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Cyclization reactions of 1-[3′-hydroxy-2′-(hydroxymethyl)prop-1′-enyl]pyrimidine nucleobases: intramolecular Michael additions to the C(5)=C(6) bonds and intramolecular dehydrations

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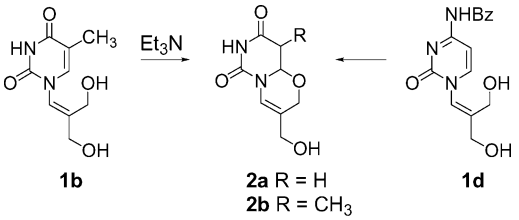
The tendency of a series of acyclic nucleoside analogues **1a**–**f** to undergo intramolecular cyclization reactions was investigated. All compounds, when treated with NaOD, were in equilibrium with the bicyclic compounds **2a**–**f**, arising from Michael addition of a hydroxy group to the C(5)=C(6) bonds. Derivatives of 2,4-pyrimidinediones (**1a**,**b**) had the highest tendency to undergo intramolecular Michael addition when treated with triethylamine, whereas the cyclization of 4-amino-2-pyridones (**1c**–**f**) proceeded best with acid. The exocyclic double bond of **1** was essential for the cyclization to occur. Commonly used *N*-protecting groups as the benzoyl- and the dibutylaminomethylene group enhanced cyclization. Under acidic anhydrous conditions **1b** and **1e** cyclized to the 2,4′-anhydro compounds **3b** and **3e**.

Introduction

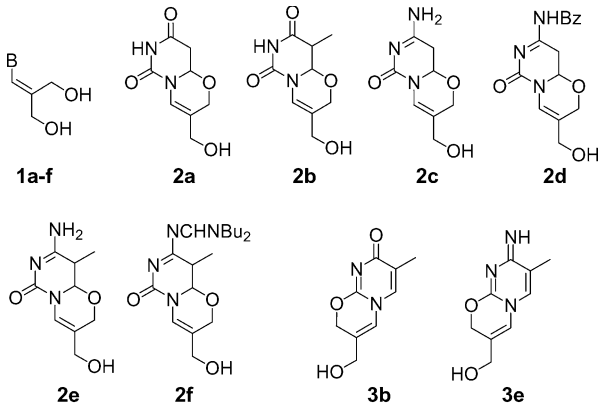
Analogues of nucleosides and oligonucleotides are of current interest as potential antiviral and antitumor agents. In a search for new compounds of this type we prepared an acyclic, achiral nucleoside analogue 1-[3′-hydroxy-2′-(hydroxymethyl)prop-1′-enyl]thymine **1b** (Scheme 1), conformationally restricted by an enamide bond.[1] Molecular modeling and geometry calculations indicated that **1b** could a good candidate, *e.g.* be able to mimic thymidine in both A- and B-type helices.[2] However, the binding affinity of oligonucleotides containing **1b** towards complementary DNA and RNA strands was reduced compared to native DNA (ΔT_m −2 to −6.5 °C per modification).[3] Furthermore, the coupling efficiency (phosphoramidite chemistry) of the next monomer following the modification was quite low (80–85% DMT efficiency). In order to investigate the potential of this type of nucleoside analogue in more detail, we prepared a series of analogues and found, unexpectedly, that the cytosine derivative **1d** formed the bicyclic uracil derivative **2a** during synthesis, and that the thymine derivative **1b** formed the bicyclic compound **2b** when treated with triethylamine (Scheme 1).[4]



Scheme 1 Michael reactions of **1b** and **1d**.

This type of intramolecular Michael addition could, at least partly, be responsible for the reduced coupling efficiency mentioned above, and we decided to make a detailed investigation of the tendency of the compounds **1** (all *N*(1)-substituted pyrimidine nucleobases) to form **2**. During this investigation we found that **1b** also formed another type of bicyclic compound **3b** when treated with trifluoroacetic acid under anhydrous conditions (Fig. 1).

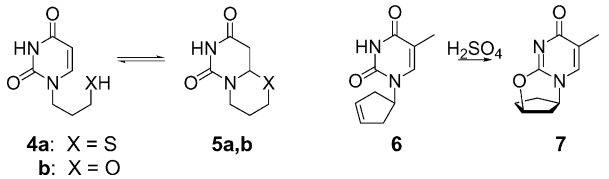
Many reactions of pyrimidine nucleobases are initiated by addition of a nucleophilic reagent to the C(5)=C(6) bonds, *e.g.* the bisulfite ion catalyzed deamination of cytosine and



a B = uracil
b B = thymine
c B = cytosine
d B = *N*-4-benzoylcytosine
e B = 5-methylcytosine
f B = *N*-4-(dibutylaminomethylene)-5-methylcytosine

Fig. 1

deuterium labeling at C(5) in uracil and cytosine derivatives.[5] Intramolecular additions are known for compounds with –SH as the nucleophile, *e.g.* **4a** (Scheme 2)[6] and 5′-thiouridine.[7] For nucleobases with –OH as the nucleophile, *e.g.* **4b** and uridine, only the open form has been observed, although incorporation of deuterium at C(5) in the nucleobases indicates an equilibrium with the cyclonucleosides, *e.g.* **5b**.[8] The formation of **2a** is to our knowledge the first example where a cyclonucleoside has been isolated with –OH as the nucleophile. 2,2′-, 2,3′- and 2,5′-anhydropyrimidine nucleosides are intermediates in



