Pergamon

Tetrahedron 58 (2002) 9593–9603

TETRAHEDRON

Chemical synthesis of ^{13}C labeled anti-HIV nucleosides as mass-internal standards

Yoshio Saito,[a] Thomas A. Zevaco[b] and Luigi A. Agrofoglio[a,*]

[a]*Institut de Chimie Organique et Analytique, CNRS UMR 6005, Université d'Orléans, BP 6759, 45100 Orléans, France*
[b]*Forschungszentrum Karlsruhe GmbH, Inst. Techn. Chemie, Bereich Chemisch-Physikalische Verfahren, D-7601 Karlsruhe, Germany*

Received 5 July 2002; revised 6 September 2002; accepted 30 September 2002

Abstract—Synthesis of [$^{13}C_5$]-labeled anti-HIV nucleosides, e.g. d4T, ddI, ddA, is described. The methodology used has been optimized due to the very high cost of the starting compound. The key step of this approach was the stereoselective dehomologation of 1,2:5,6-di-*O*-isopropylidene-3-oxo-α-D-glucofuranose (**2**) with periodic acid and sodium borohydride, which gave optically pure ribose derivative as the exclusive product. Nucleoside derivatives **6a–c** were obtained from ribosylation of **5** with persilylated nucleobases under Vorbrüggen conditions. Deoxygenation of **9a–c** under Corey-Winter conditions afforded the desired labeled nucleoside analogues **12a–c**.

1. Introduction

Videx® or didanosine (ddI, 2′,3′-dideoxyinosine) or one of its metabolites ddA (2′,3′-dideoxyadenosine) can be associated with stavudine (d4T, 2′,3′-didehydro-3′-deoxythymidine) in the treatment of HIV therapy.[1–3] Determining the intracellular levels of a nucleoside and its anabolites is crucial to understand its mechanism of action, to describe its pharmacokinetic profile, and also to provide valuable information about malabsorption, drug–drug interactions, compliance, therapeutic drug level monitoring.

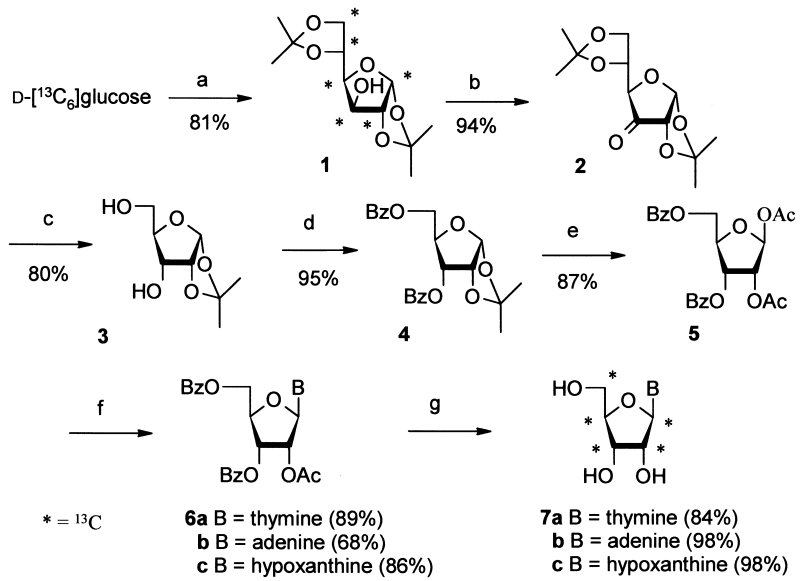
Quantitative bioanalytical chemistry has been revolutionized in the past 10 years by the development of liquid interfaces for tandem mass spectrometry (MS–MS),[4] used to study nucleosides.[5] The immense selectivity of this technique stems for MS–MS detection. Others and us have used that method of choice for quantification of many nucleoside reverse transcriptase inhibitors in biological fluid.[6–9] To assess the precision (% CV), the accuracy (% bias), and in general to validate a MS–MS method, the use of an appropriate internal standard is necessary. Its selection represents a critical aspect of method development because it influences repeatability and reproducibility especially with electrosray mass spectrometry compared to HPLC/UV. In order to tackle this, synthetic labeled derivatives are the best choice as they have the same ionization and fragmentation pattern similar to those of the unlabeled parent, but with a different molecular weight.[10] A unique mass spectrometric scanning procedure, which allowed simultaneous MS–MS product ion analysis of both the analyte and the internal standard, can enhance precision and accuracy in these low level detections. Therefore, it was of interest to synthesize the corresponding ^{13}C mass-labeled (M+5) d4T, ddA, and ddI, respectively, in anticipation of bio-analytical analyses by mass spectrometry. We have previously reported a preliminary account of ^{13}C labeled nucleoside as starting material for monomers of DNA or RNA oligomers.[11] In this paper, we report the experimental details of those hitherto unknown $^{13}C_5$ labeled d4T, ddA, and ddI, and we discuss their NMR characteristics. Thus, our efforts aimed at reproducible experimental procedures giving high-yield products with respect to the isotope-containing precursors. One of the major problems in the chemical syntheses of ^{13}C enriched nucleoside derivatives is the very high cost of the starting compound that requires optimized reactions.

2. Result and discussion

The published methods recently reviewed by Lagoja et al.[12a] or Milecki[12b] and reported by our group for uniformly $^{13}C_5$-labeled nucleosides start from the D-[$^{13}C_6$]glucose. Thus, commercially D-[$^{13}C_6$]glucose was derived into [$^{13}C_6$]-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (**1**) in 98% yield from the consumed D-glucose (Scheme 1); the unreacted glucose was recovered by filtration and re-used. After treatment of **1** with PDC, the key step of this approach was the stereoselective dehomologation[13] of 1,2:5,6-di-*O*-isopropylidene-3-oxo-α-D-glucofuranose (**2**). This ketone was submitted through a one-pot sequential transformation with periodic acid and sodium borohydride, to the optically

Keywords: labeled; HIV; nucleosides.
* Corresponding author. Tel.: +33-2-3849-4582; fax: +33-2-3851-0728; e-mail: luigi.agrofoglio@univ-orleans.fr

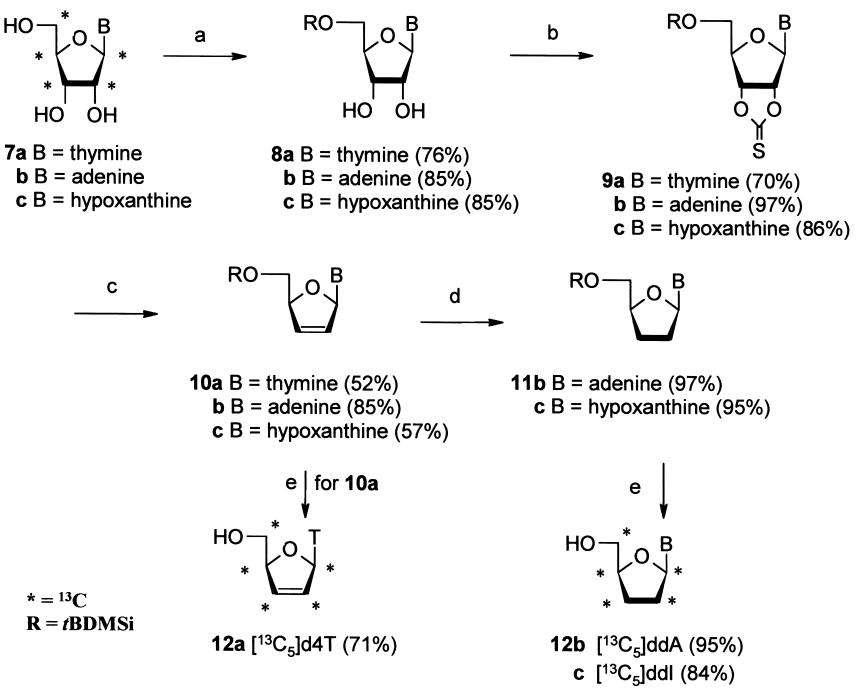
0040–4020/02/$ - see front matter
PII: S0040-4020(02)01246-2



Scheme 1. *Reagents*: (a) $ZnCl_2$, phosphoric acid, acetone; (b) PDC, Ac_2O, CH_2Cl_2; (c) H_5IO_6, EtOAc, then $NaBH_4$, EtOH; (d) BzCl, Py; (e) H_2SO_4, AcOH, Ac_2O; (f) thymine (for **6a**), N^6-benzoyladenine (for **6b**), or hypoxanthine (for **6c**), HMDS, $(NH_4)_2SO_4$, then TMSOTf, DCE; (g) NH_3/MeOH.

pure ribose derivative **3**[14] as the exclusive product in 80% yield. The stereoselectivity of the reduction step is probably due to the electronic effect as well as the steric hindrance of the oxygen of the isopropylidene group, which prevents the hydride attacking from the same side of the isopropylidene group. After benzoylation of **3**, treatment of **4** with Ac_2O/H_2SO_4, afforded the 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-β-ribo-furanose (**5**) after recrystallization from 2-propanol-cyclohexane. Nucleoside derivatives **6a–c** were obtained by condensation of **5** with persilylated nucleobases (thymine, adenine, hypoxanthine) under Vorbrüggen conditions.[15] The deprotection of *O*-acyl groups from **6a–c** was achieved with NH_3–MeOH. In all cases, de-acylated nucleosides **7a–c** were directly used in the next reaction.

