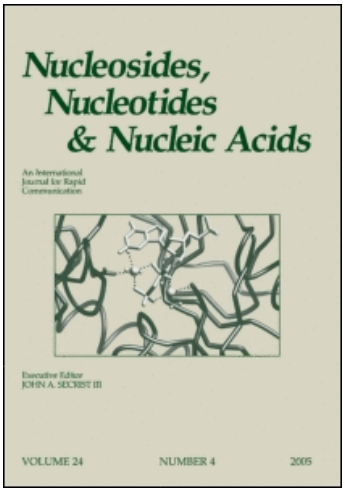
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

SYNTHESIS AND IN VITRO CYTOTOXIC AND ANTIVIRAL ACTIVITIES OF 1-(2,5,6-TRIDEOXY-6-HALOGENOHEPT-5-ENOFURANURONONITRILE)THYMINE AND DERIVATIVES

Jean M. J. Tronchet[a]; Frederic Chalard[a]; Elisabeth Rivara-Minten[a]; Michel Seman[b]; Erik De Clercq[c]; Jan Balzarini[c]; Pierre Dilda[b]
[a] Department of Organic Pharmaceutical Chemistry, Sciences II, University of Geneva, Geneva, Switzerland [b] Research Department, Laboratoires Mayoly Spindler, Chatou Cedex, France [c] Rega Institute for Medical Research, Leuven, Belgium

Online publication date: 18 April 2002

To cite this Article Tronchet, Jean M. J. , Chalard, Frederic , Rivara-Minten, Elisabeth , Seman, Michel , De Clercq, Erik , Balzarini, Jan and Dilda, Pierre(2002) 'SYNTHESIS AND IN VITRO CYTOTOXIC AND ANTIVIRAL ACTIVITIES OF 1-(2,5,6-TRIDEOXY-6-HALOGENOHEPT-5-ENOFURANURONONITRILE)THYMINE AND DERIVATIVES', Nucleosides, Nucleotides and Nucleic Acids, 21: 3, 191 — 206
To link to this Article: DOI: 10.1081/NCN-120003285
URL: http://dx.doi.org/10.1081/NCN-120003285

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 21(3), 191–206 (2002)

SYNTHESIS AND IN VITRO CYTOTOXIC AND ANTIVIRAL ACTIVITIES OF 1-(2,5,6-TRIDEOXY-6-HALOGENOHEPT-5-ENOFURANURONONITRILE)THYMINE AND DERIVATIVES

Jean M. J. Tronchet,[1] Frederic Chalard,[1] Elisabeth Rivara-Minten,[1] Michel Seman,[2] Erik De Clercq,[3] Jan Balzarini,[3] and Pierre Dilda[2]

[1]Department of Organic Pharmaceutical Chemistry, University of Geneva, Sciences II, CH-1211 Geneva 4, Switzerland
[2] Laboratoires Mayoly Spindler, Research Department, 6, Avenue de l'Europe, F-78401 Chatou Cedex, France
[3]Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven

Downloaded At: 11:44 26 January 2011

ABSTRACT

All 1-(2,5,6-trideoxy-6-halogenohept-5-enofuranurononitrile)thymine and their 3′-O-TBDMS derivatives have been prepared and their configuration established. Some of these compounds are endowed with a cytotoxic or cytostatic activity in cell culture. The single most important factor affecting the cytotoxicity of these compounds is the presence on the molecule of a soft (electrofugal) halogen atom.

INTRODUCTION

The 1-cyanovinyl group, a soft electrophile, fixed onto blocked sugars or ribonucleosides has been shown to bear cytotoxic and/or antiviral properties [1,2] somewhat dependent on its electrophilicity[3]. We describe here the fixation of a positional isomer of this group, a 2-cyanovinyl, and all its common 2-halogeno derivatives on the 5′ position of a more biologically promising host molecule, the 2′-deoxythymidine. We have thus prepared a

Copyright © 2002 by Marcel Dekker, Inc. www.dekker.com

complete series of the 1-(2,5,6-trideoxy-6-halogenohept-5-enofuranurononitrile)thymine and their 3′-O-*tert*-butyldimethylsilyl derivatives, assigned their E/Z configuration and reported the spectroscopic properties of each geometrical isomer. This allowed the study of the influence of E/Z configuration and of the nature of the halogen atom upon spectroscopic and biological properties of these molecules.