Scheme 2

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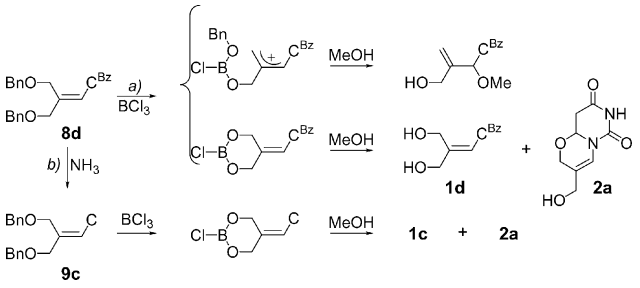
modifications of the nucleosides and have often been isolated. They have been prepared by a variety of methods, usually by conversion of the 2′-, 3′- or 5′-OH to a good leaving group.[9] An example from the carbocyclic analogues with a carbocation as the active species is the formation of **7** when **6** was treated with conc. H_2SO_4 (Scheme 2).[10]

In this paper we describe the synthesis of the new uracil analogue **1a**, an improved synthesis of the cytosine derivative **1c**, and a detailed investigation of the tendency of the analogues **1a**–**f** to cyclize to the bicyclic compounds **2a**–**f**. Examples of cyclization of **1** to the 2,4′-anhydro compounds **3** are given, and the relevance of these reactions for the use of **1** as modified nucleosides in oligonucleotide syntheses is discussed.

Results and discussion

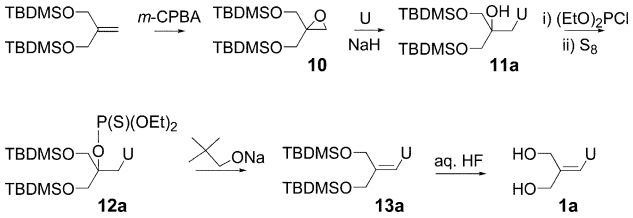
Synthesis of 1a and 1c

The cytosine analogue **1c** was prepared previously[4] from **1d**. The latter was obtained from **8d** *via* route *a*, as shown in Scheme 3. The yield was low (35%, **1d**) due to cyclization and deamination to **2a** and formation of an allylic carbocation (shown in Scheme 3) upon treatment with boron trichloride. The synthesis has now been improved by removing the *N*-benzoyl group before treatment with boron trichloride (route *b*). Cyclization could not be totally avoided, but **2a** was the only byproduct.



Scheme 3

The new uracil analogue **1a** could not be efficiently prepared by the same route, since the main product was **2a**, but **1a** could be synthesized by a similar pathway (Scheme 4) where *t*-butyldimethylsilyl (TBDMS) was used as protection group instead of benzyl. TBDMS could be removed under much milder conditions (HF in $MeCN–H_2O$ at rt) than the benzyl group. The other reactions proceeded as previously described,[1,4] but the elimination step to give **13a** was much slower with TBDMS as protecting group, probably due to steric interference. After eleven days at rt, the reaction was *ca.* 95% complete, according to ^{1}H NMR on a neutralised sample. When the reaction was run at 78 °C the starting material was consumed after 12 h, but the yield was reduced to less than 10%.

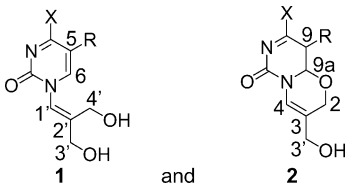


Scheme 4

Cyclization reactions

All experiments were performed in NMR tubes and the reactions followed by ^{1}H NMR spectroscopy, in most cases in a mixture of CD_3OD and DMSO-d_6 (5 : 2). NMR analysis is feasible since the spectra of compounds **1** and **2** are rather different; essential NMR data are given in Table 1. Cyclization data are summarized in Table 2, given as $t_{\frac{1}{2}}$ for incorporation of deuterium at C(5) for **1a**,**c**,**d** and at C(9) for **2a**,**b**, and as % **2a**–**f** after 1 and 24 h reaction at 22–25 °C. The $t_{\frac{1}{2}}$ values are a measure of how fast the equilibrium is established. Incorporation of deuterium in the starting material at C(5) indicates that the cyclization is reversible. The dibenzyl protected uracil derivative **9a** (Fig. 2), prepared in the same way as the thymine analogue,[1] did not incorporate deuterium at C(5) under similar conditions, *i.e.* a free hydroxy group at C(4′) is essential. The cyclization-opening

Table 1 Essential NMR data[a] for compounds



	X	R	**1** C(6)H	**2** C(9a)H	**1** C(1′)H	**2** C(4)H	**1** C(3′)H	**2** C(3′)H	**1** C(4′)H	**2** C(2)H	**1** C(5)Me	**2** C(9)Me
a	OH	H	7.55 d[b]	5.12 dd[c]	6.53 br s	7.07 s	4.20 d	4.01 AB	4.06 s	4.34 AB		
			$J_{5,6}$ 7.9	J_{AX} 5.7, J_{BX} 9.8			$J_{1',3'}$ 1.2	J_{AB} 13.8		J_{AB} 15.8		
b	OH	Me	7.39 q	4.75 d[d]	6.49 t	7.06 s	4.19 d	4.00 AB	4.05 s	4.34 AB	1.84 d	1.33 d[d]
			J 1.2	$J_{9,9a}$ 10.0	$J_{1',3'}$ 1.5		$J_{1',3'}$ 1.5	J_{AB} 13.6		J_{AB} 15.7	*J* 1.2	*J* 7.0
c	NH_{2x}	H	7.56 d[b]	5.01 d[e]	6.62 br s	7.10 s	4.19 d	3.98 br s	4.03 s	4.30 AB		
			$J_{5,6}$ 7.3	$J_{9,9a}$ 5.6			$J_{1',3'}$ 1.3			J_{AB} 15.9		
d	NHBz	H	8.02 d[b]	4.95 s[f]	6.74 br s	7.02 s	4.25 d	4.00 br s	4.07 s	4.33 AB		
			$J_{5,6}$ 7.3				$J_{1',3'}$ 1.7			J_{AB} 15		
e[g]	NH_2	Me	7.78q	4.78 s[f]	6.58 br s	7.15 s	4.28 d	3.94 br s	4.13 s	4.36 AB	2.07 d	1.32 s[f]
			J 1.2				$J_{1',3'}$ 1.5			J_{AB} 15.5	*J* 1.2	
f[h]	$NCHNBu_2$	Me	7.91q	4.69 d[d]	6.63 br s	7.07 s	4.25 d	4.01 br s	4.07 s	4.37 AB	2.03 d	1.03 s[f]
			J 1.2	$J_{9,9a}$ 10.3			$J_{1',3'}$ 1.5			J_{AB} 15.5	*J* 1.2	