The synthesis of dideoxynucleosides (ddNs) or didehydro-dideoxynucleosides (d4Ns) from nucleosides has been extensively reviewed.[16] It has been mainly achieved through a radical reaction (Barton deoxygenation),[17] a fragmentation of 2′,3′-cyclic orthoformates (Eastwood olefination)[18] or through the reductive elimination of 2′(3′)-acetoxy-3′(2′)-halogeno derivatives (Mattocks reaction).[19] We choose to apply a synthetic methodology based on the Corey–Winter reaction of 2′,3′-cyclic thionocarbonates



Scheme 2. *Reagents*: (a) TBDMSCl, imidazole, DMF; (b) TCDI, DCE; (c) triethyl phosphite; (d) H_2, Pd/C(10%), TEA(0.5%)–MeOH; (e) TBAF, THF.

Table 1. ^{1}H NMR chemical shifts (δ) of ^{13}C-labeled sugar derivatives (**1**–**5**) (in $CDCl_3$)

Comp.	H-1	H-2	H-3	H-4	H-5α	H-5β	H-6α	H-6β	Others
1	6.24; 5.63,	4.82; 4.18,	4.61; 3.99,	4.33; 3.81,	4.61; 3.99,	–	4.43; 3.84,	4.27; 3.68,	1.46 (s, CH_3), 1.41 (s, CH_3),
	5.93 (5.90)	*4.50* (4.49)	*4.30* (4.29)	*4.07* (4.03)	*4.30* (4.29)		*4.13* (4.12)	*3.97* (3.97)	1.33 (s, CH_3), 1.28 (s, CH_3)
2	6.48; 5.73,	4.68; 4.03,	–	4.68; 4.03,	4.68; 4.03,	–	4.31; 3.71,	4.27; 3.68,	1.44 (s, $2CH_3$), 1.44 (s, CH_3),
	6.10 (6.13)	*4.35* (4.37)		*4.35* (4.37)	*4.35* (4.37)		*4.01* (4.04)	*3.97* (4.01)	1.34 (s, CH_3)
3[a]	6.05; 5.30,	4.78; 4.15,	4.18; 3.71,	4.10; 3.62,	4.08; 3.56,	3.83; 3.35,	–	–	1.58 (s, CH_3), 1.38 (s, CH_3)
	5.67 (5.78)	*4.46* (4.55)	*3.94* (3.95)	*3.86* (3.85)	*3.82* (3.82)	*3.59* (3.71)			
4	6.28; 5.55,	4.99; 4.39,	5.28; 4.71,	4.94–4.17, (5.05)	4.94–4.17, (4.73)	4.94–4.17, (4.73)	–	–	8.11–7.34 (m, Ar), 1.58 (s, CH_3),
	5.91 (5.94)	*4.69* (4.63)	*4.99* (5.00)						1.46 (s, CH_3), 1.35 (s, CH_3)
5β	6.60; 5.88,	5.88; 5.21,	6.06; 5.43,	5.02; 4.44,	4.97; 4.35,	4.75; 4.17,	–	–	8.13–7.32 (m, Ar), 1.95 (s, AcO),
	6.24 (6.18)	5.54 (5.50)	5.74 (5.78)	4.73 (4.74)	4.66 (4.68)	4.46 (4.47)			2.09 (s, AcO)

Observed values, *average* (^{12}C value).
[a] Observed values, *average* (lit. value).[14]

Table 2. One bond ^{1}H–^{13}C coupling constants (in Hz, obtained from 1D ^{1}H NMR spectra) of ^{13}C-labeled sugar derivatives (**1**–**5**) (in $CDCl_3$)

Comp.	H-1	H-2	H-3	H-4	H-5α	H-5β	H-6α	H-6β
1	185	158	153	147	153	–	149	149
2	186	161	–	161	161	–	149	149
3	185	163	oc	oc	145	145	–	–
4	184	148	oc	oc	oc	oc	–	–
5β	182	163	163	165	153	158	–	–

oc—peak overlapping makes estimation of coupling constants impossible.

for the preparation of ddNs and d4Ns.[20] Careful exclusion of oxygen from the reaction mixture and use of triethyl phosphite avoided an extensive N-methylation of the base moiety when applied to the synthesis of d4T. Thus, after protection of nucleosides **7a**–**c**, the obtained 5′-*O*-silyl protected nucleosides **8a**–**c** were reacted with 1,1′-thiocarbonyl diimidazole to give the thionocarbonate derivatives **9a**–**c** (Scheme 2). Deoxygenation of **9a**–**c** under Corey–Winter conditions[20b] afforded the desired protected 2′,3′-didehydronucleosides **10a**–**c**. Deprotection of **10a** gave the labeled [$^{13}C_5$]-d4T (**12a**), meanwhile the reduction of **10b**,**c** to **11b**,**c** and subsequent deprotection afforded [$^{13}C_5$]-ddA (**12b**) and [$^{13}C_5$]-ddI (**12c**), respectively.

Assignment of ^{1}H- and ^{13}C NMR signals were made on the basis of ^{1}H, ^{13}C, ^{1}H–^{1}H COSY and ^{1}H, ^{13}C-COSY spectra. The main spectroscopic data are summarized in the following tables. Tables 1 and 3 contain chemical shift data, which are closely related to the values for the non-labeled compounds, meanwhile the Tables 2 and 4 report the one bond ^{1}H–^{13}C coupling constants for different ^{13}C-labeled nucleosides.

Tables 5 and 6 show ^{13}C–^{13}C coupling constants of various ^{13}C-labeled sugar derivatives, non-observable for the non-labeled nucleosides. The attribution of the ^{13}C signals for both the non-labeled- and ^{13}C-labeled fragments of the molecule is straightforward.

The ^{1}H-spectrum is at first sight a bit more interesting owing to the 99% ^{13}C-enrichment of the five carbon atoms of the carbocyclic moiety, the attribution of the non-labeled part of the molecule (adenosine, 2 protons) being unproblematic. As it can be taken from the ^{1}H and ^{1}H, ^{1}H-COSY spectra, the ^{1}J ^{13}C–^{1}H scalar coupling somewhat complicates the signals' attribution. An unambiguous attribution can be made for the proton located at a tertiary carbon, H-1′, which is expected to be found at lower field (Table 7).

The attribution can be then made successively along the carbocyclic backbone: proton H-1′shows a strong coupling with proton H-2′ at 2.47 ppm which in turn exhibits a strong coupling with H-3′ at 2.11 ppm. Proton H-4′ at 4.22 ppm eventually displays a coupling with protons H-3′. The most interesting spectroscopic feature is found in the non-equivalence of the geminal protons H-5′α and H-5′β of the methylene group belonging to the hydroxylic 'arm' of the carbocycle (^{2}J=12 Hz, AB coupling pattern) and the absence of strong ^{3}J coupling with the proton H-4′ as it can be taken from the ^{1}H, ^{1}H-COSY spectrum.

Table 3. ^{1}H NMR chemical shifts (δ) of $^{13}C_5$-labeled nucleosides (**6**–**12**)

