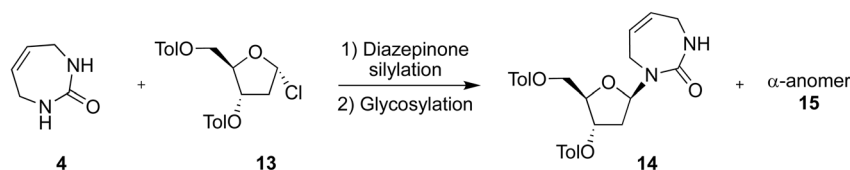
We therefore attempted direct glycosylation to obtain the target 2'-deoxy-diazepinone nucleoside. Initially, we tried the same conditions as Cristalli *et al.*<sup>10</sup> using excess *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) for silylation and



Scheme 3 Synthesis of diazepinone riboside.



Table 3 Optimization of direct glycosylation reaction conditions



Entry	Silylation conditions	Solvent/catalyst	Höffer's sugar (eq.)	Temp. (°C)	Anomeric ratio (crude) ( $\beta/\alpha$ ) <sup>a</sup>	Isolated yield (%) ( $\beta$ -anomer)
1	BSTFA (excess) <sup>a</sup> acetonitrile	CH <sub>2</sub> Cl <sub>2</sub> SnCl <sub>4</sub> (1.0 eq.)	1.1	RT	N/A	N/A
2	Et <sub>3</sub> N (1.6 eq.) TMS-Cl (1.2 eq.) benzene	ClCH <sub>2</sub> CH <sub>2</sub> Cl SnCl <sub>4</sub> (3.0 eq.)	0.85	−30	9 : 1	48
3	Et <sub>3</sub> N (2.0 eq.) TMS-Cl (1.4 eq.) benzene	ClCH <sub>2</sub> CH <sub>2</sub> Cl SnCl <sub>4</sub> (3.0 eq.)	0.85	−30	9 : 1	51
4	Et <sub>3</sub> N (1.6 eq.) TMS-Cl (1.2 eq.) benzene	ClCH <sub>2</sub> CH <sub>2</sub> Cl SnCl <sub>4</sub> (3.0 eq.)	1.2	−30	5 : 1	28
5	Et <sub>3</sub> N (1.6 eq.) TMS-Cl (1.2 eq.) benzene	ClCH <sub>2</sub> CH <sub>2</sub> Cl SnCl <sub>4</sub> (3.0 eq.)	0.85	0	10 : 1	60

<sup>a</sup> Entry 1 as per Cristalli *et al.*<sup>10</sup> <sup>b</sup> Crude anomeric ratio's measured by H NMR.

SnCl<sub>4</sub> for glycosylation. However, multiple attempts failed to provide any detectable nucleoside product in our hands. Next, we applied controlled silylation conditions,<sup>24</sup> similar to those used for coupling the acyclic urea **1** to Hoffer's chlorosugar **13** in the linear synthetic approach to access the 2'-deoxy-diazepinone nucleoside.<sup>17</sup> Gratifyingly, using similar silylation and glycosylation conditions with diazepinone **4** and Hoffer's chlorosugar **13** under general Vorbrüggen conditions (SnCl<sub>4</sub>, 1,2-dichloroethane)<sup>25</sup> yielded the desired 2'-deoxy nucleoside in a 9 : 1 anomeric ratio, providing the  $\beta$ -anomer **14** in 48% isolated yield (Table 3, entry 2). The  $\alpha$ -anomer **15** was also characterized. The anomeric identity of the  $\beta$ -anomer **14** and  $\alpha$ -anomer **15** was confirmed by 2D NOESY NMR experiments (Fig. S4 and S5†).

We then tried to optimize this glycosylation reaction (Table 3) by varying reagent amounts and temperature. First, we slightly increased the equivalents of silylation reagent, but this had little effect on crude anomeric ratio or reaction yield (Table 3, entry 3). Next, we increased the amount of Hoffer's chlorosugar to 1.2 eq., but this lowered both anomeric ratio and yield (Table 3, entry 4). Finally, we repeated entry 2 but at 0 °C rather than −30 °C, and saw a marginal increase in selectivity but moderate increase in yield, giving the target 2'-deoxy nucleoside in a 10 : 1 crude anomeric ratio, and 60% isolated yield of the  $\beta$ -anomer **14** (Table 3, entry 5).

Therefore, under controlled silylation and Vorbrüggen conditions we report the direct glycosylation of the non-aromatic diazepinone nucleobase (Scheme 4), giving access to the protected 2'-deoxy-diazepinone nucleoside **14** in excellent yield and anomeric ratio. Toluoyl-protected **14** was deprotected using aqueous ammonia in MeOH to give nucleoside **16** in 90% yield after lyophilization. However, we noticed this nucleoside appeared to be unstable in solution. <sup>1</sup>H NMR samples in D<sub>2</sub>O

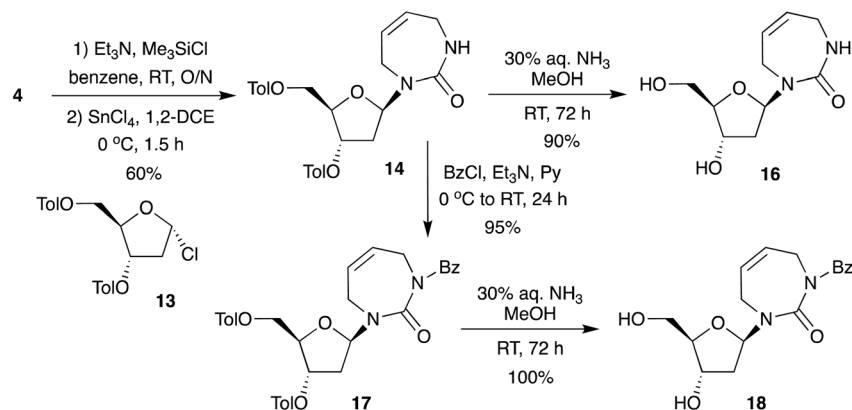
showed extra signals form within hours, clearly visible around the anomeric and 2' proton range (Fig. S1†). In addition, multiple attempts to purify by water/chloroform extraction (to remove deprotection by-products) resulted in a mixture of nucleoside products after lyophilization of the aqueous phase. Indeed, instability of non-aromatic nucleobases has been reported previously, including with 7-membered cyclic urea nucleosides.<sup>18</sup> In this process, ring-opening of the sugar *via* Schiff base formation at the glycosidic bond allows for anomeric interconversion, as well as thermodynamically driven interconversion from furanose to pyranose.<sup>11,26,27</sup> We reasoned that blocking of the diazepinone NH with an electron-withdrawing group might help to provide additional stability by reducing electron density on the glycosidic nitrogen, and so **14** was Bz-protected in quantitative yield to a form fully protected nucleoside **17**. The 5'- and 3'-toluoyl protecting groups were selectively removed under mild ammonia treatment to give *N*-Bz-protected 2'-deoxy-diazepinone nucleoside **18** (Scheme 4). While careful handling of **18** was still required, this derivative appeared more stable than **16**. Interestingly, *N*-boc-protected 2'-deoxy-diazepinone nucleoside **31** from the deoxygenation scheme (Scheme S1B†) also appeared to be stable, and the instability and acid sensitivity of **16** likely explains why we couldn't remove the *N*-boc group in Scheme S1B.†

Overall, our method provides a highly efficient route to 2'-deoxy-diazepinone nucleoside **16** in excellent yield through direct glycosylation to the sugar. This convergent synthesis over 5 steps results in overall 38% yield which is significantly improved in comparison to overall 8% yield obtained for the linear strategy recently reported.<sup>17</sup>