Concerning the antiproliferative activities, as the importance of a *tert*-butyldimethylsilyl group has been previously established [4], the preparation of these compounds allowed a comparison of the biological properties of the protected and unprotected nucleoside analogues. On the other hand, when the halogen atom is notably electrofugal, two electrophilic sites exist in the molecule, the 5′ carbon atom and the halogen atom. The biological testing of these compounds could indicate which of these electrophilic centers plays the major role in determining the antiproliferative properties of the molecules.

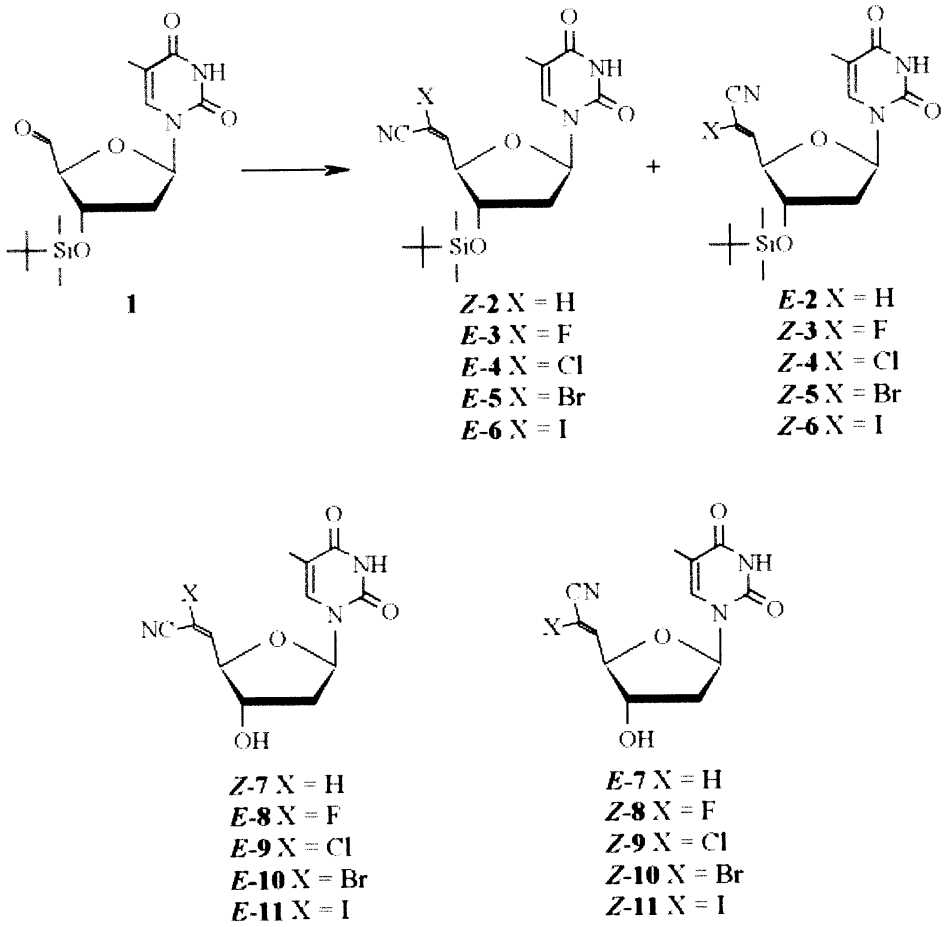
RESULTS AND DISCUSSION

Compound **1** [5,6] has been submitted to Wittig reactions with cyanomethylenetriphenylphosphorane [7,8] and the series of the common cyanohalogenomethylenetriphenylphosphoranes [9] to lead to compounds **2–6** (Scheme, Table 1).