Comp.	Solvent	H-1′	H-2′	H-3′	H-4′	H-5′α	H-5′β	others
6a	$CDCl_3$	6.60; 5.93	6.05; 5.43	5.84; 5.22	5.07; 4.48	4.87; 4.27	4.79; 4.20	8.22 (s, NH), 8.05–7.37 (m, Ar), 7.17 (s, H-6), 2.23 (AcO)
		6.26 (6.26)	5.74 (5.72)	5.53 (5.51)	4.77 (4.77)	4.57 (4.55)	4.49 (4.48)	
6b	$CDCl_3$	6.81; 6.22,	6.65; 6.05,	6.53; 5.95,	5.16; 4.67,	5.11; 4.58,	4.97; 4.39,	8.95 (s, H-2), 8.42 (s, H-8), 8.25–7.75 (m, Ar),
		6.51 (6.50)	*6.35* (6.31)	*6.24* (6.22)	*4.91* (4.92)	*4.84* (4.85)	*4.68* (4.69)	2.19 (AcO)
6c	$CDCl_3$	6.79; 6.23,	6.67; 6.04,	6.54; 5.97,	5.18; 4.69,	5.13; 4.55,	4.98; 4.41,	8.41 (s, H-2), 8.11 (s, H-8), 8.31–7.72 (m, Ar),
		6.51 (6.51)	*6.35* (6.33)	*6.25* (6.22)	*4.93* (4.92)	*4.84* (4.85)	*4.69* (4.69)	2.23 (AcO)
7a	DMSO-*d*6	6.10; 5.43,	4.31; 3.73,	4.27; 3.67,	4.11; 3.51,	3.88; 3.32,	3.84; 3.24,	7.73 (s, H-6), 1.77 (s, CH_3), 5.88 −5.76 (m, 2′-OH,
		5.77 (5.78)	*4.02* (4.05)	*3.97* (3.97)	*3.81* (3.83)	*3.60* (3.63)	*3.54* (3.55)	3′-OH), 5.13 (m 5′-OH)
7b	DMSO-*d*6	6.19; 5.54,	4.91; 4.33,	4.43; 3.84,	4.25; 3.66,	3.92; 3.32,	3.84; 3.25,	8.34 (s, H-2), 8.13 (s, H-8), 5.54–5.22 (m, 2′-OH,
		5.87 (5.88)	*4.62* (4.60)	*4.13* (4.14)	*3.95* (3.96)	*3.62* (3.67)	*3.55* (3.55)	3′-OH), 5.11 (t, 5′-OH)
7c	DMSO-*d*6	6.23; 5.59,	4.81; 4.23,	4.45; 3.86,	4.26; 3.68,	3.98; 3.38,	3.86; 3.27,	8.39 (s, H-2), 8.11 (s, H-8), 5.73–5.34 (m, 2′-OH,
		5.91 (5.91)	*4.52* (4.52)	*4.16* (4.17)	*3.87* (3.98)	*3.68* (3.69)	*3.57* (3.59)	3′-OH), 5.22 (t, 5′-OH)
8a	$CDCl_3$	6.29; 5.61,	4.50; 3.90,	4.44; 3.85,	4.21; 3.63,	4.10; 3.55,	4.06; 3.50,	7.58 (s, H-6), 1.89 (s, CH_3), 0.96 (s, *t*BuSi),
		5.95 (5.96)	*4.20* (4.22)	*4.14* (4.14)	*3.92* (3.93)	*3.82* (3.82)	*3.78* (3.80)	0.01 (s, $SiMe_2$)
8b	$CDCl_3$	6.47; 5.81,	4.86; 4.26,	4.73; 4.13,	4.55; 3.95,	4.26; 3.72,	4.13; 3.55,	8.30 (s, H-2), 8.07 (s, H-8), 0.75 (s, *t*BuSi),
		6.14 (6.05)	*4.56* (4.55)	*4.43* (4.36)	*4.25* (4.13)	*3.99* (3.99)	*3.84* (3.86)	0.01 (s, $SiMe_2$)
8c	CD_3OD	6.39; 5.72,	4.83; 4.24,	4.63; 4.04,	4.42; 3.82,	4.24; 3.68,	4.13; 3.57,	8.30 (s, H-2), 8.04 (s, H-8), 0.92 (s, *t*BuSi),
		6.05 (6.05)	*4.53* (4.55)	*4.33* (4.36)	*4.52* (4.50)	*3.96* (3.94)	*3.85* (3.86)	0.10 (s, $SiMe_2$)
9a		Not isolated		Not isolated		Not isolated		Not isolated
9b	CD_3OD	6.89; 6.17,	6.74; 6.04,	6.89; 6.17,	4.89; 4.28,	4.08; 3.56,	4.08; 3.56,	8.32 (s, H-2), 7.92 (s, H-8), 0.82 (s, *t*BuSi),
		6.53 (6.52)	*6.39* (6.37)	*6.53* (6.52)	*4.58* (4.57)	*3.82* (3.81)	*3.82* (3.81)	−0.03 (s, $SiMe_2$)
9c	$CDCl_3$	6.69; 6.02,	6.78; 6.19,	6.49; 5.82,	4.23; 3.65,	4.09; 3.52,	4.09; 3.52,	8.21 (s, H-2), 8.01 (s, H-8), 0.82 (s, *t*BuSi),
		6.35 (6.52)	*6.48* (6.46)	*6.15* (6.15)	*3.94* (3.94)	*3.80* (3.80)	*3.80* (3.80)	0.01 (s, $SiMe_2$)
10a		Not isolated		Not isolated		Not isolated		Not isolated
10b	$CDCl_3$	7.42; 6.76,	6.39; 5.62,	6.76; 6.05,	5.30; 4.68,	4.11; 3.54,	4.11; 3.54,	8.38 (s, H-2), 8.09 (s, H-8), 0.88 (s, *t*BuSi),
		7.09 (7.09)	*6.01* (6.01)	*6.40* (6.44)	*4.99* (4.97)	*3.82* (3.81)	*3.82* (3.81)	0.05 (s, $SiMe_2$)
10c	$CDCl_3$	7.38; 6.71,	6.41; 5.71,	6.76; 6.08,	5.29; 4.69,	4.09; 3.52,	4.09; 3.52,	8.38 (s, H-2), 8.18 (s, H-8), 0.88 (s, *t*BuSi),
		7.04 (7.09)	*6.06* (6.04)	*6.42* (6.42)	*4.99* (4.97)	*3.80* (3.81)	*3.80* (3.81)	0.05 (s, $SiMe_2$)
11b	$CDCl_3$	6.66; 5.99,	2.71; 2.19,	2.41;1.86, *2.13* (2.13)α *2.30*;	4.52; 3.94,	4.27; 3.71,	4.07; 3.49,	8.32 (s, H-2), 8.29 (s, H-8), 0.91 (s, *t*BuSi),
		6.32 (6.33)	*2.45* (2.44)	1.77 2.03 (2.03)β	*4.23* (4.25)	*3.99* (4.00)	*3.78* (3.77)	0.09 (s, $SiMe_2$)
11c	$CDCl_3$	6.64; 5.96,	2.79; 2.11,	2.47; 1.79, *2.13* (2.13)α 2.31;	4.54; 3.96,	4.26; 3.70,	4.05; 3.49,	8.32 (s, H-2), 8.11 (s, H-8), 0.92 (s, *t*BuSi),
		6.30 (6.33)	*2.45* (2.45)α 2.67; 1.89 *2.28* (2.28)β	1.62 *1.96* (2.00)β	*4.25* (4.25)	*3.98* (4.00)	*3.77* (3.77)	0.10 (s, $SiMe_2$)
12a [$^{13}C_5$]-d4T	$CDCl_3$	7.40; 6.90,	5.98; 5.47,	6.71; 6.19,	5.23; 4.62,	4.21; 3.67,	4.10; 3.54,	7.47 (s, H-6), 1.85 (s, CH_3)
		7.15 (7.05)	*5.72* (5.83)	*6.45* (6.35)	*4.92* (4.93)	*3.94* (3.94)	*3.82* (3.81)	
12b [$^{13}C_5$]-ddA	CD_3OD	6.61; 5.94,	2.77; 2.23,	2.41; 1.88, *2.14* (2.15)	4.55; 3.96,	4.13; 3.58,	3.92; 3.39,	8.40 (s, H-2), 8.18 (s, H-8)
		6.27 (6.28)	*2.50* (2.51)		*4.25* (4.26)	*3.85* (3.86)	*3.65* (3.65)	
12c [$^{13}C_5$]-ddI	CD_3OD	6.52; 5.85,	2.66; 2.14,	2.27; 1.73,	4.46; 3.85,	4.01; 3.44,	3.82; 3.29,	8.27 (s, H-2), 7.92 (s, H-8)
		6.18 (6.20)	*2.40* (2.43)	*2.00* (2.03)	*4.15* (4.15)	*3.72* (3.74)	*3.55* (3.57)	

Observed values, *average* (^{12}C value).

Table 4. One bond $^{1}H-^{13}C$ coupling constants (in Hz, obtained from 1D ^{1}H NMR spectra) of ^{13}C-labeled nucleosides (**6**–**12**)

Comp.	Solvent	H-1′	H-2′	H-3′	H-4′	H-5′α	H-5′β
6a	$CDCl_3$	167	155	155	148	150	148
6b	$CDCl_3$	165	156	157	149	153	149
6c	$CDCl_3$	163	155	158	151	155	150
7a	DMSO-*d*6	167	144	151	148	140	150
7b	DMSO-*d*6	162	145	147	147	147	148
7c	DMSO-*d*6	160	145	148	145	149	148
8a	$CDCl_3$	169	150	147	145	138	140
8b	$CDCl_3$	167	149	151	149	135	146
8c	CD_3OD	167	149	149	149	138	139
9a		Not isolated		Not isolated		Not isolated	
9b	CD_3OD	182	144	oc	oc	144	144
9c	$CDCl_3$	167	147	oc	145	142	142
10a		Not isolated		Not isolated		Not isolated	
10b	$CDCl_3$	168	193	177	154	141	141
10c	$CDCl_3$	168	174	171	151	141	141
11b	$CDCl_3$	166	132	134 H3′α 131 H3′β	145	141	146
11c	$CDCl_3$	170	168 H2′α oc H2′β	oc H3′α 171 H3′β	147	140	139
12a [$^{13}C_5$]-d4T	$CDCl_3$	123	128	130	153	135	140
12b [$^{13}C_5$]-ddA	CD_3OD	169	134	133	147	140	140
12c [$^{13}C_5$]-ddI	CD_3OD	169	130	134	153	142	oc

oc—peak overlapping makes estimation of coupling constants impossible.

Table 5. ^{13}C NMR chemical shifts (δ), multiplicities and one bond $^{13}C-^{13}C$ coupling constants (Hz) of ^{13}C-labeled sugar derivatives (**1**–**5**) (in $CDCl_3$)

Comp.	C1	C2	C3	C4	C5	C6
1	107.2, d, (33.5)	86.9, dd, (44.1, 33.5)	77.1, dd, (44.1, 36.8)	82.9, dd, (48.1, 36.8)	75.5, dd, (48.1, 34.4)	69.5, d, (34.4)
2	108.1, d, (36.9)	84.3–81.4, m, (oc)	214.2, t, (36.3)	84.3–81.4, m, (oc)	84.3–81.4, m, (oc)	69.6, d, (32.7)
3	104.6, d, (33.8)	82.5–80.5, m, (oc)	72.3, dd, (41.1, 40.3)	82.5–80.5, m, (oc)	61.7, d (43.3)	–
4	104.4, d, (33.2)	77.9–75.0, m, (oc)	73.2, t, (41.1)	77.9–75.0, m, (oc)	63.17, d, (44.4)	–
5β	97.6, d, (44.5)	77.5–75.1, m, (oc)	72.9, t, (41.2)	77.5–75.1, m, (oc)	64.10, d, (44.3)	–

oc—peak overlapping makes estimation of coupling constants impossible.