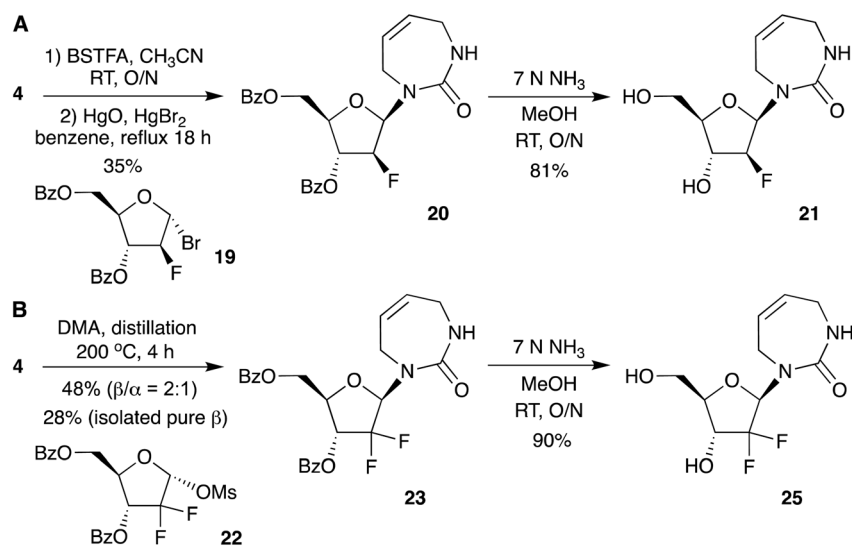
Motivated by the efficient synthesis of the protected 2'-deoxy nucleoside **14** *via* direct glycosylation, we next explored whether this strategy could also be extended to 2'-fluorinated analogues.







Scheme 4 Synthesis of 2'-deoxyribo-diazepinone nucleoside by direct glycosylation.



Scheme 5 Synthesis of fluorinated diazepinone nucleosides by direct glycosylation.

We started from commercially available 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl- $\alpha$ -D-arabinofuranose and 2-deoxy-2,2-difluoro-1-*O*-methanesulfonyl-3,5-di-*O*-benzoyl-ribo-furanose as glycosyl donors (Scheme 5).

For the 2-fluoroarabino-1-bromo derivative **19** (Scheme 5A), glycosylation did not proceed under the optimized  $\text{SnCl}_4$  catalyzed conditions that worked for Hoffer's chlorosugar (**13** to **14** in Scheme 4). However, the reaction proceeded well with the mercury-catalyzed conditions employed for glycosylation with the 1-bromo ribofuranose (**9** to **10** in Scheme 3). The 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl- $\alpha$ -D-arabinofuranose was converted to its 1-bromo derivative **19** using 30% HBr in AcOH in quantitative yield.<sup>28</sup> Bromosugar **19** was then coupled with **4** via mercury-catalyzed glycosylation in refluxing benzene to afford the protected 2'-fluoroarabino nucleoside **20** as the pure  $\beta$ -anomer in 35% isolated yield. No  $\alpha$ -anomer was detected. We also isolated the minor *O*-glycoside by-product, as observed previously for mercury-catalyzed glycosylation reactions.<sup>16</sup> Protected nucleoside **20** was then deprotected in methanolic ammonia to furnish

the 2'-fluoroarabino-diazepinone nucleoside **21**.  $^1\text{H}$  NMR studies of **21** in  $\text{D}_2\text{O}$  showed complete stability out to at least 48 hours (Fig. S2†), in contrast to 2'-deoxyribo-diazepinone **16**, which showed partial degradation within hours. The 2'-fluorination of nucleosides (as well as oligonucleotides containing them) is a known strategy to stabilize them to acidic conditions.<sup>12,29,30</sup>

Next, to access the 2'-deoxy-2',2'-difluoro nucleoside analogue, we followed the conditions reported in the patent literature (Scheme 5B).<sup>11</sup> Condensation of **4** with 2-deoxy-2,2-difluoro sugar **22** was achieved by vigorous distillation in DMA, without a catalyst. This gave protected 2'-deoxy-2',2'-difluoro diazepinone nucleoside in 48% yield with 2 : 1  $\beta$  selectivity. Separation of anomers was challenging as reported previously, but we isolated the pure  $\beta$ -anomer **23** in 28% yield. Compound **23** was deprotected using 7 N  $\text{NH}_3$  in MeOH to give nucleoside **25**, in 90% yield. Similar to 2'-fluoroarabino nucleoside **21**, we also observed that the difluoro analogue **25** was more stable than 2'-deoxyribo-diazepinone **16** (Fig. S3†).

## Conclusions

Cyclic ureas are a broadly useful class of synthetic intermediates and bioactive molecules. In particular, diazepinone-based nucleosides, which are attractive deaminase inhibitors, have proved synthetically challenging. We present efficient synthetic methods to access the 7-membered 1,3-diazepinone cyclic urea, as well as direct glycosylation approaches to access diazepinone-based nucleosides. These strategies can be applied to the synthesis of other cyclic urea derivatives, as well as other diverse nucleoside analogues by direct glycosylation with modified sugars.

We first developed an improved 3-step synthesis of 1,3-diazepinone **4** starting from readily available 1,3-diallyl urea, *via* a highly efficient RCM reaction, with 70% overall yield. Next, we pursued a convergent direct glycosylation approach to install the diazepinone on different 2'-substituted sugars. This provided direct access to the ribo- and 2'-deoxyribo-diazepinone nucleosides (**11** and **16**), as well as the more stable 2'-fluoroarabino-, and 2'-deoxy-2',2'-difluoro-diazepinone nucleosides (**21** and **25**), with excellent anomeric selectivity. Overall, our work provides efficient synthetic routes to multiple potent diazepinone-based CDA inhibitors. These compounds are also key synthetic intermediates required to synthesize diazepinone-containing oligonucleotides as potential APOBEC3 inhibitors,<sup>6–8</sup> work which is ongoing in our laboratory.

## Experimental section

### General information

All chemicals, reagents and solvents were purchased from commercial sources (Sigma-Aldrich, Fisher Scientific, Chem-Impex, Biosynth) and were used as received, unless otherwise stated. Anhydrous solvents were purchased from Sigma-Aldrich and were further dried over activated 3 Å molecular sieves. All reactions were performed in oven-dried round bottomed flasks fitted with rubber septa under argon atmosphere, unless otherwise noted. Automated flash column chromatography was performed on an Interchim PuriFlash system equipped with a UV-vis detectors using prepacked silica gel cartridges or was performed manually using silica gel (230–400 mesh, EMD Millipore). Analytical thin-layer chromatography (TLC) was performed using silica gel (60 F<sub>254</sub>) coated aluminum plates (EMD Millipore), and spots were visualized by exposure to ultraviolet light (UV), and/or stained with 10% H<sub>2</sub>SO<sub>4</sub> in ethanol or ninhydrin, followed by brief heating. <sup>1</sup>H, <sup>13</sup>C, <sup>19</sup>F, DEPT-135, and 2D NMR spectra were acquired on a Bruker Avance III HD 500 MHz NMR instrument. Chemical shifts are reported in ppm ( $\delta$  scale) relative to the solvent signal and coupling constant (*J*) values are reported in hertz. Data are presented as follows: chemical shift, multiplicity (*s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, *br* = broad, *dd* = doublet of doublets), coupling constant in Hz, and integration. Structural assignments were made using a combination of 1D and 2D NMR spectra, including gradient selected COSY, HSQC, HMBC, and NOESY experiments (particularly for nucleoside anomer assignment) as needed. High-resolution mass spectra (HRMS)

were recorded on a Thermo Scientific Orbitrap Velos Pro mass spectrometer coupled with a Thermo Scientific Accela 1250 UPLC and an autosampler using electrospray ionization (ESI) in the positive mode.