[a] δ_H (CD_3OD : DMSO-d_6, 5 : 2 v/v). C(5)H in **1** and C(9)H in **2** are deuterated in most experiments; the spin coupling between H and D is not resolved. The data for **2b**, **2e** and **2f** are for the main isomer (*trans*). [b] d → s when deuterated at C(5). [c] dd → s when deuterated at C(9). [d] d → s when deuterated at C(9). [e] Monodeuterated (*trans*) at C(9). [f] Deuterated at C(9). [g] δ_H (CD_3OD : DMSO-d_6 : TFA-d, 5 : 2 : 1 v/v/v). [h] δ_H ($CDCl_3$: DMSO-d_6 : TFA-d, 5 : 2:0.1 v/v/v).

Table 2 Data for cyclization of compounds **1**, opening of **2a** and **2b**, and $t_{\frac{1}{2}}$ for incorporation of deuterium at C(5) of **1** and C(9) of **2**, obtained by integration of characteristic ^{1}H NMR signals of **1** and **2**[a]

	TFA (10 or 100 µl)			Et_3N (20 µl)			NaOD (10 µl 40% in D_2O)		
		Mol% **2** after			Mol% **2** after			Mol% **2** after	
Compound	$t_{\frac{1}{2}}$/min	1 h	24 h	$t_{\frac{1}{2}}$/min	1 h	24 h	$t_{\frac{1}{2}}$/min	1 h	24 h
1a	–[b]	<5[c]	31	20	92	93	<5	55	54[d]
2a	–[b]	100[c]	92	5	93	93	<5	55	51[d]
1b		0[c]	0		10	37		5	5
$\mathbf{1b}_{DMT}$					20, 27[e]	38, 65[e]			
2b		100[c]	100	135	33	36	<10	5	5
1c	120[d]	42[f]	>50[g]	180	12	16	<5	32	18[h]
1d		100[f,g]		<10	61	<10[i]		<5[i]	
1e		<3[c]	6[j]		0	0		<5	<5
1f		21[f,k]	85		0	0		<5	<5[l]

[a] *Ca.* 3×10^{-2} min CD_3OD : DMSO-d_6, 5 : 2 v/v, with addition of TFA-d (10 µl or 100 µl), Et_3N (20 µl) or NaOD (10 µl 40% in D_2O). [b] No deuterium incorporated. [c] 100 µl TFA-d. [d] Decomposition is observed. [e] In $CDCl_3$. [f] 10 µl TFA-d. [g] Decomposition and deamination to **2a** are observed. [h] A mixture of **1a** (11%), **2a** (13%), **1c** (57%) and **2c** (18%) is observed. [i] Decomposition and solvolysis are observed, the main product being **1c**. [j] 44% after 1 week. [k] In $CDCl_3$: DMSO-d_6, 5 : 2 v/v. [l] Decomposition and solvolysis are observed, the main product being **1e**.

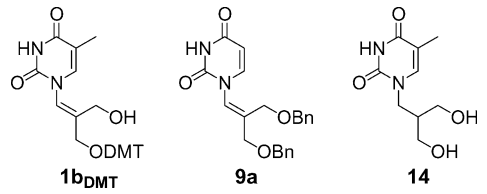


Fig. 2

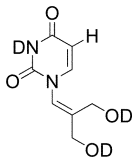
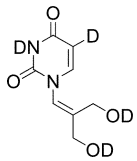
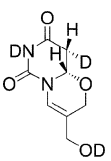
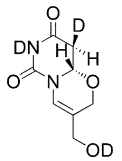
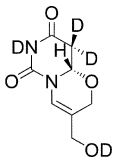
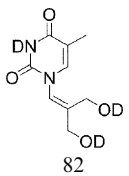
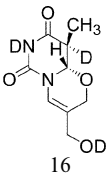
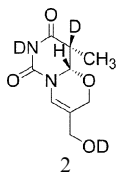
reactions cannot be completely stereoselective *cis–cis* or *trans–trans*, since no deuterium would be incorporated in this case. Incorporation of deuterium in the products **2** (Tables 3 and 4) allows deduction about the mechanism, since *cis* addition must give products with C(9)H and C(9a)H *cis* (*J* 5–6) and *trans* addition products with C(9)H and C(9a)H *trans* (*J* 10). The 5-methyl analogues give products with different δ values for *cis* and *trans* isomers.

The exocyclic double bond of **1** is essential for the cyclization to occur. When a saturated analogue[2] **14** (Fig. 2) was treated with NaOD, Et_3N or TFA in the same way as **1** no bicyclic compound was observed, as was the case with a similar compound **4b**.[8] The exocyclic double bond apparently preorganizes **1** sufficiently for cyclization to occur by placing the OH group close to the C(5)=C(6) bond (confirmed by NOE[2]).

In general, derivatives of 2,4-pyrimidinediones (**1a**,**b**) have the highest tendency to undergo intramolecular Michael addition when treated with triethylamine, whereas the cyclization of 4-amino-2-pyridones (**1c**–**f**) proceeds best with acid, the active species probably being the *N*(3) protonated form. The additions are not completely stereoselective, but predominantly *cis*, contrary to the addition of bisufite which is *trans*.[5] Derivatives of uracil and cytosine undergo cyclization much faster than the 5-methyl analogues, probably due to less steric hindrance. Four types of experiments were performed in CD_3OD : DMSO-d_6 (5 : 2): addition of NaOD, of Et_3N, of TFA-d, and no addition. During synthesis of oligonucleotides modified with **1**,[3] the DMT protecting groups are removed with CCl_3COOH in CH_2Cl_2 thus making cyclization possible; *i.e.* experiments with addition of acid are simulating the conditions during oligonucleotide synthesis.