Fig. 1 (panel A and B) represents the expanded regions of the sugar of [$^{13}C_5$]-ddA in the ^{13}C (proton decoupled) and (off-resonance decoupled) are presented. The interpretation of the ^{1}H, ^{13}C-COSY spectrum corroborates the attribution made for the different protons.

In Fig. 2, a representation of [$^{13}C_5$]-ddA shows some calculated and found NMR coupling constants.

3. Conclusion

In summary, the syntheses of hitherto unknown [$^{13}C_5$]-ddA, [$^{13}C_5$]-ddI and [$^{13}C_5$]-d4T have been accomplished from D-[$^{13}C_6$]-glucose. The nucleosides obtained were fully characterized by spectroscopic methods and especially by NMR. ^{1}H and ^{13}C NMR spectra of [$^{13}C_5$]-nucleosides as well as other physico-chemical data are

Table 6. ^{13}C NMR chemical shifts (δ), multiplicities and one bond $^{13}C-^{13}C$ coupling constants (Hz) of ^{13}C-labeled nucleosides (**6**–**12**)

Comp.	Solvent	C1′	C2′	C3′	C4′	C5′
6a	$CDCl_3$	87.6, d, (42.8)	74.5, dd, (42.8, 38.1)	68.7, dd, (38.1, 37.5)	84.3, dd, (42.0, 37.5)	61.1, d, (42.0)
6b	$CDCl_3$	88.1, d, (42.3)	73.7, dd, (42.3, 38.5)	71.2, dd, (38.5, 36.3)	84.5, dd, (41.7, 36.3)	62.2, d, (41.7)
6c	$CDCl_3$	86.8, d, (42.6)	74.6, dd, (42.6, 37.5)	70.5, dd, (37.5, 36.3)	85.9, dd, (41.5, 36.3)	62.1, d, (41.5)
7a	DMSO-*d*6	87.4, d, (43.0)	73.2, dd, (43.0, 37.5)	69.8, dd, (38.1, 37.5)	84.7, dd, (41.8, 38.1)	60.9, d, (41.8)
7b	DMSO-*d*6	87.9, d, (42.1)	73.4, dd, (42.1, 37.5)	70.6, dd, (37.5, 36.0)	85.9, dd, (41.1, 36.0)	61.7, d, (41.1)
7c	DMSO-*d*6	87.3, d, (42.0)	74.1, dd, (42.0, 37.5)	70.3, dd, (37.5, 36.0)	85.5, dd, (41.2, 36.0)	61.7, d, (41.2)
8a	CD_3OD	89.6, d, (43.0)	75.7, dd, (43.0, 38.1)	71.7, dd, (38.8, 38.1)	86.4, dd, (43.0, 38.8)	64.3, d, (43.0)
8b	$CDCl_3$	89.2, d, (41.8)	75.6, dd, (41.8, 36.9)	70.4, dd, (40.0, 36.9)	85.7, dd, (43.0, 40.0)	62.8, d, (43.0)
8c	CD_3OD	90.0, d, (42.5)	76.6, dd, (42.5, 37.4)	71.4, dd, (39.0, 37.4)	86.5, dd, (43.1, 39.0)	63.9, d, (43.1)
9a		Not isolated		Not isolated		Not isolated
9b	$CDCl_3$	90.2, d, (42.0)	88.6–86.8, m, (oc)	88.6–86.8, m, (oc)	88.6–86.8, m, (oc)	62.2, d, (42.5)
9c	$CDCl_3$	90.4, d, (40.0)	88.5–86.1, m, (oc)	88.5–86.1, m, (oc)	88.5–86.1, m, (oc)	62.5, d, (44.2)
10a		Not isolated		Not isolated		Not isolated
10b	$CDCl_3$	89.8, d, (40.0)	135.7, dd, (69.0, 40.0)	126.3, dd, (69.0, 43.0)	89.6, dd, (43.0, 42.4)	66.0, d, (42.4)
10c	$CDCl_3$	88.6, d, (44.2)	135.1, dd, (69.0, 44.2)	125.2, dd, (69.0, 41.7)	88.1, dd, (42.4, 41.7)	64.9, d, (42.4)
11b	CD_3OD	86.8, d, (35.7)	34.0, dd, (35.7, 31.5)	25.7, dd, (33.3, 31.5)	83.8, dd, (41.8, 33.3)	65.1, d, (41.8)
11c	$CDCl_3$	85.6, d, (35.7)	33.7, dd, (35.7, 32.1)	25.0, dd, (33.3, 32.1)	82.4, dd, (43.6, 33.3)	64.0, d, (43.6)
12a [$^{13}C_5$]-d4T	$CDCl_3$	89.8, d, (39.4)	134.7, dd, (69.6, 39.4)	126.2, dd, (69.6, 41.2)	87.2, dd, (41.2, 41.2)	63.3, d, (41.2)
12b [$^{13}C_5$]-ddA	CD_3OD	87.6, d, (36.0)	33.8, dd, (36.0, 31.0)	26.7, dd, (35.0, 31.0)	83.7, dd, (41.0, 35.0)	64.7, d, (41.0)
12c [$^{13}C_5$]-ddI	CD_3OD	87.3, d, (35.6)	33.9, dd, (35.6, 32.2)	26.3, dd, (33.5, 32.2)	83.8, dd, (42.3, 33.5)	64.2, d, (42.3)

oc—peak overlapping makes estimation of coupling constants impossible.

Table 7. ^{1}H- and ^{13}C NMR analysis of [$^{13}C_5$]-ddA

Location	H-1′	H-2′	H-3′	H-4′	H-5′α and H-5′β
δC (ppm)	87.6	33.8	26.7	83.7	64.7
$^{1}J_{C-C}$ (Hz)	$J_{1'-2'}$: 36	$J_{2'-3'}$: 31	$J_{3'-4'}$: 35	$J_{4'-5'}$: 41	
$^{1}J_{C-H}$ (Hz)	169	134	133	147	140
δH (Hz)[a]	6.25	2.47	2.11	4.22	3.82/3.29
Mult.	d	m	m	d	ddd
^{2}J (Hz)	–	–	–	–	$J_{5'a-5'b}$: 12
^{3}J (Hz)	–			–	$J_{5'a-4'}$: 4 $J_{5'b-4'}$: 7
^{4}J (Hz)	–			–	$J_{5'a-Cx}$: 2 $J_{5'b-Cx}$: 3

The ^{1}H- and ^{13}C NMR spectra were recorded on a Varian Inova$_{Unity}$ 400 spectrometer (^{1}H: 399.81 MHz, ^{13}C: 100.54 MHz) in (D_4)-methanol, shift values δ in ppm relative to $SiMe_4$ as internal reference; *J* in Hz.
[a] Average.

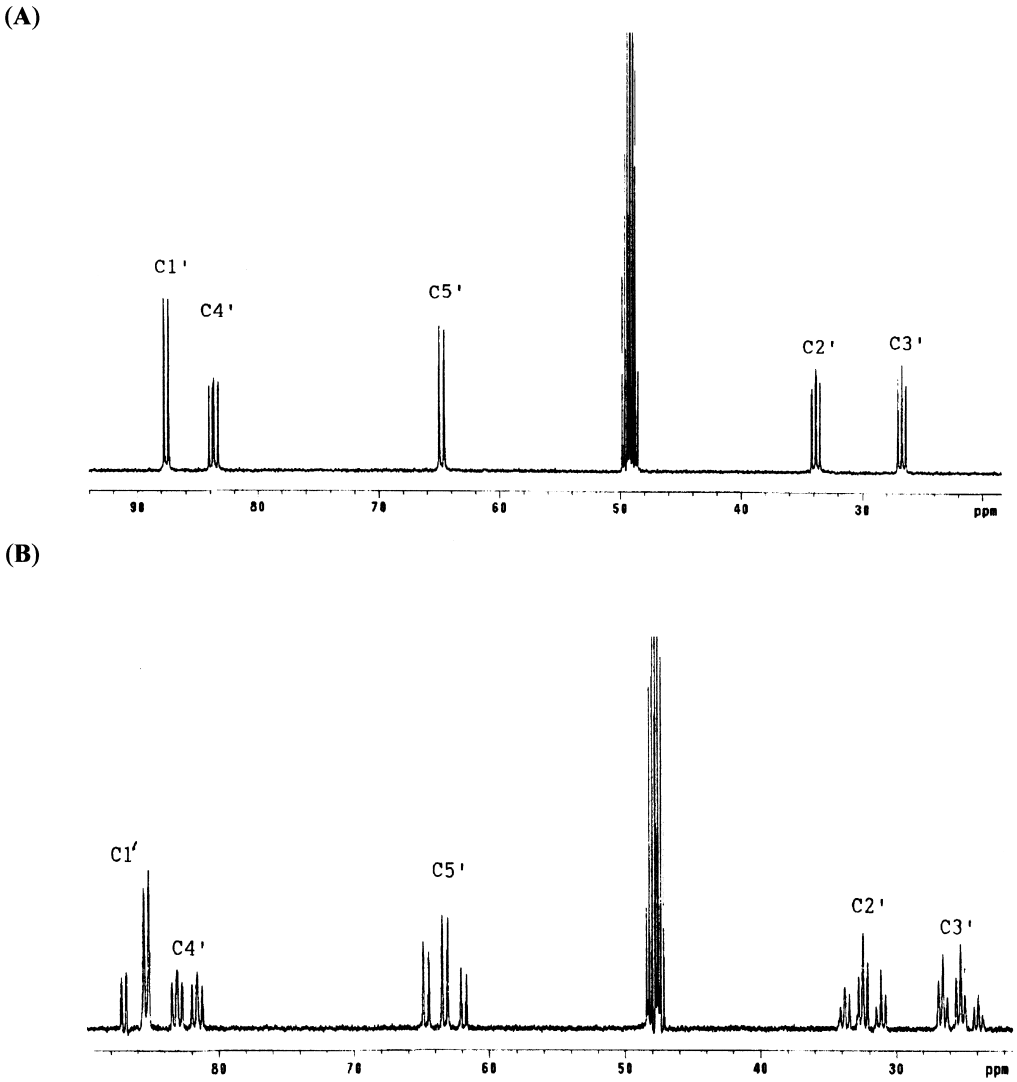


Figure 1. The expanded regions of the sugar moiety of [$^{13}C_5$]-ddA in the ^{13}C-proton decoupled (panel A) and in the ^{13}C-off resonance decoupled (panel B).