### Synthesis of 1,3-diazepinone

***N,N'*-Carbonylbis(*N*-allylbenzamide) (2).** A solution of diallyl urea **1** (5.0 g, 36 mmol) in anhydrous pyridine (140 mL) was placed under argon atmosphere and cooled to 0 °C. Et<sub>3</sub>N (25 mL, 179 mmol) was slowly added followed by drop-wise addition of benzoyl chloride (21 mL, 178 mmol) over 30 min. The resulting slurry was stirred at 0 °C for 30 min, warmed to room temperature and stirred for 2 days. The reaction mixture was concentrated under reduced pressure, and the residue was dried under high vacuum. The dark brown residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (250 mL) and washed with 2 N HCl (250 mL) and saturated aqueous NaCl solution (250 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated to provide an orange solid. The solid was dissolved in a minimum volume of CH<sub>2</sub>Cl<sub>2</sub>/hexanes (1 : 1) mixture and subjected to flash chromatography, initially eluting with the same mixture and then with CH<sub>2</sub>Cl<sub>2</sub>. Fractions containing the product were pooled and evaporated to provide a pale-yellow solid. Recrystallization from EtOAc/hexanes (1 : 5) mixture provided the bis-benzoylated diallyl urea **2** (10.7 g, 86%) as colorless needles. TLC: *R*<sub>f</sub> = 0.65 (25% EtOAc/hexanes).

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.55–7.50 (m, 2H, H-Bz), 7.41 (apparent d, *J* = 4.4 Hz, 8H, H-Bz), 5.89–5.81 (m, 2H, H-5, H-8), 5.20 (dd, *J* = 17.1, 1.3 Hz, 2H, H-6/H-9), 5.15 (dd, *J* = 10.2, 1.2 Hz, 2H, H-6/H-9), 3.98 (d, *J* = 6.1 Hz, 4H, H-4, H-7) ppm; <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  171.04 (2C, Bz-C=O), 158.39, (C=O, C-2), 135.49 (2C, C-Ph), 132.44 (2C, CH-5, CH-8), 132.13 (2C, CH-Ph), 128.75 (4C, CH-Ph), 128.05 (4C, CH-Ph), 118.99 (2C, CH-6, CH-9), 50.10 (2C, CH<sub>2</sub>-4, CH<sub>2</sub>-7) ppm; HRMS (ESI): *m/z* Calcd. for C<sub>21</sub>H<sub>21</sub>N<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup> 349.1547; found 349.1536.

**(2-Oxo-4,7-dihydro-1*H*-1,3-diazepine-1,3(2*H*)-diyl)bis(phenylmethanone) (3).** Compound **2** (2.50 g, 7.20 mmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (1.5 L) and this solution degassed using 10 × fill cycles of vacuum and argon. The solution was then heated to 40 °C under an argon atmosphere. After 15 min, Zhan 1b catalyst (0.053 g, 0.072 mmol, 0.01 eq) was then added to the solution, which turned pale green. The solution was stirred for 1 h at which point TLC indicated reaction had reached completion (*R*<sub>f</sub> starting material = 0.67, *R*<sub>f</sub> product = 0.51, 25% EtOAc/hexanes, visualized by UV). Further compound **2** (2.50 g, 7.20 mmol) was added to the reaction mixture and fully dissolved before addition of more Zhan 1b catalyst (0.053 g, 0.072 mmol, 0.01 eq). The reaction mixture was stirred for another 1 h and the addition of compound **2** followed by Zhan 1b catalyst repeated three further times (total 12.5 g, 35.9 mmol of compound **2** added) with the final addition cycle being stirred for 1.5 h to allow full reaction completion, confirmed by TLC (*R*<sub>f</sub> product = 0.50, 25% EtOAc/hexanes, visualized by UV). The reaction mixture went from pale green to brown over the course of the reaction. The solvent was evaporated *in vacuo* to a small volume (~100 mL) and filtered



through a pad of silica gel (10 × 4 cm) and the pad was further eluted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL). The combined fractions were evaporated *in vacuo* to provide a light brown solid, which was recrystallized overnight from EtOAc/hexanes (9:1) mixture, providing the pure bis-benzoylated 1,3-diazepinone **3** (9.40 g) as a white crystalline solid. The filtrate was evaporated *in vacuo* and the solid residue was recrystallized, giving additional pure product (1.60 g) as a white crystalline solid. Total yield: 11.0 g, 96%. TLC: *R*<sub>f</sub> = 0.50 (25% EtOAc/hexanes).

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.53–7.50 (m, 4H, H-Bz), 7.46–7.43 (m, 2H, H-Bz), 7.38–7.35 (m, 4H, H-Bz), 6.00 (t, *J* = 2.3 Hz, 2H, H-5, H-6), 4.82 (d, *J* = 2.2 Hz, 4H, H-4, H-7) ppm; <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 172.11 (2C, Bz-C=O), 159.88 (C=O, C-2), 134.94 (2C, C-Ph), 132.03 (2C, CH-5, CH-6), 128.60 (4C, CH-Ph), 127.71 (4C, CH-Ph), 126.20 (2C, CH-Ph), 42.93 (2C, CH<sub>2</sub>-4, CH<sub>2</sub>-7) ppm; HRMS (ESI) *m/z*: Calcd. for C<sub>19</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup> 321.1234; found 321.1227.

**1,3,4,7-Tetrahydro-2H-1,3-diazepin-2-one (4).** A solution of *N,N'*-bis-benzoyl-1,3-diazepinone **3** (2.75 g, 8.58 mmol) in 1,4-dioxane (20 mL) was cooled in an ice-water bath (1,4-dioxane starts to freeze around 10 °C) and 8 N HCl (10 mL) was slowly added. The resulting white slurry was stirred at 10 °C for 5 min, allowed to warm to room temperature and then slowly heated to 45 °C and stirred for 48 h; the reaction mixture became a clear solution overnight. The reaction mixture was cooled to 0 °C and neutralized to pH 7 by slow addition of aqueous 4 N NaOH. The resulting mixture was evaporated under reduced pressure to yield a white solid, which was further dried under high vacuum. The solid residue was triturated with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1) mixture (3 × 200 mL) and filtered. The filtrate was passed through a short pad of silica gel (8 × 4 cm) and the pad was further eluted with the same solvent mixture (3 × 100 mL). The combined fractions were evaporated *in vacuo* to provide the product as a white crystalline solid, which was contaminated with the by-product benzoic acid. Recrystallization from MeOH provided the pure 1,3-diazepinone **4** (0.83 g, 86%) as a white crystalline solid. TLC: *R*<sub>f</sub> = 0.60 (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ 5.95 (s, 2H, H-1, H-3), 5.76 (t, *J* = 2.7 Hz, 2H, H-5, H-6), 3.53–3.51 (m, 4H, H-4, H-7) ppm; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 5.80 (t, *J* = 2.8 Hz, 2H), 4.84 (br s, 2H), 3.74 (s, 4H) ppm; <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 126 MHz): δ 164.44 (C=O, C-2), 127.35 (2 × CH, CH-5, CH-6), 40.36 (2 × CH<sub>2</sub>, CH<sub>2</sub>-4, CH<sub>2</sub>-7) ppm; <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 165.65, 126.90 (2C), 41.70 (2C) ppm; HRMS (ESI) *m/z*: Calcd. for C<sub>5</sub>H<sub>9</sub>N<sub>2</sub>O [M + H]<sup>+</sup> 113.0709, found 113.0704.