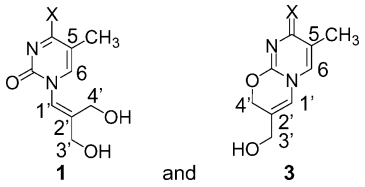
1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]uracil (1a). As expected from attempts to prepare **1a** this compound cyclizes easily to **2a**. When a solution of **1a** in CD_3OD : DMSO-d_6 (5 : 2) is treated with NaOD or Et_3N an equilibrium with **2a** is rapidly established (Tables 2 and 3). Deuterium is incorporated at C(5) in **1a**, the addition is mainly *cis*, but two deuterium atoms are rapidly incorporated at C(9) of **2a**. The product distribution

Table 3 Product distribution for cyclization of **1a** and **1b**[a]

					Reaction condtitions
24	34	5	0	37	NaOD[b]
17	4	51	1	27	Et_3N[c]
69	0	19	7	5	TFA-d[d]
52	4	17	23	4	No addition[e]
					
82		16	2		Et_3N[f]

[a] % in CD_3OD (500 µl) + DMSO-d_6 (200 µl). [b] After 5 min with addition of NaOD (10 µl 40% in D_2O). [c] After 5 min with addition of Et_3N (20 µl). [d] After 24 h with addition of TFA-d (100 µl). [e] After 24 h (no addition). [f] After 2 h with addition of Et_3N (20 µl).

Table 4 ^{1}H NMR data[a] for compounds



1 and **3**

	C(6)H		C(1′)H		C(3′)H		C(4′)H		C(5)Me	
	1	**3**	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**
b	7.26 q *J* 1.2	7.29 q *J* 1.5	6.54 br s	6.86 s	4.54 br s	4.99 s	4.41 s	5.06	2.01 d *J* 1.2	2.03 d *J* 1.5
e	7.50 q *J* 1.2	7.52 s	6.56 br s	6.84 s	4.52 d $J_{1',3'}$ 1.2	4.97 s	4.40 s	5.06 s	2.13 d *J* 1.2	2.16 s

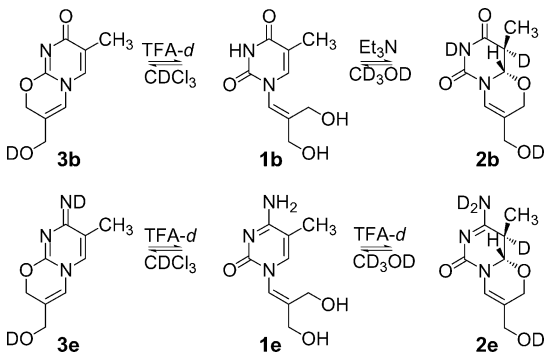
[a] δ_H ($CDCl_3$: TFA-d, 5 : 2 v/v)

after 5 min is shown in Table 3. Treatment with TFA also caused cyclization of **1a**, but with a marked difference in the rate and product distribution. The reaction is much slower, much more *trans* isomer is formed, and no deuterium is incorporated at C(5) of the starting material, indicating that equilibrium has not been established. This cyclization is not necessarily effected solely by TFA. A solution of **1a** in CD_3OD : DMSO is not stable, **2a** is formed but with *trans* as the main isomer. When solutions of the bicyclic compound **2a** were treated with NaOD or Et_3N **1a** was formed, deuterium was incorporated, and the same ratio **1a** : **2a** was observed as when **1a** was the starting material; *i.e.* an equilibrium has been established. On the other hand, **2a** is stable in CD_3OD : DMSO-d_6 (5 : 2), also after addition of TFA, and deuterium is not incorporated.

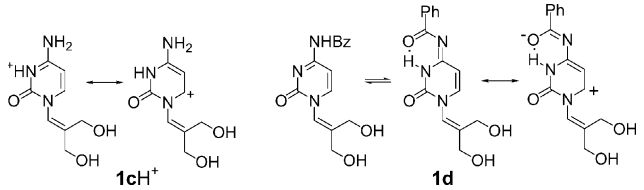
1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]thymine (1b). Compound **1b** is, like **1a**, in equilibrium with **2b** when treated with NaOD or Et_3N in CD_3OD : DMSO-d_6 (5 : 2) (Table 2). The reaction rate and the position of the equilibrium are independent of the concentration of Et_3N and the cyclization is not completely stereoselective, but *cis* addition is predominant. A typical product distribution is shown in Table 3. The same ratio **1b** : **2b** and *cis* : *trans* **2b** was observed when pure *trans* **2b**[4] was treated with NaOD or Et_3N, confirming that an equilibrium has been established. Both **1b** and **2b** are stable in CD_3OD : DMSO-d_6 (5 : 2) also after addition of TFA and deuterium is not incorporated at C(9) of **2b**. Mono-DMT protected **1b** (**1b**$_{DMT}$,[3] Fig. 2) was treated with Et_3N both in CD_3OD : DMSO-d_6 (5 : 2) and in $CDCl_3$, and it was found that more **2b**$_{DMT}$ was formed in the less polar solvent (Table 2).