identical with those of authentic samples except for the expected additional signal multiplicity arising from $^{13}C-^{1}H$ and $^{13}C-^{13}C$ couplings, respectively. Labeled d4T and ddI have been used as internal standard for the validation of the quantification procedure, on cell extract, by MS–MS. Data will be reported elsewhere.

4. Experimental

4.1. General

Commercially available chemicals and solvents were reagent grade and used as received. Dry tetrahydrofuran, pyridine and dichloromethane were obtained from

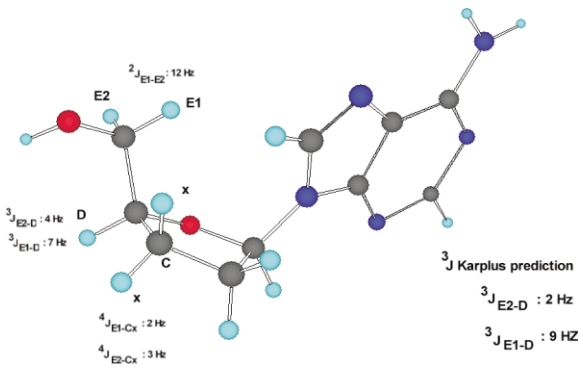


Figure 2. Some calculated and found NMR coupling constants for [$^{13}C_5$]-ddA. An optimal configuration for [$^{13}C_5$]-ddA was simulated using the semi-empirical method AM1 (Software package Chemdraw ultra 6.0/Chem 3D Pro 5.0). Note that the assignments have been represented by Cx for H-3′α and β, D for H-4′, E1 and E2 for H-5′α and H-5′β, respectively. Atoms: gray=C, pale blue=H, dark blue=N, red=O.

distillation over CaH_2 or Na, *N*,*N*-dimethylformamide over BaO. The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation and/or spraying with 20% H_2SO_4 in EtOH, followed by charring at 150°C. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). Melting point were recorded on a Büchi (Dr Tottoli) and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX 250 Fourier Transform spectrometer at 250 MHz for ^1H and 62.9 MHz for ^{13}C, respectively, using tetramethylsilane as the internal standard; signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on Perkin–Elmer SCIEX API-300 (heated nebullizer) spectrometer. HRMS were performed by the CRMPO, University of Rennes 1–Fr.

The characteristic ^1H, ^{13}C data for compounds **1**–**12** are collected and shown in Tables 1–7. References in the experimental part refer to the unlabeled homologues except for compound **3**.

4.1.1. [$^{13}C_6$]-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (1). To a well-stirred suspension of anhydrous [$^{13}C_6$]-α-D-glucose (5.0 g, 26.9 mmol) in anhydrous acetone (50 mL) was added pulverized anhydrous zinc chloride (4.0 g) followed by 0.25 g of 85% phosphoric acid. This mixture was stirred 30 h at room temperature, and the unreacted sugar was collected and washed with a little acetone. The filtrate and washings were cooled and made slightly alkaline with 2.5N NaOH. The insoluble inorganic material was removed by filtration and washed with acetone. The almost colorless filtrate and washings were concentrated under reduced pressure and the residue was diluted with water (10 mL), extracted with CH_2Cl_2 (3×50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Crystallization of the residue from hexane gave the desired compound as colorless needles (4.14 g, 58%). The recovered unreacted sugar was converted to the title compound by the same way. Combined yield gave 81% (5.72 g) of the protected glucofuranose (**1**)[21]; R_f 0.45 (1:1 hexanes–EtOAc); $[\alpha]_D^{25}=-12°$ (*c* 1.0, $CHCl_3$), lit.[22] $[\alpha]_D^{25}=-13°$ (*c* 5.0, $CHCl_3$); mp 106–107°C; ^{13}C NMR ($CDCl_3$) [see Table 5], others δ 114.3 (*C*(Me)$_2$), 111.6 (*C*(Me)$_2$), 28.7 (*C*H$_3$), 28.7 (*C*H$_3$), 28.1 (*C*H$_3$), 27.1 (*C*H$_3$); MS: *m*/*z* 267 $[M+H]^+$. Anal. calcd for ($^{13}C_6$)$C_6H_{20}O_6$: C, 56.38; H, 7.57. Found: C, 56.41; H, 7.58.

4.1.2. [$^{13}C_6$]-1,2:5,6-Di-*O*-isopropylidene-3-oxo-α-D-glucofuranose (2). To a stirred solution of protected sugar **1** (2.50 g, 9.6 mmol) in anhydrous CH_2Cl_2 (20 mL) were added pyridinium dichromate (PDC, 2.17 g, 5.7 mmol) and acetic anhydride (3 mL, 31.7 mmol), the mixture was then refluxed for 1.5 h. The solvent was evaporated under reduced pressure and EtOAc (50 mL) was added. The mixture was applied to a silica gel pad and eluted with EtOAc. The combined eluant was concentrated under reduced pressure and co-evaporated with toluene (2×25 mL) to give **2** as a colorless solid[23] (2.33 g, 94%); R_f 0.65 (1:9 hexanes–EtOAc); mp 127–129°C; IR (neat) 1728 cm^{-1} (C=O); ^{13}C NMR ($CDCl_3$) [see Table 5], others δ 135.5 (*C*(Me)$_2$), 132.6 (*C*(Me)$_2$), 27.9 (*C*H$_3$), 27.3 (*C*H$_3$), 25.6 (*C*H$_3$), 25.5 (*C*H$_3$); MS: *m*/*z* 265 $[M+H]^+$. Anal. calcd for ($^{13}C_6$)$C_6H_{18}O_6$: C, 56.81; H, 6.86. Found: C, 56.72; H, 6.89.

4.1.3. [$^{13}C_5$]-1,2-*O*-Isopropylidene-α-D-ribofuranose (3). A solution of the dried ketonic compound **2** (2.65 g, 10 mmol) and H_5IO_6 (2.75 g, 12 mmol) in anhydrous EtOAc (28 mL) was stirred at room temperature for 2 h. The reaction mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in EtOH (abs., 20 mL), and $NaBH_4$ (1 g, 26.4 mmol) was added in small portions with vigorous stirring. Stirring was continued for 30 min then 10% acetic acid was added for neutralization, and volatiles were evaporated. The residue was dissolved in EtOAc, and the organic phase was washed with H_2O, dried over $MgSO_4$ and volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography (9:1 CH_2Cl_2–MeOH) to give **3**[14,20a,22] (1.52 g, 80%) as a colorless solid; R_f 0.35 (1:9 hexanes–EtOAc); mp 86–87°C lit.[24] mp 85.5–86°C; IR (neat) 3300 cm^{-1} (HO); $[\alpha]_D^{25}=-12°$ (*c* 2.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) [see Table 5], others δ 113.5 (*C*(Me)$_2$), 27.4 (*C*H$_3$), 27.2 (*C*H$_3$); MS: *m*/*z* 196 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_3H_{14}O_5$: C, 51.79; H, 7.23. Found: C, 51.82; H, 7.20.

4.1.4. [$^{13}C_5$]-3,5-Di-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose (4). To a stirred solution of diol **3** (1.45 g, 7.4 mmol) in anhydrous pyridine (10 mL) at 0°C was added dropwise benzoyl chloride (3.0 mL, 26.12 mmol) and the mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure and the residue extracted with EtOAc, washed with saturated $NaHCO_3$, and dried over Na_2SO_4. After concentration under vacuum, the residue was purified by silica gel column chromatography (7:3 hexanes–EtOAc) to give **4** (2.79 g, 95%) as a colorless oil. Analytical quality was obtained by recrystallization of **4** from ether to a colorless solid; R_f 0.60 (8:2 hexanes–EtOAc); mp 82–84°C dec; $[\alpha]_D^{25}=+122.4°$ (*c* 0.85, $CHCl_3$) [lit.[26] mp 83–85°C; $[\alpha]_D^{25}=-122.4°$ (*c* 0.9, $CHCl_3$) for the (−)-^{12}C-enantiomer]; ^{13}C NMR ($CDCl_3$) [see Table 5], others δ 166.2 (*C*=O), 133.5 (*C*H), 129.7 (quat C), 129.1 (*C*H), 128.3 (*C*H), 100.9 (*C*(Me)$_2$), 26.0 (C(*Me*)$_2$); MS: *m*/*z* 404 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_{17}H_{22}O_7$: C, 66.74; H, 5.49. Found: C, 66.82; H, 5.51.