### Synthesis of 2'-deoxyribo-diazepinone nucleoside

**((2R,3S,5R)-3-((4-Methylbenzoyl)oxy)-5-(2-oxo-2,3,4,7-tetrahydro-1H-1,3-diazepin-1-yl)tetrahydrofuran-2-yl)methyl 4-methylbenzoate (14).** A mixture of diazepinone **4** (0.72 g, 6.42 mmol) in anhydrous benzene (20 mL) at room temperature and under argon was treated with Et<sub>3</sub>N (1.45 mL, 10.4 mmol) followed by Me<sub>3</sub>SiCl (1.0 mL, 7.87 mmol). The reaction mixture was stirred under argon overnight. The resulting white precipitate was filtered off with a sintered funnel, washed with benzene (3 × 3 mL), and the filtrate solvents evaporated under

reduced pressure, keeping the bath at room temperature. The silylated product was obtained as a clear oil (1.18 g), which was used immediately in the glycosylation reaction with minimal handling.

The above silylated diazepinone compound (1.18 g, 6.42 mmol) was dissolved in 1,2-DCE (45 mL) to give a colorless solution and cooled to 0 °C using an ice bath. Freshly distilled SnCl<sub>4</sub> (2.0 mL, 17 mmol) was added dropwise, followed by Hoffer's chlorosugar **13** (2.20 g, 5.60 mmol). This gave a clear yellow solution which was stirred at 0 °C for 1.5 h, at which point the mixture had turned dark brown. Pyridine (7.5 mL) and water (40 mL) were added, and the reaction mixture was stirred at room temperature for 1 h. After diluting with water (50 mL), the reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 150 mL). The combined organic fractions were filtered through a bed of Celite, washed with saturated aqueous NaCl (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to dryness. The resulting dark brown residue was dissolved in a minimum volume of CH<sub>2</sub>Cl<sub>2</sub> and purified by flash column chromatography using a silica gel column (SiliaSep, 40 g, gradient elution with 0–20% acetone/CH<sub>2</sub>Cl<sub>2</sub>) to give the pure β-anomer compound **14** (1.58 g, 60%) as a white foamy solid. TLC: *R*<sub>f</sub> = 0.40 (8% acetone/CH<sub>2</sub>Cl<sub>2</sub>).

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.94–7.92 (m, 4H, H-Ph), 7.26–7.23 (m, 4H, H-Ph, overlapping), 6.17 (dd, *J* = 9.1, 5.8 Hz, 1H, H-1'), 5.70–5.66 (m, 1H, H-5/6), 5.65–5.60 (m, 1H, H-5/6), 5.53–5.50 (m, 1H, H-3'), 4.65 (dd, *J* = 11.9, 3.6 Hz, 1H, H-5'), 4.54 (dd, *J* = 11.9, 3.7 Hz, 1H, H-5'), 4.50 (br. s, 1H, H-3), 4.37 (dd, *J* = 6.2, 3.6 Hz, 1H, H-4'), 3.84–3.77 (m, 2H, H-4, H-7), 3.71–3.63 (m, 2H, H-4, H-7), 2.42 (s, 6H, CH<sub>3</sub>-Tol), 2.31–2.20 (m, 2H, H-2') ppm; <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 166.31 (C=O-Tol), 166.30 (C=O-Tol), 165.57 (C-2), 144.36 (C-Ph), 144.17 (C-Ph), 129.92 (2 × CH-Ph), 129.72 (2 × CH-Ph), 129.39 (2 × CH-Ph), 129.32 (2 × CH-Ph), 127.27 (CH-5/6), 127.14 (C-Ph), 126.80 (C-Ph), 125.96, (CH-5/6), 86.71 (CH-1'), 80.88 (CH-4'), 75.16 (CH-3'), 64.68 (CH<sub>2</sub>-5'), 43.58 (CH<sub>2</sub>-7), 40.11 (CH<sub>2</sub>-4), 35.20 (CH<sub>2</sub>-2'), 21.84 (CH<sub>3</sub>-Tol), 21.83 (CH<sub>3</sub>-Tol) ppm; HRMS (ESI) *m/z*: Calcd. for C<sub>26</sub>H<sub>29</sub>N<sub>2</sub>O<sub>6</sub> [M + H]<sup>+</sup> 465.2020; found 465.2018.

**1-((2R,4S,5R)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,3,4,7-tetrahydro-2H-1,3-diazepin-2-one (16).** Compound **14** (0.33 g, 0.71 mmol) was dissolved in MeOH (30 mL) and aqueous NH<sub>4</sub>OH (3 mL) was added. The reaction vessel was tightly sealed and stirred gently at room temperature for 72 h. TLC (*R*<sub>f</sub> starting material = 0.92, *R*<sub>f</sub> product = 0.25, 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, both visualized by UV and ninhydrin stain) indicated reaction completion. Solvents were evaporated and the solid residue dissolved in water (5 mL) and immediately flash frozen in liquid N<sub>2</sub> and lyophilized. The solid residue was redissolved in H<sub>2</sub>O (5 mL), filtered through a syringe filter, immediately flash frozen and lipolyzed to provide the target nucleoside **16** (0.15 g, 90%) as an off-white foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 6.00–5.95 (m, 1H, H-5/6), 5.97 (dd, *J* = 8.3, 6.4 Hz, 1H, overlapping, H-1'), 5.93–5.88 (m, 1H, H-5/6), 4.33 (dt, *J* = 6.7, 3.4 Hz, 1H, H-3'), 3.86–3.72 (m, 6H), 3.69 (dd, *J* = 12.2, 5.4 Hz, 1H, H-4'), 2.20 (ddd, *J* = 15.2, 8.2, 7.2 Hz, 1H, H-2'), 2.03 (ddd, *J* = 14.2, 6.4, 3.2 Hz, 1H, H-2'); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 6.02 (br s, 1H, NH), 5.82–5.77 (m, 1H, H-5/6), 5.77 (dd, *J* = 8.6, 6.1 Hz, 1H, overlapping, H-1'), 5.73–5.69 (m, 1H, H-



5/6), 5.0 (br s, 1H), 4.73 (br s, 1H), 4.05 (br d,  $J = 2.8$  Hz, 1H, H-3'), 3.67–3.49 (m, 5H), 3.46–3.30 (m, 2H), 1.86 (ddd,  $J = 15.0$ , 8.5, 6.5 Hz, 1H, H-2'), 1.71 (ddd,  $J = 13.2$ , 6.1, 2.7 Hz, 1H, H-2');  $^{13}\text{C}$  NMR (126 MHz,  $\text{D}_2\text{O}$ )  $\delta$  166.02 (C-2), 128.05 (CH-5/6), 126.80 (CH-5/6), 86.30 (CH-1'), 84.65 (CH<sub>2</sub>-4'), 70.92 (CH-3'), 61.68 (CH<sub>2</sub>-5'), 41.75 (CH<sub>2</sub>-4/7), 39.89 (CH<sub>2</sub>-4/7), 36.06 (CH<sub>2</sub>-2'); HRMS (ESI):  $m/z$  Calcd. for  $\text{C}_{10}\text{H}_{17}\text{N}_2\text{O}_4$  [ $\text{M} + \text{H}$ ]<sup>+</sup> 229.1183; found 229.1180.