The thymine nucleoside analogue (**1b**) did not cyclize with acid in CD_3OD : DMSO-d_6 (5 : 2), but this solvent system is a poor mimic of the acid treatment during oligonucleotide synthesis which is performed in dichloromethane and the experiment was therefore repeated in $CDCl_3$: TFA-d (5 : 2). Surprisingly, a new compound **3b** was formed. The transformation was nearly complete after 3 days, but the product was sensitive to water and partly reversed to **1b**, and only a mixture of **1b** and **3b** could be isolated. The compound is assigned the structure **3b** based on ^{1}H NMR data (given in Table 4), and the assignment was confirmed by FAB$^+$. The different cyclization reactions are shown in Scheme 5. Two mechanisms may be proposed for the formation of **3**. The compounds **1** could be in equilibrium with the TFA esters and the trifluoroacetate group thus acting as leaving group, or the reacting species could be the stabilized allylic cation. The instability of the compounds **3** supports the carbocation hypothesis.

1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]cytosine (1c) and *N*-4-benzoyl-1-[3′-hydroxy-2′-(hydroxymethyl)prop-1′-enyl]-cytosine (1d). Compound **1c**, like the uracil analogue **1a**, partly cyclizes to **2c** when a solution in CD_3OD : DMSO-d_6 (5 : 2) is treated with NaOD, Et_3N or TFA-d, the latter being most effective. The N(3) protonated form is probably the active species in the presence of TFA (Scheme 6). The 8-amino compound **2c** is formed initially but deamination to the uracil derivative **2a** is observed upon standing. Deuterium is incorporated at C(5) of **1c** and the main product is the *cis* isomer as for the other analogues (Table 2 and 5). Pure **2c** could not be isolated due to deamination to **2a**.



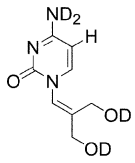
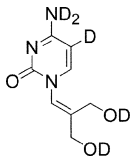
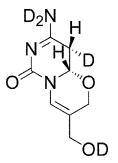
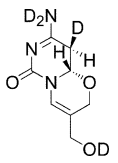
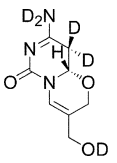
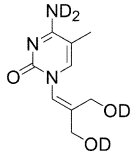
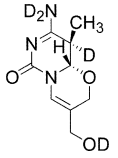
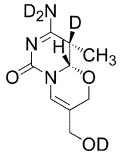
Scheme 5



Scheme 6

The benzoyl group is often used as *N*-protecting group in oligonucleotide synthesis, and the propensity of the *N*-4-benzoylcytosine analogue **1d** to cyclize was investigated. The compound was unstable when NaOD was added. With TFA **1d** cyclized completely within 1 h compared to 42% for **1c**. The same trend is observed with Et_3N, much more **2d** than **2c** is formed, and the reaction is much faster ($t_{\frac{1}{2}}$ < 10 min for **1d** but 180 min for **1c**, Table 2). The 4-imino form (Scheme 6) could be responsible for the enhanced cyclization. *N*-4-Benzoyl-2′-deoxycytidine is known to exist in the 4-amino form, whereas *N*-4-benzoyl-5-methyl-2′-deoxycytidine exists predominantly in the tautomeric 4-imino form.[11] Consequently, the *N*-4-benzoyl-5-methylcytosine analogue could not be prepared by benzoylation

Table 5 Product distribution for cyclization of **1c** and **1e**[a]

					Reaction conditions
4	65	0	0	31	NaOD[b]
86	6	7	0	1	Et_3N[c]
72	8	11	0	9	TFA-d[d]
81	14	2.35	2.35	0.3	No addition[e]
					
72		14	4		TFA-d[f]

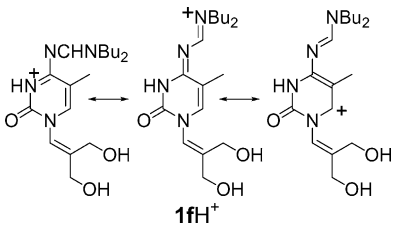
[a] % in CD_3OD (500 µl) + DMSO-d_6 (200 µl). [b] After 20 min with addition of NaOD (10 µl 40% in D_2O). [c] After 20 min with addition of Et_3N (20 µl). [d] After 20 min with addition of TFA-d (10 µl). [e] After 24 h (no addition). [f] After 3 days with addition of TFA-d (100 µl).

of **1e**. The thymine derivative **2b** was isolated instead, *i.e.* **1e** cyclized and deaminated during attempted derivatisation.

1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]-5-methylcytosine (1e) and *N*-4-(dibutylaminomethylene)-1-[3′-hydroxy-2′-(hydroxymethyl)prop-1′-enyl]-5-methylcytosine (1f). A solution of the 5-methylcytosine analogue **1e** in CD_3OD : DMSO-d_6 (5 : 2) is stable towards Et_3N and only a small amount of **2e** is formed when treated with NaOD. Addition of TFA caused slow cyclization to **2e**, the main product being the *cis* addition product (Tables 2 and 5). The reaction is dependent on the concentration of TFA, and the following amounts of **2e** were observed after one week: 5% (10 µl), 33% (50 µl) and 44% (100 µl TFA). Compound **2e** was stable in solution, but deaminated partly to the thymine analogue **2b** during isolation.

Under anhydrous conditions ($CDCl_3$: TFA-d, 5 : 2) **1e**, like **1b**, was transformed to a compound different from **2e**. The compound was assigned the structure **3e** (Scheme 5) based on ^{1}H NMR data (given in Table 4), and the assignment was confirmed by FAB$^+$. Compound **3e** is sensitive to water and reverts easily to **1e**, and only a mixture of **1e** and **3e** could be isolated.

The dibutylaminomethylene group is used as *N*-protecting group in oligonucleotide synthesis, and the propensity of **1f** to cyclize was investigated. The results with Et_3N and NaOD in CD_3OD : DMSO-d_6 (5 : 2) are the same as for **1e** (Table 2), but **1f** is much more sensitive to acid than **1e**. The dibutylaminomethylene group is electron donating and stabilizes protonated **1f** thus promoting Michael addition to the C(5)=C(6) double bond (Scheme 7).