4.1.5. [$^{13}C_5$]-1,2-Di-*O*-acetyl-3,5-di-*O*-benzoyl-β-D-ribofuranose (5). Sulfuric acid (1.5 mL) was added dropwise to a stirred solution of isopropylidene sugar **4** (4.23 g, 10.5 mmol) in AcOH (40 mL) and Ac_2O (15 mL). After storage overnight at room temperature, the mixture was poured into ice-water, extracted with $CHCl_3$, washed with saturated $NaHCO_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (7:3 hexanes–EtOAc) to give **5** (4.08 g, 87%) as needles after recrystallization from 2-propanol-cyclohexane. R_f 0.52 (75:25 hexanes–EtOAc); mp 127–128°C, lit.[25] mp 126–127°C; ^{13}C NMR ($CDCl_3$) [see Table 5], others δ 171.1 (*C*═O), 170.5 (*C*═O), 168.4 (*C*═O), 168.2 (*C*═O), 133.5 (*C*H), 130.1 (quat C), 127.8 (*C*H), 127.3 (*C*H), 21.2 (CH_3), 20.9 (CH_3); MS: *m/z* 448 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_{18}H_{22}O_9$: C, 62.86; H, 4.96. Found: C, 62.78; H, 5.01.

4.1.6. [$^{13}C_5$]-1-(2-*O*-Acetyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl)-5-methyluracil (6a). *General Procedure for coupling*. Dry thymine (704 mg, 5.60 mmol) and $(NH_4)_2SO_4$ (0.1 g) were stirred in refluxing HMDS (30 mL) under nitrogen until homogeneous solution and was then evaporated under vacuum to give the silylated thymine. A solution of protected sugar **5** (1.0 g, 2.24 mmol) in anhydrous dichloroethane (DCE, 8 mL) was added to the silylated thymine, followed by the dropwise addition of TMSOTf (1.05 mL, 5.43 mmol) and the resulting solution was refluxed for 3.5 h. It was quenched with saturated $NaHCO_3$, extracted with CH_2Cl_2 (2×50 mL), washed successively with saturated $NaHCO_3$ and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (20:1 CH_2Cl_2–MeOH) to yield **6a** (1.15 g, 89%) as a colorless oil. R_f 0.53 (10:1 CH_2Cl_2–MeOH); $[\alpha]_D^{25}$=+32° (*c* 1.0, $CHCl_3$); MS: *m/z* 514 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_{21}H_{24}N_2O_9$: C, 61.80; H, 4.71; N, 5.45. Found: C, 62.11; H, 4.68; N, 5.73.

4.1.7. [$^{13}C_5$]-1-(2-*O*-Acetyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl)adenine (6b). A mixture of **5** (690 mg, 1.54 mmol), silylated adenine (1.44 g, 4.63 mmol) and TMSOTf (725 μL, 3.75 mmol) in DCE (10 mL) was refluxed for 3.5 h under Ar. After workup and silica gel column chromatography (20:1 CH_2Cl_2–MeOH), **6b** was obtained as a colorless oil (655 mg, 68%). R_f 0.62 (20:1 CH_2Cl_2–MeOH); MS: *m/z* 523 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_{21}H_{23}N_5O_7$: C, 60.72; H, 4.43; N, 13.40. Found: C, 60.84; H, 4.31; N, 13.75.

4.1.8. [$^{13}C_5$]-1-(2-*O*-Acetyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl)hypoxanthine (6c). A mixture of **5** (285 mg, 0.638 mmol), silylated hypoxanthine (448 mg, 1.59 mmol) and TMSOTf (309 μL, 1.59 mmol) in DCE (7.0 mL) was refluxed for 2.5 h under Ar. After workup and silica gel column chromatography (15:1 CH_2Cl_2–MeOH), **6c** was obtained as a colorless solid (288 mg, 86%). R_f 0.61 (15:1 CH_2Cl_2–MeOH); MS: *m/z* 523 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_{21}H_{22}N_4O_8$: C, 60.61; H, 4.23; N, 10.70. Found: C, 60.83; H, 4.18; N, 11.05.

4.1.9. D-[$^{13}C_5$]-5-Methyluridine (7a). *General procedure for de-O-acylation*. A solution of **6a** (255 mg, 0.497 mmol) in saturated methanolic ammonia (5.0 mL) was stirred at room temperature for 48 h. The flask was opened and left under the hood for 5 h to allow ammonia gas to escape. The solution was carefully evaporated, the remaining solid was triturated with ethyl ether, leaving a colorless powder of **7a** (110 mg, 84%); R_f 0.30 (4:1 CH_2Cl_2–MeOH); UV (H_2O) λ_{max} 273.0 nm; ^{13}C NMR (DMSO-*d6*) [see Table 6], others δ 165.2 (*C*═O), 158.4 (*C*═O), 135.1 (C5), 108.4 (quat C), 15.9 (CH_3); MS: *m/z* 264 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_5H_{14}N_2O_6$·0.5H_2O: C, 45.96; H, 5.55; N, 10.29. Found: C, 45.94; H, 5.58; N, 10.32.

4.1.10. D-[$^{13}C_5$]-Adenosine (7b). A solution of **6b** (202 mg, 0.323 mmol) in saturated methanolic ammonia (7.5 mL) was stirred at room temperature for 48 h. After concentration, **7b** was obtained as a colorless solid (98.6 mg, >98%); R_f 0.10 (10:1 CH_2Cl_2–MeOH); UV (H_2O) λ_{max} 261.0 nm; ^{13}C NMR (DMSO-*d6*) [see Table 6], others δ 156.8 (quat C), 153.5 (C2), 149.8 (quat C), 142.5 (C8), 120.5 (quat C); MS: *m/z* 273 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_5H_{13}N_5O_4$·0.3H_2O: C, 45.89; H, 4.93; N, 25.23. Found: C, 45.93; H, 4.85; N, 25.26.

4.1.11. D-[$^{13}C_5$]-Inosine (7c). A solution of **6c** (175 mg, 0.334 mmol) in saturated methanolic ammonia (5.0 mL) was stirred at room temperature for 48 h. After concentration, **7c** was obtained as a colorless solid (93.6 mg, >98%); R_f 0.10 (10:1 CH_2Cl_2–MeOH); UV (H_2O) λ_{max} 250.0 nm; ^{13}C NMR (DMSO-*d6*) [see Table 6], others δ 156.9 (C═O), 148.5 (quat C), 147.3 (C2), 139.1 (C8), 124.8 (quat C); MS: *m/z* 274 $[M+H]^+$. Anal. calcd for ($^{13}C_5$)$C_5H_{12}N_4O_5$: C, 45.79; H, 4.43; N, 20.50. Found: C, 45.82; H, 4.38; N, 20.63.

4.1.12. D-[$^{13}C_5$]-5-Methyl 5′-*O*-*tert*-butyldimethylsilyl-uridine (8a). To a solution of **7a** (580 mg, 1.54 mmol) and imidazole (34.2 mg, 1.05 mmol) in anhydrous DMF (2.0 mL) at 0°C was added *tert*-butyldimethylsilyl chloride (75.9 mg, 0.503 mmol) with stirring. The mixture was warmed to room temperature and subsequently stirred for 17 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to give **8a**[20b] (103 mg, 76%) as a colorless waxy solid. R_f 0.53 (10:1 CH_2Cl_2–MeOH); ^{13}C NMR ($CDCl_3$) [see Table 6], others δ 166.3 (C═O), 152.6 (C═O), 137.4 (C6), 111.5 (quat C), 26.5 ((CH_3)$_3$CSi), 19.3 (*C*H$_3$), 12.6 (*C*H$_3$Si), −5.2 (*C*H$_3$Si); MS: *m/z* 378 $[M+H]^+$.

4.1.13. D-[$^{13}C_5$]-5′-*O*-*tert*-Butyldimethylsilyl adenosine (8b). To a solution of **7b** (87.9 mg, 0.323 mmol) and imidazole (52.9 mg, 0.777 mmol) in anhydrous DMF (2.0 mL) at 0°C was added *tert*-butyldimethylsilyl-chloride (58.4 mg, 0.388 mmol) with stirring. The mixture was warmed to room temperature and subsequently stirred for 14 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (5:1 CH_2Cl_2–MeOH) to give **8b**[27] (105 mg, 85%) as a colorless solid; R_f 0.30 (15:1 CH_2Cl_2–MeOH); mp 184–186°C, lit.[27] mp 185–186°C; ^{13}C NMR ($CDCl_3$) [see Table 6], others δ 156.9 (quat C), 154.0 (C2), 150.8 (quat C), 140.3 (C8), 121.0 (quat C), 27.2 ((CH_3)$_3$CSi), 10.8 (*C*H$_3$Si), −4.7 (*C*H$_3$Si); MS: *m/z* 387 $[M+H]^+$.

4.1.14. D-[$^{13}C_5$]-5′-*O*-*tert*-Butyldimethylsilyl inosine (8c). To a solution of inosine **7c** (87.9 mg, 0.323 mmol) and imidazole (52.9 mg, 0.777 mmol) in anhydrous DMF (2.0 mL) at 0°C was added *tert*-butyldimethylsilyl chloride (58.4 mg, 0.388 mmol) with stirring. The mixture was warmed to room temperature and subsequently stirred for 14 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (5:1 CH_2Cl_2–MeOH) to give **8c**[20b] (105 mg, 85%) as a colorless waxy solid; R_f 0.35 (15:1 CH_2Cl_2–MeOH); ^{13}C NMR (CD_3OD) [see Table 6], others δ 152.3 (C=O), 148.9 (quat C), 143.9 (C2), 137.3 (C8), 125.7 (quat C), 23.2 ((CH_3)$_3$CSi), 13.3 (CH_3Si), −3.8 (CH_3Si); ESIMS *m/z* 388 $[M+H]^+$.