**(2R,3S,5R)-5-(3-Benzoyl-2-oxo-2,3,4,7-tetrahydro-1H-1,3-diazepin-1-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate (17).** A solution of diazepinone derivative **14** (0.47 g, 1.0 mmol) in anhydrous pyridine (12 mL) was cooled to 0 °C under argon atmosphere. Et<sub>3</sub>N (0.45 mL, 3.23 mmol) was slowly added followed by drop-wise addition of benzoyl chloride (0.25 mL, 2.15 mmol). The resulting reaction mixture was warmed to room temperature and stirred for 48 h. Solvents were evaporated under reduced pressure, and after drying under high vacuum, the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (50 mL) and washed with saturated aqueous NaCl solution (50 mL). The organic portion was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and evaporated. The resulting yellow residue was dissolved in a minimum volume of  $\text{CH}_2\text{Cl}_2$  and purified by flash column chromatography using a silica gel column (Siliasep, 25 g, gradient elution with 10–80% EtOAc/hexanes) to provide the *N*-Bz protected compound **17** (0.54 g, 95%) as a pale-yellow solid. TLC:  $R_f = 0.50$  (35% EtOAc in hexanes).

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.97 (d,  $J = 8.0$  Hz, 2H, H-Ph), 7.88 (d,  $J = 8.0$  Hz, 2H, H-Ph), 7.56–7.53 (m, 2H, H-Ph), 7.48–7.44 (m, 1H, H-Ph), 7.40–7.35 (m, 2H, H-Ph), 7.29 (d,  $J = 8.0$  Hz, 2H, H-Ph), 7.22 (d,  $J = 8.0$  Hz, 2H, H-Ph), 6.19 (dd,  $J = 9.4$ , 5.4 Hz, 1H, H-1'), 5.79 (dt,  $J = 10.8$ , 3.3 Hz, 1H, H-5), 5.70–5.65 (m, 1H, H-6), 5.56–5.54 (m, 1H, H-3'), 4.74 (dd,  $J = 12.0$ , 3.3 Hz, 1H, H-5'), 4.65 (br d,  $J = 18.5$  Hz, 1H, H-4), 4.57 (dd,  $J = 12.0$ , 3.6 Hz, 1H, H-5'), 4.35 (q,  $J = 3.1$  Hz, 1H, H-4'), 4.31 (br d,  $J = 18.5$  Hz, 1H, H-4), 4.10–4.06 (m, 1H, H-7), 3.96 (dd,  $J = 17.0$ , 5.0 Hz, 1H, H-7), 2.45 (s, 3H, CH<sub>3</sub>-Tol), 2.40 (s, 3H, CH<sub>3</sub>-Tol), 2.31 (ddd,  $J = 14.0$ , 5.4, 1.5 Hz, 1H, H-2'), 2.18 (ddd,  $J = 14.1$ , 9.4, 6.6 Hz, 1H, H-2') ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.05 (C=O-Bz), 166.23 (C=O-Tol), 166.20 (C=O-Tol), 159.71 (C=O-C-2), 144.53 (C-Ph), 144.45 (C-Ph), 135.22 (C-Ph), 131.62 (CH-Ph), 129.86 (2C, CH-Ph), 129.68 (2 × CH-Ph), 129.52 (2 × CH-Ph), 129.34 (2 × CH-Ph), 128.57 (2 × CH-Ph), 128.32 (CH-5/6), 127.38 (2 × CH-Ph), 126.98 (C-Ph), 126.52 (C-Ph), 124.03 (CH-5/6), 85.64 (CH-1'), 81.52 (CH-4'), 74.94 (CH-3'), 64.34 (CH<sub>2</sub>-5'), 43.27 (CH<sub>2</sub>-4), 39.29 (CH<sub>2</sub>-7), 35.44 (CH<sub>2</sub>-2'), 21.85 (CH<sub>3</sub>-Tol), 21.82 (CH<sub>3</sub>-Tol) ppm; HRMS (ESI):  $m/z$  Calcd. for  $\text{C}_{33}\text{H}_{32}\text{N}_2\text{O}_7\text{Na}$  [ $\text{M} + \text{Na}$ ]<sup>+</sup> 591.2102; found 591.2102.

**1-Benzoyl-3-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,3,4,7-tetrahydro-2H-1,3-diazepin-2-one (18).** The protected diazepinone 2-deoxyribose **17** (0.57 g, 1.0 mmol) was dissolved in MeOH (50 mL) at room temperature and treated with 30% aqueous ammonia solution (5 mL). The reaction flask was sealed, and the suspension was stirred at room temperature for 72 h. After the reaction was complete, solvents were evaporated under reduced pressure, keeping the bath temperature at 25 °C. The residue was co-evaporated from  $\text{H}_2\text{O}$  (5 mL) to remove the methyl toluate and then lyophilized

from  $\text{H}_2\text{O}$  (5 mL) to provide the *N*-Bz protected nucleoside derivative **18** (0.34 g, 100%) as an off-white solid. TLC: 10% MeOH in  $\text{CH}_2\text{Cl}_2$ ;  $R_f = 0.50$ .

$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  7.65–7.61 (m, 1H, H-Ph), 7.58–7.56 (m, 2H, H-Ph), 7.54–7.51 (m, 2H, H-Ph), 6.03–5.96 (m, 1H, H-5/6), 5.99 (dd,  $J = 8.0$ , 6.5 Hz, 1H, overlapping, H-1'), 5.89–5.85 (m, 1H, H-5/6), 4.48 (br s, 2H, H-4/7), 4.38 (td,  $J = 6.6$ , 3.2 Hz, 1H, H-3'), 4.20 (br d,  $J = 3.7$  Hz, 2H, H-4/7), 3.90 (ddd,  $J = 7.5$ , 5.1, 3.8 Hz, 1H, H-4'), 3.78 (dd,  $J = 12.3$ , 4.0 Hz, 1H, H-5'), 3.72 (dd,  $J = 12.3$ , 5.2 Hz, 1H, H-5'), 2.20 (ddd,  $J = 14.4$ , 7.8, 6.6 Hz, 1H, H-2'), 2.07 (ddd,  $J = 14.1$ , 6.3, 3.2 Hz, 1H, H-2') ppm;  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.56–7.53 (m, 2H), 7.46–7.44 (m, 1H), 7.40–7.37 (m, 2H), 5.95 (t,  $J = 7.1$  Hz, 1H), 5.91–5.87 (m, 1H), 5.83–5.80 (m, 1H), 4.57–4.53 (m, 1H), 4.38–4.35 (m, 1H), 4.38–4.35 (m, 1H), 4.14–4.09 (m, 1H), 4.07–4.02 (m, 1H), 3.80 (dd,  $J = 7.5$ , 3.7 Hz, 1H), 3.75 (m, 1H), 3.71 (dd,  $J = 11.5$ , 3.7 Hz, 1H), 2.08 (dt,  $J = 13.8$ , 7.3 Hz, 1H), 2.01 (ddd,  $J = 13.7$ , 6.6, 3.8 Hz, 1H) ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  172.90 (C=O-Bz), 160.29 (C-2), 133.85 (C-Ph), 132.21 (CH-Ph), 128.94 (2 × CH-Ph), 127.02 (CH-Ph), 126.86 (2 × CH-Ph), 123.86 (CH-Ph), 85.79 (CH-1'), 85.74 (CH-4'), 70.84 (CH-3'), 61.50 (CH<sub>2</sub>-5'), 43.61 (CH<sub>2</sub>-4), 39.71 (CH<sub>2</sub>-7), 36.43 (CH<sub>2</sub>-2') ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.05 (C=O-Bz), 159.53 (C-2), 135.18 (C-Ph), 131.62 (CH-Ph), 128.57 (2 × CH-Ph), 128.23 (CH-Ph), 127.48 (2 × CH-Ph), 124.26 (CH-Ph), 86.47 (CH-1'), 85.74 (CH-4'), 71.69 (CH-3'), 62.77 (CH<sub>2</sub>-5'), 43.36 (CH<sub>2</sub>-4), 41.04 (CH<sub>2</sub>-7), 38.14 (CH<sub>2</sub>-2') ppm; HRMS (ESI):  $m/z$  Calcd. for  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{Na}$  [ $\text{M} + \text{Na}$ ]<sup>+</sup> 355.1264; found 355.1265.