1fH$^+$

Scheme 7

Conclusion

The nucleoside derivatives **1a**–**f** were found to undergo reversible intramolecular Michael addition to form the bicyclic compounds **2a**–**f** when treated with acids or bases. The C(5)–Me derivatives (**1b** and **1e**) were less inclined to cyclize than their C(5) unsubstituted analogues (**1a** and **1c**). Acids promoted cyclization of the 4-amino-2-pyridone derivatives (**1c**–**f**), whereas cyclization of the 2,4-pyrimidinedione derivatives (**1a**,**b**) proceeded best with triethylamine. The exocyclic double bond of **1** was essential for the cyclization to occur. Two commonly used *N*-protecting groups in oligonucleotide synthesis, the benzoyl- and the dibutylaminomethylene group both made Michael addition more feasible. Under acidic anhydrous conditions **1b** and **1e** cyclized to the 2,4′-anhydro compounds **3b** and **3e**. The Michael addition and formation of the anhydro compounds might very well have consequences for the use of **1** as modified nucleosides in oligonucleotide syntheses. These cyclization reactions could occur during the acidic DMT removal step prior to coupling of the next monomer. This would, as observed,[3] result in a reduced coupling efficiency of the next monomer. However, preliminary attempts to synthesize oligonucleotides containing purine analogues of **1**, which are unable to cyclize in the same way as **1a**–**f**, have been disappointing, and further experiments are needed to reveal other side reactions responsible for the low yields experienced during oligonucleotide synthesis.

Experimental

Compounds **1b**,**d**,**e**, **2a**,**b** and **8d** were prepared as described previously.[1,4] Chemicals were from Aldrich and solvents were HPLC grade from LABSCAN. TLC was performed on Merck 5554 silica 60 aluminum sheets and dry column vacuum chromatography[12] on Merck 15111 silica 60 (0.015–0.040 mm). NMR spectra (δ_H and δ_C with Me_4Si as reference, *J* in Hz) were recorded on a Varian Mercury 300 MHz spectrometer at 25 °C and FAB$^+$ on a Jeol HX 110/110 mass spectrometer with *m*-NBA as matrix.

1-[3′-Benzyloxy-2′-(benzyloxymethyl)prop-1′-enyl]cytosine (9c)

To a solution of **8d** (0.29 g, 0.6 mmol) in THF (10 ml) and MeOH (10 ml) was added conc. aq. NH_3 (20 ml), and the mixture was stirred overnight at rt, followed by concentration *in vacuo*. The residue was recrystallized from ethyl acetate to give **9c** as colourless crystals (0.18 g, 80%) and used without further purification.δ_H (DMSO-d_6) 8.23 and 7.74 (2 × 1 H, 2 × br s, NH), 7.54 (1 H, d, *J* 7.5, 6-H), 7.35–7.22 (10 H, m, Ar), 6.69 (1 H, br s, N–CH=C), 5.78 (1 H, d, *J* 7.5, 5-H), 4.49 and 4.39 (2 × 2 H, 2 × s, PhCH_2), 4.12 (2 H, d, *J* 1.0, BnOCH_2), 3.99 (2 H, s, BnOCH_2); *m*/*z* (FAB$^+$) 378.2 (M + H$^+$ calc. 378.2).

1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]cytosine (1c)

To a stirred solution of **9c** (0.378 g, 1 mmol) in dry CH_2Cl_2 (30 ml) under N_2 at 0 °C was added dropwise BCl_3 (1 M in CH_2Cl_2, 10 ml, 10 mmol). After stirring for 1 h at 0 °C, MeOH : CH_2Cl_2 (1 : 1, v/v, 5 ml) was added, and the solvents removed *in vacuo*. The residue was immediately dissolved in MeOH : CH_2Cl_2 (1 : 1, v/v, 20 ml) and solid $NaHCO_3$ was added to pH 6–7. The solids were removed by filtration and washed with MeOH : CH_2Cl_2 (1 : 1, v/v, 25 ml). The combined filtrates were concentrated *in vacuo*, and the residue partitioned between H_2O (25 ml) and $CHCl_3$ (25 ml). Evaporation of the aqueous phase yielded a mixture of **1c**·HCl and **2a** as colourless crystals (*ca.* 3 : 1 estimated from ^{1}H NMR). The mixture was dissolved in MeOH (10 ml) and a saturated solution of NaOH in MeOH was added, by which only **1c** precipitated (0.128 g, 65%), mp > 250 °C (dec) (Found: C, 46.9; H, 5.55; N, 19.9. $C_8H_{211}N_3O_3\cdot\frac{1}{2}H_2O$ requires C, 46.6; H, 5.85; N, 20.35%.); δ_H (DMSO-d_6) 7.51 (1H, d, *J* 7, 6-H), 7.24 and 7.15 (2 × 1H, 2 × br s, NH), 6.56 (1H, br s, N–CH=C), 5.71 (1H, d, *J* 7, 5-H), 4.97 and 4.96 (2 × 1H, 2 × t, 2 × *J* 5, OH), 4.06 (2H, dd, *J* 5 and 1.5, (CH_2C=CH), 3.87 (2H, d, *J* 5, (CH_2C=CH)); δ_C (DMSO-d_6) 166.0, 155.0, 145.6, 134.9, 124.6, 93.4, 60.9, 55.6; *m*/*z* (FAB$^+$) 198.1 (M + H$^+$ calc. 198.1).