4.1.15. D-[$^{13}C_5$]-5-Methyl-2′,3′-*O*-thiocarbonylene-5′-*O*-*tert*-butyldimethylsilyluridine (9a). A mixture of **8a** (103 mg, 0.273 mmol) and 1,1′-thiocarbonyl diimidazole (72.9 mg, 0.409 mmol) in 1,2-dichloroethane (4.0 mL) was refluxed for 3 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (20:1 CH_2Cl_2–MeOH) to afford **9a**[20b] (80.0 mg, 70%) as a colorless glassy material. This product was engaged directly into the next step without any further characterization. R_f 0.70 (10:1 CH_2Cl_2–MeOH).

4.1.16. D-[$^{13}C_5$]-2′,3′-*O*-Thiocarbonylene-5′-*O*-*tert*-butyldimethylsilyl adenosine (9b). A mixture of **8b** (193 mg, 0.50 mmol) and 1,1′-thiocarbonyl diimidazole (178 mg, 1.00 mmol) in 1,2-dichloroethane (5.5 mL) was refluxed for 3 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to afford **9b**[20b] (208 mg, 97%) as a colorless solid; R_f 0.37 (15:1 CH_2Cl_2–MeOH); mp 198–200°C, lit.[20b] mp 198–199°C; ^{13}C NMR (CD_3OD) [see Table 6], others δ 189.9 (C=S), 157.7 (quat C), 152.3 (C2), 148.3 (quat C), 140.1 (C8), 118.9 (quat C), 24.7 ((CH_3)$_3$CSi), 17.3 (CH_3Si), −3.8 (CH_3Si); MS: *m/z* 429 $[M+H]^+$.

4.1.17. D-[$^{13}C_5$]-2′,3′-*O*-Thiocarbonylene-5′-*O*-*tert*-butyldimethylsilyl inosine (9c). A mixture of **8c** (78.2 mg, 0.202 mmol) and 1,1′-thiocarbonyl diimidazole (54.0 mg, 0.303 mmol) in 1,2-dichloroethane (5.0 mL) was refluxed for 3 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (20:1 CH_2Cl_2–MeOH) to afford **9c**[20b] (74.5 mg, 86%) as a colorless waxy solid; R_f 0.47 (15:1 CH_2Cl_2–MeOH); ^{13}C NMR ($CDCl_3$) [see Table 6], others δ 190 (C=S), 159.4 (C=O), 155.8 (quat C), 148.7 (quat C), 146.6 (C2), 138.3 (C8), 126.2 (quat C), 26.5 ((CH_3)$_3$CSi), 12.6 (CH_3Si), −5.2 (CH_3Si); MS: *m/z* 430 $[M+H]^+$.

4.1.18. D-[$^{13}C_5$]-(2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-*tert*-butyldimethylsilylthymidine (10a). A solution of **9a** (74.2 mg, 0.177 mmol) in triethyl phosphite (3.0 mL) was refluxed for 3 h. After completion of the reaction, the excess triethyl phosphite was removed in vacuo. The residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to afford **10a**[20b] (31.7 mg, 52%) as a colorless waxy solid. This product was engaged directly into the next step without any further characterization. R_f 0.47 (8:1 CH_2Cl_2–MeOH).

4.1.19. D-[$^{13}C_5$]-(2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-*tert*-butyldimethylsilyladenosine (10b). A solution of **9b** (208 mg, 0.488 mmol) in triethyl phosphite (6.0 mL) was refluxed for 3 h. After completion of the reaction, the excess triethyl phosphite was removed in vacuo. The residue was purified by silica gel column chromatography (30:1 CH_2Cl_2–MeOH) to afford **10b**[20b] (145 mg, 85%) as a colorless solid; R_f 0.49 (8:1 CH_2Cl_2–MeOH); mp 118–120°C, lit.[20b] mp 117–121°C; ^{13}C NMR ($CDCl_3$) [see Table 6], others δ 153.7 (C2), 142.5 (C8), 133.2 (quat C), 26.2 ((CH_3)$_3$CSi), 12.3 (CH_3Si), −4.9 (CH_3Si); MS: *m/z* 353 $[M+H]^+$.

4.1.20. D-[$^{13}C_5$]-(2′,3′-Didehydro-2′,3′-dideoxy-5′-*O*-*tert*-butyldimethylsilylinosine (10c). A solution of **9c** (63.3 mg, 0.148 mmol) in triethyl phosphite (4.5 mL) was refluxed for 3 h. After completion of the reaction, the excess triethyl phosphite was removed in vacuo. The residue was purified by silica gel column chromatography (20:1 CH_2Cl_2–MeOH) to afford **10c**[20b] (30.1 mg, 57%) as a colorless waxy solid; R_f 0.38 (10:1 CH_2Cl_2–MeOH); ^{13}C NMR ($CDCl_3$) [seeTable 6], others δ 157.4 (C=O), 146.9 (C2), 143.1 (C8), 24.1 ((CH_3)$_3$CSi), 13.8 (CH_3Si), −4.9 (CH_3Si); MS: *m/z* 354 $[M+H]^+$.

4.1.21. D-[$^{13}C_5$]-2′,3′-Dideoxy-5′-*O*-*tert*-butyldimethylsilyladenosine (11b). A mixture of **10b** (145 mg, 0.415 mmol) and 10% Pd/C (15.0 mg) in 0.5% triethylamine–MeOH (15 mL) was stirred under hydrogen atmosphere for 3 h. The reaction mixture was filtered through a celite pad and washed with MeOH (10 mL×2), the filtrate and the washings were combined. After evaporation of the solvent, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to afford **11b** (141 mg, 97%) as a colorless waxy solid; R_f 0.71 (5:1 CH_2Cl_2–MeOH); ^{13}C NMR (MeOD-*d*3) [see Table 6], others δ 156.9 (C=O), 154.2 (C2), 140.7 (quat C), 137.2 (C8), 118.6 (quat C), 22.3 ((CH_3)$_3$CSi), 13.5 (CH_3Si), −3.7 (CH_3Si); MS *m/z* 355 $[M+H]^+$.

4.1.22. D-[$^{13}C_5$]-2′,3′-Dideoxy-5′-*O*-*tert*-butyldimethylsilylinosine (11c). A mixture of **10c** (29.9 mg, 0.0846 mmol) and 10% Pd/C (10.0 mg) in 0.5% triethylamine–MeOH (7.5 mL) was stirred under hydrogen atmosphere for 3 h. The reaction mixture was filtered through a celite pad and washed with MeOH (10 mL×2), the filtrate and the washings were combined. After evaporation of the solvent, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to afford **11c** (28.4 mg, 95%) as a colorless waxy solid; R_f 0.35 (10:1 CH_2Cl_2–MeOH); ^{13}C NMR ($CDCl_3$) [see Table 6], others δ 161.8 (C=O), 149.7 (quat C), 147.2 (C2), 141.4 (C8), 126.2 (quat C); 23.4 ((CH_3)$_3$CSi), 11.9 (CH_3Si), −5.1 (CH_3Si); MS: *m/z* 356 $[M+H]^+$.

4.1.23. D-[$^{13}C_5$]-2′,3′-Didehydro-2′,3′-dideoxythymidine (d4T, 12a). To a solution of **10a** (31.7 mg, 0.092 mmol) in THF (5.0 mL) at 0°C was added TBAF (1.0 M solution in THF, 184 μL, 0.184 mmol) with stirring. The mixture was warmed to room temperature and subsequently stirred for 40 min. After evaporation of the solvent, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to give **12a**[28] (15.0 mg, 71%) as a

colorless powder; R_f 0.23 (10:1 CH_2Cl_2–MeOH); mp 164–166°C (lit.[28] mp 165–166°C); ^{13}C NMR ($CDCl_3$) [see Table 6], others δ 163.9 (C═O), 150.6 (C═O), 136.8 (C6), 110.7 (quat C), 12.3 (CH_3); HRESIMS *m/z* 252.0863 calcd for $(^{13}C_5)C_5H_{12}N_2O_4Na$ $[M+Na]^+$, found 252.0865.

4.1.24. D-[$^{13}C_5$]-2′,3′-Dideoxyadenosine (ddA, 12b). To a solution of **11b** (183 mg, 0.52 mmol) in THF (15 mL) at 0°C was added TBAF (1.0 M solution in THF, 1.04 mL, 1.04 mmol) with stirring. The mixture was warmed to room temperature and subsequently stirred for 40 min. After evaporation of the solvent, the residue was purified by silica gel column chromatography (10:1 CH_2Cl_2–MeOH) to give **12b**[29] (118 mg, 95%) as a colorless powder; R_f 0.57 (5:1 CH_2Cl_2–MeOH); mp 186–188°C, lit.[29] mp 185–187°C; ^{13}C NMR (CD_3OD) [see Table 6], others δ 157.3 (quat C), 153.5 (C2), 149.8 (quat C), 141.0 (C8), 120.5 (quat C); HRESIMS *m/z* 263.1135 calcd for $(^{13}C_5)C_5H_{13}N_5O_2Na$ $[M+Na]^+$, found 263.1134; R_f 0.57 (5:1 CH_2Cl_2–MeOH).