### Synthesis of 2'-fluorinated diazepinone nucleosides

**((2R,3R,4S,5R)-3-(Benzoyloxy)-4-fluoro-5-(2-oxo-2,3,4,7-tetrahydro-1H-1,3-diazepin-1-yl)tetrahydrofuran-2-yl)methyl benzoate (20).** To a suspension of the 1,3-diazepinone **4** (0.50 g, 4.46 mmol) in anhydrous  $\text{CH}_3\text{CN}$  (20 mL) was added excess BSTFA (5.60 mL) and the mixture was stirred at room temperature for 2 h. Solvent and excess reagent were removed by evaporation under reduced pressure keeping the bath temperature at 20 °C, and the residue was dried under high vacuum for 1 h to provide the persilylated cyclic urea **8** as a clear oil.

A solution of the above persilylated cyclic urea **8** in dry benzene (10 mL) was rapidly added to a refluxing mixture of  $\text{HgO}$  (1.80 g) and  $\text{HgBr}_2$  (1.80 g) in dry benzene (110 mL) under dry  $\text{N}_2$  atmosphere. After 10 min, a solution of 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl- $\alpha$ -D-arabinofuranosyl bromide **19** in dry benzene (10 mL) was added and refluxing continued for 18 h. After cooling to room temperature, the reaction mixture was filtered through a pad of Celite, and the filter cake was washed with EtOAc. The combined filtrates were washed with saturated aqueous  $\text{NaHCO}_3$  (2 × 200 mL) and water (200 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography using a silica gel column (Siliasep, 80 g, gradient elution with 0–100% EtOAc/hexanes) to provide the protected nucleoside **20** (0.72 g, 35%) as a white foamy solid. TLC:  $R_f = 0.35$  (60% EtOAc/hexanes).

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.10–8.03 (m, 4H, Ph), 7.63–7.55 (m, 2H, Ph), 7.48–7.43 (m, 4H, Ph), 5.99 (dd,  $J = 25.6$ ,



3.3 Hz, 1H, H-1'), 5.99 (dd,  $J = 25.6, 3.3$  Hz, 1H, H-1'), 5.80–5.75 (m, 1H, H-6), 5.73–5.69 (m, 1H, H-5), 5.57 (dd,  $J = 18.9, 3.3$  Hz, 1H, H-3'), 5.23 (dd,  $J = 50.8, 3.2$  Hz, 1H, H-2'), 4.75 (dd,  $J = 11.9, 4.0$  Hz, 1H, H-5'), 4.67 (dd,  $J = 11.9, 4.7$  Hz, 1H, H-5'), 4.48 (t,  $J = 3.0$  Hz, 1H, NH), 4.32 (q,  $J = 4.1$  Hz, 1H, H-4'), 4.01–3.91 (m, 2H, H-7), 3.85–3.75 (m, 2H, H-4) ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.36 (C), 165.44 (C), 164.72 (C), 133.98 (CH-Ph), 133.36 (CH-Ph), 130.05 ( $2 \times$  CH-Ph), 129.90 ( $2 \times$  CH-Ph), 129.81 (C-Ph), 128.75 ( $2 \times$  CH-Ph), 128.58 ( $2 \times$  CH-Ph), 127.09 (CH-6), 126.84 (CH-5), 95.0 (d,  $J = 190.9$  Hz, CH-2'), 86.2 (d,  $J = 16.0$  Hz, CH-1'), 79.06 (CH-4'), 77.3 (d,  $J = 30.3$  Hz, CH-3'), 63.76 ( $\text{CH}_2$ -5'), 43.52 ( $\text{CH}_2$ -4), 42.0 (d,  $J = 7.3$  Hz,  $\text{CH}_2$ -7) ppm;  $^{19}\text{F}$  NMR (470 MHz,  $\text{CDCl}_3$ ):  $\delta$  -201.05 ppm; HRMS (ESI):  $m/z$  Calcd. for  $\text{C}_{24}\text{H}_{24}\text{FN}_2\text{O}_6$   $[\text{M} + \text{H}]^+$  455.1613; found 455.1612.

**1-((2*R*,3*S*,4*R*,5*R*)-3-Fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (21).** Compound **20** (0.39 g, 0.86 mmol) was treated with a solution of  $\text{NH}_3$  in MeOH (7 N, 40 mL). The reaction vessel was tightly sealed and stirred at room temperature for 18 h. Solvent was evaporated, and the residue dissolved in a minimum volume of 5% MeOH/ $\text{CH}_2\text{Cl}_2$  mixture and purified by flash column chromatography (Siliasep, 12 g, equilibrated with 1%  $\text{Et}_3\text{N}$  in  $\text{CH}_2\text{Cl}_2$ , gradient elution with 0–10% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to provide the protected nucleoside. The product was dissolved in  $\text{H}_2\text{O}$  (5 mL), flash frozen in liquid  $\text{N}_2$  and lyophilized overnight. The lyophilization was repeated four times to remove  $\text{Et}_3\text{N}$ , giving the target nucleoside as a glassy translucent solid **21** (0.17 g, 81%).

$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  6.00–5.96 (m, 1H, H-5), 5.93–5.90 (m, 1H, H-4), 5.88 (dd,  $J = 18.1, 5.2$  Hz, 1H, overlapping, H-1'), 5.07 (ddd,  $J = 53.0, 5.1, 3.7$  Hz, 1H, H-2'), 4.33 (ddd,  $J = 22.8, 6.3, 3.7$  Hz, 1H, H-3'), 3.96–3.85 (m, 3H, H-5', H-7), 3.82–3.76 (m, 4H, H-4', H-5', H-3) ppm;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  6.10 (t,  $J = 2.9$  Hz, 1H), 5.79–5.75 (m, 1H), 5.73–5.69 (m, 1H), 5.63 (dd,  $J = 21.2, 4.6$  Hz, 1H), 5.63 (d,  $J = 5.1$  Hz, 1H, overlapping), 4.88–4.85 (m, 1.5H), 4.77 (dd,  $J = 4.6, 3.0$  Hz, 0.5H), 4.03 (ddd,  $J = 22.5, 7.9, 5.0$  Hz, 1H), 3.76–3.66 (m, 2H), 3.60–3.54 (m, 3H), 3.53–3.47 (m, 2H) ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  165.44 (C=O, C-2), 127.88 (CH-4/5), 127.28 (CH-4/5), 96.95 (d,  $J = 190.5$  Hz, CH-2'), 84.36 (d,  $J = 16.5$  Hz, CH-1'), 80.08 (d,  $J = 6.1$  Hz, CH-4'), 73.98 (d,  $J = 24.7$  Hz, CH-3'), 60.46 ( $\text{CH}_2$ -5'), 41.88 (d,  $J = 6.1$  Hz,  $\text{CH}_2$ -7), 41.65 ( $\text{CH}_2$ -4) ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  163.89 (C=O, C-2), 127.27 (CH-4/5), 126.86 (CH-4/5), 97.86 (d,  $J = 189.4$  Hz, CH-2'), 84.18 (d,  $J = 16.3$  Hz, CH-1'), 81.45 (d,  $J = 5.0$  Hz, CH-4'), 74.01 (d,  $J = 23.7$  Hz, CH-3'), 60.60 ( $\text{CH}_2$ , C-5'), 41.90 ( $\text{CH}_2$ -4), 41.69 (d,  $J = 6.2$  Hz,  $\text{CH}_2$ -7), ppm;  $^{19}\text{F}$  NMR (470 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  -197.70 ppm; HRMS (ESI):  $m/z$  Calcd. for  $\text{C}_{10}\text{H}_{16}\text{FN}_2\text{O}_4$   $[\text{M} + \text{H}]^+$  247.1089; found 247.1090.