2,2-Di(*t*-butyldimethylsilyloxymethyl)oxiran (10)

To a solution of 3-(*t*-butyldimethylsilyloxy)-2-(*t*-butyldimethylsilyloxymethyl)propen[13] (3.17 g, 10 mmol) in $CHCl_3$ (60 ml) was added *m*-CPBA (70% in water, 3.1 g, 12.5 mmol), and the mixture was refluxed for 3 h under N_2, cooled to rt and extracted with 10% aq. $Na_2S_2O_5$ (50 ml), sat. aq. $NaHCO_3$ (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo* to a colourless oil (3.23 g, 97%). The compound was used without purification. δ_H ($CDCl_3$) 3.77 (4H, AB system, Δ 9 Hz, *J* 11.5, CH_2OSi), 2.73 (2H, s, oxiran), 0.89 (18H, s, *t*-butyl), 0.07 and 0.06 (2 × 6H, 2 × s, CH_3); δ_C ($CDCl_3$) 63.0, 59.9, 48.7, 25.8, 18.3, −5.4; *m*/*z* (GC-MS) 317 ([M − CH_3]$^+$ calc. 317), 274, 245.

1-[3′-(*t*-Butyldimethylsilyloxy)-2′-(*t*-butyldimethylsilyloxymethyl)-2′-hydroxypropyl]uracil (11a)

To a suspension of uracil (3.03 g, 27 mmol) in dry DMF (120 ml) was added NaH (60% in oil, 0.64 g, 16 mmol) under N_2. After stirring for 1 h at rt **10** (2.13 g, 6.4 mmol) was added and the mixture heated to reflux for 24 h. After cooling sat. aq. NH_4Cl (200 ml) and EtOAc–heptane (1 : 1 v/v, 300 ml) were added and the mixture stirred for 15 min. The precipitate was removed by filtration and the aq. phase was extracted with EtOAc–heptane (3 × 100 ml). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. The residue, pale yellow crystals (2.33 g, 81%) was used without purification. An analytical sample was obtained by recrystallization from EtOAc–heptane, colourless crystals, mp 138–141 °C (Found: C, 53.7; H, 9.1; N, 6.2. $C_{20}H_{40}N_2O_5Si_2$ requires C, 54.0; H, 9.1; N, 6.3%.); δ_H (DMSO-d_6) 11.2 (1H, br s, NH), 7.46 (1H, d, *J* 8.0, 6-H), 5.50 (1H, d, *J* 8.0, 5-H), 4.58 (1H, s, OH), 3.73 (2H, s, CH_2N), 3.42 and 3.30 (2 × 2H, 2 × s, CH_2O), 0.84 (18H, s, *t*-butyl), 0.00 (12H, s, CH_3Si); δ_C (DMSO-d_6) 163.7, 151.5, 100.1, 74.4, 64.0, 50.0, 25.9, 18.0, −5.5; *m*/*z* (FAB$^+$) 445.2 (M + H$^+$ calc. 445.3).

1-[3′-(*t*-Butyldimethylsilyloxy)-2′-(*t*-butyldimethylsilyloxymethyl)-2′-(diethoxythiophosphoryloxy)propyl]uracil (12a)

To a stirred solution of **11a** (4.45 g, 10 mmol) in dry pyridine (30 ml) under N_2 at rt was added dropwise diethyl phosphorochloridite (1.70 ml, 12 mmol). After 1 h S_8 (640 mg, 20 mmol) was added and the mixture stirred overnight. The reaction mixture was concentrated *in vacuo*, and the residue dissolved in EtOAc (100 ml), filtered, and extracted with sat. aq. $NaHCO_3$ (2 × 50 ml). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo* to give the crude product that was purified by dry column vacuum chromatography (0–40% EtOAc in hexane, 5% increments) to give **12a** as a pale yellow oil that slowly crystallized (5.37 g, 90%), mp 55–58 °C. The compound was used without further purification. The compound is very soluble in hexane, and attempts to recrystallize it failed. δ_H ($CDCl_3$) 8.53 (1H, s, NH), 7.70 (1H, d, *J* 8.2, 6-H), 5.66 (1H, d, *J* 8.2, 5-H), 4.32 (2H, s, NCH_2), 4.14–3.85 (8H, m, TBDMSOCH_2 + POCH_2), 1.30 (6H, t, *J* 7.0, POCCH_3), 0.90 (18H, s, *t*-butyl), 0.07 and 0.06 (2 × 6H, 2 × s, CH_3Si); δ_C ($CDCl_3$) 163.7, 151.3, 146.4, 101.5, 88.1 (d, J_{PC} 9), 64.5 (d, J_{PC} 6), 62.5 (d, J_{PC} 6), 48.6 (d, J_{PC} 3), 25.9, 18.4, 16.0 (d, J_{PC} 8), −5.4, −5.4; δ_P 58.90; *m*/*z* (FAB$^+$) 597.3 (M + H$^+$ calc. 597.3).

1-[3′-(*t*-Butyldimethylsilyloxy)-2′-(*t*-butyldimethylsilyloxymethyl)prop-1′-enyl]uracil (13a)

To dry *t*-amyl alcohol (0.88 g, 10 mmol) in dry toluene (30 ml) was added NaH (60% in oil, 0.40 g, 10 mmol) and the mixture was stirred under N_2 at rt for 1 h. A solution of **12a** (1.13 g, 2.0 mmol, dried by co-evaporation with dry toluene) in dry toluene (10 ml) was added and the mixture stirred at rt for 11 days. The mixture was quenched with 4 M aq. acetic acid (3.0 ml) and the solvents removed *in vacuo*. The crude product in EtOAc (50 ml) was extracted with aq. $NaHCO_3$ (5%, 20 ml) and H_2O (20 ml). The aq. phases were extracted with EtOAc (20 ml), and the combined organic phases dried ($MgSO_4$) and the solvent removed *in vacuo*. Crystallization of the crude yellow product (0.785 g, 92%) from hexane (6 ml) gave **13a** as colourless crystals (0.425 g, 50%), mp 137–138 °C (Found: C, 56.2; H, 9.2; N, 6.5. $C_{20}H_{38}N_2O_4Si_2$ requires C, 56.3; H, 9.0; N, 6.6%.); δ_H ($CDCl_3$) 8.77 (1H, s, NH), 7.45 (1H, d, *J* 7.9, 6-H), 6.60 (1H, br s, N–CH=C), 5.69 (1H, dd, *J* 7.9 and 2.0, 5-H), 4.27 (2H, d, *J* 1.5, TBDMSOCH_2), 4.10 (2H, s, TBDMSOCH_2), 0.92 and 0.88 (2 × 9H, 2 × s, *t*-butyl), 0.10 and 0.06 (2 × 6H, 2 × s, CH_3Si); δ_C($CDCl_3$) 163.4, 149.9, 145.5, 137.7, 122.5, 101.7, 62.8, 57.6, 26.0, 25.9, 18.5, 18.3, −5.3, −5.4; *m*/*z* (FAB$^+$) 427.3 (M + H$^+$ calc. 427.2).