4.1.25. D-[$^{13}C_5$]-2′,3′-Dideoxyinosine (ddI, 12c). To a solution of **11c** (27.1 mg, 0.0763 mmol) in THF (4.5 mL) at 0°C was added TBAF (1.0 M solution in THF, 153 μL, 0.153 mmol) with stirring. The mixture was warmed to room temperature and subsequently stirred for 40 min. After evaporation of the solvent, the residue was purified by silica gel column chromatography (6:1 CH_2Cl_2–MeOH) to give **12c**[30] (15.4 mg, 84%) as a colorless powder; R_f 0.53 (5:1 CH_2Cl_2–MeOH); mp 183–185°C, lit.[30] mp 184–186°C; ^{13}C NMR (CD_3OD) [see Table 6], others δ 156.7 (C═O), 148.3 (quat C), 144.3 (C2), 138.1 (C8), 123.5 (quat C); HRESIMS *m/z* 264.0975 calcd for $(^{13}C_5)C_5H_{12}N_4O_3Na$ $[M+Na]^+$, found 264.0973.

Acknowledgements

This work was supported in part grant by the Agence Nationale de Recherches sur le SIDA (ANRS), and by the SIDACTION-ECS.

References

1. Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Nusinoff Lehrman, S.; Bolegnesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl Acad. Sci. USA* **1986**, *83*, 8333–8337.
2. Preey, C. M.; Noble, S. *Drugs* **1999**, *58*, 1099–1135, and references cited therein.
3. Molina, J.-M.; Chene, G.; Ferchal, F.; Journot, V.; Pellegrin, I.; Sombardier, M.-N.; Rancinan, C.; Cotte, L.; Madelaine, I.; Debord, T.; Decazes, J.-M. *J. Inf. Dis.* **1999**, *180*, 351–358.
4. (a) Gelpi, E. *J. Chromatogr., A* **1995**, *703*, 59–80. (b) Maurer, H. H. *J. Chromatogr., B: Biomed. Sci. Appl.* **1998**, *713*, 3–25.
5. (a) Font, E.; Lasanta, S.; Rosario, O.; Rodriguez, J. F. *Nucleosides Nucleotides* **1998**, *17*, 845–853. (b) Jajoo, H. K.; Bennett, S. M.; Kornhauser, D. M. *J. Chromatogr.* **1992**, *577*, 299–304. (c) Esmans, E. L.; Broes, D.; Hoes, I.; Lemière, F.; Vanhoutte, K. *J. Chromatogr., A* **1998**, *794*, 109–127.
6. (a) Pruvost, A.; Becher, F.; Bardouille, P.; Guerrero, C.; Creminon, C.; Delfraissy, J.-F.; Goujard, C.; Grassi, J.; Benech, H. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1401–1408. (b) Becher, F.; Pruvost, A.; Goujard, C.; Guerreiro, C.; Delfraissy, J.-F.; Grassi, J.; Benech, H. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 555–565.
7. (a) Font, E.; Rosario, O.; Santana, J.; Garcia, H.; Sommadossi, J.-P.; Rodriguez, J. F. *Antimicrob. Agents Chemother.* **1999**, *43*, 2964–2968. (b) Rodriguez, J. F.; Rodriguez, J. L.; Santana, J.; Garcia, H.; Rosario, O. *Antimicrob. Agents Chemother.* **2000**, *44*, 3097–3100.
8. (a) Cahours, X.; Dessans, H.; Morin, Ph.; Dreux, M.; Agrofoglio, L. A. *J. Chromatogr., A* **2000**, *895*, 101–109. (b) Cahours, X.; Tran, T. T.; Mesplet, N.; Kieda, C.; Morin, Ph.; Agrofoglio, L. A. *J. Pharm. Biomed. Anal.* **2001**, *26*, 819–827. (c) Cahours, X.; Morin, Ph.; Dessans, H.; Agrofoglio, L. A. *Electrophoresis* **2002**, *23*, 88–92.
9. (a) St Claire, R. L. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1625–1634. (b) Fung, E. N.; Cai, Z.; Burnette, T. C.; Sinhababu, A. K. *J. Chromatogr., B: Biomed. Sci. Appl.* **2001**, *754*, 285–295.
10. (a) La François, C. J.; Fujimoto, J.; Sowers, L. C. *Chem. Res. Toxicol.* **1998**, *11*, 75–83. (b) Dizdaroglu, M. *FEBS Lett.* **1993**, *315*, 1–6. (c) Hamberg, M.; Zhang, L. Y. *Anal. Biochem.* **1995**, *229*, 336–344. (d) Polson, A. G.; Crain, P. F.; Pomerantz, S. C.; McCloshey, J. A.; Bass, B. L. *Biochemistry* **1991**, *30*, 11507–11514. (e) Schwandt, H. J.; Wu, A. T.; Finn, C.; Sadee, W. *Nucleic Acid Chem.* **1978**, *2*, 901–915.
11. Agrofoglio, L. A.; Jacquinet, J.-C.; Lancelot, G. *Tetrahedron Lett.* **1997**, *38*, 1411–1412.
12. (a) Lagoja, I. M.; Herdewijn, P. *Synthesis* **2002**, *3*, 301–314, and references cited therein. (b) Milecki, J. *J. Label. Compd. Radiopharm.* **2002**, *45*, 307–337, and references cited therein.
13. Xie, M.; Berges, D. A.; Robins, M. *J. Org. Chem.* **1996**, *61*, 5178–5179.
14. Milecki, J.; Zamaratski, E.; Maltseva, T. V.; Földesi, A.; Adamiak, R. W.; Chattopadhyaya, J. *Tetrahedron* **1999**, *55*, 6603–6622.
15. Niedballa, U.; Vorbrüggen, H. *Angew. Chem. Int. Ed. Engl.* **1970**, *9*, 461–462.
16. Huryn, D. M.; Okabe, M. *Chem. Rev.* **1992**, *92*, 1745–1768, and references cited therein.
17. (a) Prisbe, E. J.; Martin, J. C. *Synth. Commun.* **1985**, *15*, 401–409. (b) Kim, C.-H.; Marquez, V. E.; Broder, S.; Mitsuya, H.; Driscoll, J. S. *J. Med. Chem.* **1987**, *30*, 862–866. (c) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.* **1987**, *30*, 1270–1278. (d) Sekine, M.; Nakanishi, T. *J. Org. Chem.* **1990**, *55*, 924–928.
18. (a) Crank, G.; Eastwood, F. W. *Aust. J. Chem.* **1964**, *17*, 1392–1398. (b) Josan, J. S.; Eastwood, F. W. *Aust. J. Chem.* **1968**, *21*, 2013–2020.
19. Mattocks, A. R. *J. Chem. Soc.* **1964**, 1918–1930.
20. (a) Corey, E. J.; Winter, R. A. E. *J. Am. Chem. Soc.* **1963**, *85*, 2677–2678. (b) Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. *J. Org. Chem.* **1989**, *54*, 2217–2225.
21. (a) Quant, S.; Wechselberger, R. W.; Wolter, M. A.; Wörner, H.-H.; Schell, P.; Engels, J. W.; Griesinger, C.; Schwalbe, H. *Tetrahedron Lett.* **1994**, *35*, 6649–6652. (b) Glen, W. L.; Myers, G. S.; Grant, G. R. *J. Chem. Soc.* **1951**, 2568–2572. (c) Stevens, J. D. *Methods Carbohydr. Chem.* **1972**, *6*, 123–128.
22. Murray, D. H.; Prokop, J. In *Synthetic Procedures in Nucleic*

Acid Chemistry. Zorbach, W., Tipson, S., Eds.; Interscience: New York, 1968; p 193.
23. Baker, D. C.; Horton, D. R.; Tindall, C. G. *Carbohydr. Res.* **1972**, *24*, 192–197.
24. Sinhababu, A. K.; Bartel, R. L.; Pochopin, N.; Borchardt, R. T. *J. Am. Chem. Soc.* **1985**, *107*, 7628–7632.
25. Tsutsumi, H.; Kawai, Y.; Ishido, Y. *Carbohydr. Res.* **1979**, *73*, 293–296.
26. Ma, T.; Pai, S. B.; Zhu, Y. L.; Lin, J. S.; Shanmuganathan, K.; Du, J.; Wang, C.; Kim, H.; Newton, M. G.; Cheng, Y. C.; Chu, C. K. *J. Med. Chem.* **1996**, *39*, 2835–2843.
27. (a) Beaton, G.; Jones, A. S.; Walker, R. T. *Tetrahedron* **1988**, *44*, 6419–6428. (b) Robins, M. J.; Lewandowska, E.; Wnuk, S. F. *J. Org. Chem.* **1988**, *63*, 7375–7381.
28. Mansuri, M. M.; Starrett, J. E.; Ghazzouli, I.; Hitchcock, M. J.; Sterzycki, R. Z.; Brankovan, V.; Lin, T.-S.; August, E. M.; Prusoff, W. H.; Sommadossi, J.-P.; Martic, J. C. *J. Med. Chem.* **1989**, *32*, 461–466.
29. Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.* **1984**, *25*, 367–370.
30. Saito, I.; Ikehira, H.; Kasatami, R.; Watanabe, M.; Matsuura, T. *J. Am. Chem. Soc.* **1986**, *108*, 3115–3117.