**1-((2*R*,3*R*,5*R*)-3-(Benzoyloxy)-4,4-difluoro-5-(2-oxo-2,3,4,7-tetrahydro-1*H*-1,3-diazepin-1-yl)tetrahydrofuran-2-yl)methyl benzoate (23).** A two-neck round-bottom flask was charged with diazepinone **4** (0.24 g, 2.14 mmol) and 3,5-di-*O*-benzoyl-2-deoxy-2,2-difluoro-1-*O*-methanesulfonyl- $\alpha$ - $\text{D}$ -ribofuranoside (Biosynth) (1.60 g, 3.50 mmol), and anhydrous DMA (20 mL). The flask was fitted with a distillation condenser and a rubber septum sealed with Teflon tape. The reaction mixture was heated at 200 °C while the DMA slowly distilled out. After about half of the DMA

was distilled out, additional diazepinone (0.96 g, 8.56 mmol) dissolved in warm DMA (70 mL) was slowly added over 2 h, while DMA was continuously distilled out slowly. After the addition was complete, the reaction mixture was refluxed for 30 min, and then cooled to room temperature. The solvent was evaporated under reduced pressure and the residue was dried under high vacuum. The residue was dissolved in a minimum volume of  $\text{CH}_2\text{Cl}_2$  and purified by flash column chromatography using a silica gel column (Siliasep, 40 g, gradient elution with 10–100% EtOAc/hexanes) to provide the nucleoside as a mixture of  $\alpha$ - and  $\beta$ -anomers ( $\beta/\alpha = 2:1$ ) (0.79 g, 48%) as a pale-yellow viscid solid. This material was further purified by flash column chromatography using a silica gel column (Siliasep, 40 g, gradient elution with 10–20%  $\text{CH}_3\text{CN}$ /toluene) to provide the pure  $\beta$ -nucleoside **23** (0.46 g, 28%) as a clear viscid solid. TLC:  $R_f = 0.30$  ( $\beta$ -anomer),  $R_f = 0.28$  ( $\alpha$ -anomer) (12%  $\text{CH}_3\text{CN}$ /toluene).

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.08–8.03 (m, 4H, Ph), 7.63–7.60 (m, 1H, Ph), 7.58–7.55 (m, 1H, Ph), 7.48–7.45 (m, 2H, Ph), 7.44–7.41 (m, 2H, Ph), 5.99 (dd,  $J = 11.8, 10.7$  Hz, 1H, H-1'), 5.76–5.69 (m, 2H, H4 and H5), 5.55 (ddd,  $J = 13.7, 6.4, 5.5$  Hz, 1H, H-3'), 4.78 (dd,  $J = 12.2, 3.6$  Hz, 1H, H-5'), 4.68 (br s, 1H, NH), 4.57 (dd,  $J = 12.2, 4.2$  Hz, 1H, H-5'), 4.39 (dt,  $J = 7.1, 3.6$  Hz, 1H, H-4'), 3.94–3.80 (m, 3H, H-3/7), 3.73–3.68 (m, 1H, H3/7) ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  166.11 (C=O-Bz), 165.06 (C=O-Bz), 164.86 (C-2), 134.12 (CH-Ph), 133.51 (CH-Ph), 130.22 ( $2 \times$  CH-Ph), 129.81 ( $2 \times$  CH-Ph), 129.53 (C-Ph), 128.76 ( $2 \times$  CH-Ph), 128.64 ( $2 \times$  CH-Ph), 128.38 (C-Ph), 127.01 (CH-4/5), 126.16 (CH-4/5), 121.66 (t,  $J = 260.3$  Hz, C-2'), 86.25 (dd,  $J = 37.4, 20.6$  Hz, CH-1'), 75.81 (d,  $J = 5.4$  Hz, CH-4'), 72.08 (dd,  $J = 32.5, 17.7$  Hz, CH-3'), 62.91 ( $\text{CH}_2$ -5'), 43.35 ( $\text{CH}_2$ -4), 42.31 (d,  $J = 5.1$  Hz,  $\text{CH}_2$ -7) ppm;  $^{19}\text{F}$  NMR (470 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  -115.43 (d,  $J = 247.0$ ), -116.75 (d,  $J = 246.9$ ) ppm; HRMS (ESI):  $m/z$  Calcd. for  $\text{C}_{24}\text{H}_{23}\text{F}_2\text{N}_2\text{O}_6$   $[\text{M} + \text{H}]^+$  473.1519; found 473.1523.

**1-((2*R*,4*R*,5*R*)-3,3-Difluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (25).** Compound **23** (0.24 g, 0.50 mmol) was treated with a solution of  $\text{NH}_3$  in MeOH (7 N, 30 mL). The reaction vessel was tightly sealed and stirred at room temperature for 18 h. Solvent was evaporated, and the residue dissolved in a minimum volume of 3% MeOH/ $\text{CH}_2\text{Cl}_2$  mixture and purified by flash column chromatography (Siliasep, 12 g, equilibrated with 0.2%  $\text{Et}_3\text{N}$  in  $\text{CH}_2\text{Cl}_2$ , gradient elution with 0–10% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to provide the protected nucleoside. The product was dissolved in  $\text{H}_2\text{O}$  (3 mL), flash frozen in liquid  $\text{N}_2$  and lyophilized overnight. The lyophilization was repeated giving the target nucleoside as a white solid **25** (0.12 g, 90%).

$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  5.99–5.92 (m, 1H, H-4, H-5), 5.78 (dd,  $J = 14.6, 6.1$  Hz, 1H, H-1'), 4.25 (ddd,  $J = 20.3, 11.6, 8.7$  Hz, 1H, H-3'), 3.96 (d,  $J = 11.8$  Hz, 1H, H-5'), 3.87–3.77 (m, 6H, H-4', H5', H-4, H-7) ppm;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  6.41 (t,  $J = 3.1$  Hz, 1H), 6.02 (dd,  $J = 6.6$  Hz, 1H), 5.81–5.75 (m, 2H), 5.60 (dd,  $J = 14.5, 7.3$  Hz, 1H), 4.97 (t,  $J = 5.4$  Hz, 1H), 4.02–3.93 (m, 1H), 3.72–3.51 (m, 7H) ppm;  $^{13}\text{C}$  NMR (126 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  164.36 (C=O, C-2), 127.13 (CH-4/5), 126.02 (CH-4/5), 122.00 (dd,  $J = 261.0, 252.4$  Hz, CH-2'), 85.81 (dd,  $J = 41.5, 22.3$  Hz, CH-1'), 77.23 (d,  $J = 8.9$  Hz, CH-4'), 69.30 (d,  $J = 27.5, 18.5$  Hz, CH-3'),



58.75 (CH<sub>2</sub>-5'), 40.82 (d, *J* = 4.0 Hz, CH<sub>2</sub>-7), 40.59 (CH<sub>2</sub>-4) ppm; <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>): δ 163.45 (C=O, C-2), 127.62 (CH-4/5), 126.58 (CH-4/5), 123.70 (dd, *J* = 259.6, 253.2 Hz, CH-2'), 85.85 (dd, *J* = 39.8, 22.0 Hz, CH-1'), 78.84 (d, *J* = 9.0 Hz, CH-4'), 69.87 (d, *J* = 26.7, 18.5 Hz, CH-3'), 59.48 (CH<sub>2</sub>-5'), 41.62 (CH<sub>2</sub>-4), 41.25 (d, *J* = 3.0 Hz, CH<sub>2</sub>-7) ppm; <sup>19</sup>F NMR (470 MHz, DMSO-*d*<sub>6</sub>): δ -113.68 (d, *J* = 237.0), -115.16 (d, *J* = 237.2) ppm; HRMS(ESI): *m/z* Calcd. for C<sub>10</sub>H<sub>15</sub>F<sub>2</sub>N<sub>2</sub>O<sub>4</sub> [M + H]<sup>+</sup> 265.0994; found 265.0996.