1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]uracil (1a)

To a solution of **13a** (0.085 g, 0.2 mmol) in dry CH_3CN (1.0 ml) was added 40% aq. HF (0.025 ml, 1.1 mmol). After 2 h at rt the precipitate was isolated by centrifugation, washed with cold CH_3CN (3 × 0.2 ml) and dried *in vacuo* to give pure **1a** as colourless crystals (0.038 g, 95%), mp 149–151 °C. The compound was prone to cyclization to **2a** and recrystallization attempts failed. (Found: C, 48.3; H, 4.9; N, 14.0. $C_8H_{10}N_2O_4$ requires C, 48.5; H, 5.1; N, 14.1%.); δ_H (DMSO-d_6) 11.36 (1H, br s, NH), 7.53 (1H, d, *J* 7.9, 6-H), 6.44 (1H, s, N–C=CH), 5.61 (1H, d, *J* 7.9, 5-H), 5.06 (1H, t, *J* 5.3, OH), 5.00 (1H, t, *J* 5.3, OH), 4.09 (2H, dd, *J* 1.5 and 5.3, CH_2O), 3.92 (2H, d, *J* 5.3, CH_2O); δ_C (DMSO-d_6) 163.6, 150.2, 145.5, 138.2, 121.7, 100.9, 60.6, 55.8; *m*/*z* (FAB$^+$) 199.1 (M + H$^+$ calc. 199.1).

***N*-4-(Dibutylaminomethylene)-1-[3′-hydroxy-2′-(hydroxymethyl)prop-1′-enyl]-5-methylcytosine (1f)**

To a suspension of **1e** (0.211 g, 1 mmol) in dry DMF (5 ml) was added dibutylformamide dimethylacetal[14] (0.81 g, 4 mmol). The mixture was stirred at rt under N_2 until the starting material was consumed (24 h, TLC). The solvent was removed *in vacuo*, and the residue purified by dry column vacuum chromatography (0–20% MeOH in EtOAc, containing 1% Et_3N, 2% increments) to give pure **1f** (0.310 g, 88%) as a pale yellow oil that slowly crystallized, mp 109–112 °C (Found: C, 60.3; H, 8.5; N, 15.45. $C_{18}H_{30}N_4O_3\cdot\frac{1}{3}H_2O$ requires C, 60.1; H, 8.7; N, 15.6%.); δ_H (DMSO-d_6) 8.62 (1H, s, N=CH–N), 7.55 (1H, q, *J* 0.9, 6-H), 6.60 (1H, s, N–C=CH), 4.99 and 4.97 (2 × 1H, two overlapping t, *J* 5, 2 × OH), 4.10 (2H, br d, *J* 5, CH_2OH), 3.91 (2H, d, *J* 5,

CH_2OH), 3.53 and 3.44 (2 × 2H, 2 × t, *J* 7.3, NCH_2), 1.91 (3H, d, *J* 0.9, CH_3-5), 1.65–1.52 (4H, m, NCH_2CH_2), 1.41–1.22 (4H, m, CH_2CH_3), 0.92 and 0.91 (2 × 3H, 2 × t, *J* 7.3, CH_2CH_3); δ_C (DMSO-d_6) 170.4, 156.9, 154.8, 143.6, 135.7, 124.1, 108.1, 60.7, 55.5, 51.2, 44.8, 30.3, 28.3, 19.4, 19.0, 13.5, 13.4, 13.3; *m/z* (FAB^-) 349.2 ($M - H^+$ calc. 349.2).

Other compounds

The following compounds have not been isolated in a pure state. ^1H NMR data are given in Table 1 for compounds **2c**–**f** and in Table 4 for compounds **3b** and **3e**.

8-Amino-6-oxo-3-(hydroxymethyl)-9,9*a*-dihydro-2*H*,6*H*-pyrimido[6,1-*b*][1,3]oxazine (**2c**) from **1c** with TFA.

8-(Benzoylamino)-6-oxo-3-(hydroxymethyl)-9,9*a*-dihydro-2*H*, 6*H*-pyrimido[6,1-*b*][1,3]oxazine (**2d**) from **1d** with triethylamine or TFA.

8-Amino-6-oxo-3-(hydroxymethyl)-9-methyl-9,9*a*-dihydro-2*H*, 6*H*-pyrimido[6,1-*b*][1,3]oxazine (**2e**) from **1e** with TFA.

8-(Dibutylaminomethyleneamino)-6-oxo-3-(hydroxymethyl)-9-methyl-9,9*a*-dihydro-2*H*,6*H*-pyrimido[6,1-*b*][1,3]oxazine (**2f**) from **1f** with TFA.

2,4′-Anhydro-1-[3′-Hydroxy-2′-(hydroxymethyl)prop-1′-enyl]-thymine (**3b**) from **1b** with TFA in $CDCl_3$; *m/z* (FAB^+) 195.1 ($M + H^+$ calc. 195.1).

2,4′-Anhydro-1-[3′-hydroxy-2′-(hydroxymethyl)prop-1′-enyl]-5-methylcytosine (**3e**) from **1e** with TFA in $CDCl_3$; *m/z* (FAB^+) 194.0 ($M + H^+$ calc. 194.1).

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