## Data availability

The data underlying this study are available in the published article and its online ESI.†

## Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was supported by a grant from the National Institute of Allergy and Infectious Diseases of the NIH (R01 AI150478). AKH was supported by a fellowship from the PhRMA foundation.

## References

- 1 T. Cacciamani, A. Vita, G. Cristalli, S. Vincenzetti, P. Natalini, S. Ruggieri, A. Amici and G. Magni, *Arch. Biochem. Biophys.*, 1991, **290**, 285–292.
- 2 A. Frances and P. Cordelier, *Mol. Ther.*, 2020, **28**, 357–366.
- 3 C. Serdjebi, G. Milano and J. Ciccolini, *Expert. Opin. Drug Metab. Toxicol.*, 2015, **11**, 665–672.
- 4 M. Petljak, L. B. Alexandrov, J. S. Brammeld, S. Price, D. C. Wedge, S. Grossmann, K. J. Dawson, Y. S. Ju, F. Iorio, J. M. C. Tubio, C. C. Koh, I. Georgakopoulos-Soares, B. Rodriguez-Martin, B. Otlu, S. O'Meara, A. P. Butler, A. Menzies, S. G. Bhosle, K. Raine, D. R. Jones, J. W. Teague, K. Beal, C. Latimer, L. O'Neill, J. Zamora, E. Anderson, N. Patel, M. Maddison, B. L. Ng, J. Graham, M. J. Garnett, U. McDermott, S. Nik-Zainal, P. J. Campbell and M. R. Stratton, *Cell*, 2019, **176**, 1282–1294.
- 5 S. Revathidevi, A. K. Murugan, H. Nakaoka, I. Inoue and A. K. Munirajan, *Cancer Lett.*, 2021, **496**, 104–116.
- 6 M. V. Kvach, F. M. Barzak, S. Harjes, H. A. M. Schares, G. B. Jameson, A. M. Ayoub, R. Moorthy, H. Aihara, R. S. Harris, V. V. Filichev, D. A. Harki and E. Harjes, *Biochemistry*, 2019, **58**, 391–400.
- 7 A. Maiti, A. K. Hedger, W. Myint, V. Balachandran, J. K. Watts, C. A. Schiffer and H. Matsuo, *Nat. Commun.*, 2022, **13**, 7117.
- 8 A. K. Hedger, W. Myint, J. M. Lee, D. Suchenski-Loustaunau, V. Balachandran, A. M. Shaqra, N. Kurt-Yilmaz, J. K. Watts, H. Matsuo and C. A. Schiffer, *bioRxiv*, 2024, preprint, 2024.2009.2005.611238, DOI: [10.1101/2024.09.05.611238](https://doi.org/10.1101/2024.09.05.611238).
- 9 P. S. Liu, V. E. Marquez, J. S. Driscoll, R. W. Fuller and J. J. McCormack, *J. Med. Chem.*, 1981, **24**, 662–666.
- 10 G. Cristalli, R. Volpini, S. Vittori, E. Camaioni, G. Rafaiani, S. Potenza and A. Vita, *Nucleosides Nucleotides*, 1996, **15**, 1567–1580.
- 11 S. Belyakov, B. Duvall, D. Ferraris, G. Hamilton and M. Vaal, *WO2010/118006*, 2010.
- 12 D. Ferraris, B. Duvall, G. Delahanty, B. Mistry, J. Alt, C. Rojas, C. Rowbottom, K. Sanders, E. Schuck, K.-C. Huang, S. Redkar, B. B. Slusher and T. Tsukamoto, *J. Med. Chem.*, 2014, **57**, 2582–2588.
- 13 V. E. Marquez, P. S. Liu, J. A. Kelley and J. S. Driscoll, *J. Org. Chem.*, 1980, **45**, 485–489.
- 14 M. Kim, K. Gajulapati, C. Kim, H. Y. Jung, J. Goo, K. Lee, N. Kaur, H. J. Kang, S. J. Chung and Y. Choi, *Chem. Commun.*, 2012, **48**, 11443–11445.
- 15 O. R. Ludek, G. K. Schroeder, C. Liao, P. L. Russ, R. Wolfenden and V. E. Marquez, *J. Org. Chem.*, 2009, **74**, 6212–6223.
- 16 V. E. Marquez, P. S. Liu and J. K. Linevsky, *J. Org. Chem.*, 1982, **47**, 1712–1717.
- 17 H. M. Kurup, M. V. Kvach, S. Harjes, G. B. Jameson, E. Harjes and V. V. Filichev, *Org. Biomol. Chem.*, 2023, **21**, 5117–5128.
- 18 J. A. Kelley, J. S. Driscoll, J. J. McCormack, J. S. Roth and V. E. Marquez, *J. Med. Chem.*, 1986, **29**, 2351–2358.
- 19 N. Gimeno, P. Formentín, J. H. G. Steinke and R. Vilar, *Eur. J. Org. Chem.*, 2007, **2007**, 918–924.
- 20 G. Vasquez, G. C. Freestone, W. B. Wan, A. Low, C. L. De Hoyos, J. Yu, T. P. Prakash, M. E. Østergaard, X.-h. Liang and S. T. Crooke, *Nucleic Acids Res.*, 2021, **49**, 1828–1839.
- 21 P. M. J. Jung, A. Burger and J.-F. Biellmann, *J. Org. Chem.*, 1997, **62**, 8309–8314.
- 22 H. Ueda and Y. Ueno, *Bioorg. Med. Chem.*, 2022, **60**, 116690.
- 23 H. Liu, J. Gao and E. T. Kool, *J. Org. Chem.*, 2005, **70**, 639–647.
- 24 K. Ebenryter-Olbinska, J. Karolak-Wojciechowska and E. Sochacka, *Carbohydr. Res.*, 2014, **392**, 7–15.
- 25 U. Niedballa and H. Vorbruggen, *J. Org. Chem.*, 1974, **39**, 3654–3660.
- 26 Y. Jian, G. Lin, L. Chomicz and L. Li, *J. Am. Chem. Soc.*, 2015, **137**, 3318–3329.
- 27 T.-X. Xiang, R. Niemi, P. Bummer and B. D. Anderson, *J. Pharm. Sci.*, 2003, **92**, 2027–2039.
- 28 C. J. Wilds and M. J. Damha, *Nucleic Acids Res.*, 2000, **28**, 3625–3635.
- 29 J. K. Watts, A. Katolik, J. Viladoms and M. J. Damha, *Org. Biomol. Chem.*, 2009, **7**, 1904–1910.
- 30 V. E. Marquez, C. K. H. Tseng, H. Mitsuya, S. Aoki, J. A. Kelley, H. Ford Jr, J. S. Roth, S. Broder, D. G. Johns and J. S. Driscoll, *J. Med. Chem.*, 1990, **33**, 978–985.