Compounds ***E*-2** and ***Z*-2** were directly isolated from the reaction mixture whereas the formation of **4–6** was accompanied by some dehalogenation leading to ***E*-2** (3–10%) which could not be removed by chromatography. Compounds **4–6** were then de-*O*-silylated to **9–11** respectively, then re-*O*-silylated after chromatographic removal of ***E*-2**. The separation of *E* and *Z* isomers was performed on the 3′-*O*-silylated derivatives. In the case of the fluoro derivatives **3**, they could not be separated from some excess of reagent. They had to be de-*O*-silylated then re-*O*-silylated to carry out the separation of the geometrical isomers, the geometrical isomers of deprotected compounds **8–11** exhibiting very similar chromatographic behaviors. This constitutes one of the reasons for not using a one pot oxidation/olefination of unprotected 2′-deoxythymidine as recently described in the case of a Wittig reaction using benzoylmethylenetriphenylphosphorane [10]. Furthermore, the one pot reaction is not compatible with the cyanohalogenomethylenetriphenylphosphoranes and afforded a poor yield of **2** (24%) when using cyanomethylenetriphenylphosphorane. A modification of the procedure, implying the completion of the oxidation step before the olefination led to yields equal (**2**, 46%) or inferior (**9**, 20%, **11**, 26%) to those obtained using the multistep procedure.

The *E-Z* configurational assignments were straightforward for compounds **2** and **3** where the *trans* vicinal couplings $J_{5',6'}$ and $J_{5',F}$ respectively were much larger than the *cis* ones. For **4**, **5**, and **6**, the assignments were

Downloaded At: 11:44 26 January 2011



Scheme.

based on the fact that H-5′ should be more shielded when *cis* to the cyano group [11]. This was confirmed by the effect of the configuration on the ^{13}C NMR shielding of C-5′ and C-6′ for compounds bearing a large halogen atom, C-5′ being more shielded and C-6′ deshielded in *E* isomers [12]. All these

Table 1. Synthesis of Unsaturated Nitriles **2–6**

Compound	**2**	**2(a)**	**3**	**4**	**5**	**6**
Yield	54	68	ca. 20	72	77	74
%E	89	94	ca. 80	74	64	59

[a] With addition of benzoic acid.

Downloaded At: 11:44 26 January 2011

considerations using chemical shifts are reinforced by the fact that compounds **4–6** all adopt globally the same conformation about the C-4′-C-5′ bond, H-4′ and H-5′ being almost antiperiplanar (8.0 Hz $< J_{4',5'} >$ 9.0 Hz). Finally, the configuration of **6** was definitively established by the NMR ^{13}C-^{1}H coupling (13) between the cyano group and H-5′ (13.9 Hz for ***E*-6** and 7.3 Hz for ***Z*-6**) confirming the assignments of the whole series.

The nucleoside analogues have been evaluated for their cytotoxic and cytostatic concentrations against a variety of human (CEM, NCI-H460, HT29, Caki-1, HL60) or murine (L1210) cancer cell lines and normal human cell line (MRC5) (Table 2). Regarding these cytotoxic and antiproliferative tests, **1** and other non-halogenated compounds (***Z*-2**, ***E*-2**, (***Z*+*E*)-7**) did not show any significant inhibitory activity. Except for the fluoride derivatives (***E*-3**, ***Z*-3**), the halogenated 3′-*O*-silylated nucleosides exhibited both cytotoxic and cytostatic properties on most of the cancer cell lines tested. Moreover, the ratio between IC_{50} for non-tumoral (MRC5) and murine leukemia L1210 and human lymphoblast CEM tumoral cells which is representative of an in vitro selectivity is fairly good for these compounds (***E*-4**, ***E*-5**, ***E*-6**, ***Z*-4**, ***Z*-5**, ***Z*-6**). The ***Z*-5** bromide derivative is 5.3 and 10.6 times more active than its *E* isomer on NCI-H460 and HL-60 cancer cell lines, respectively. Caki-1 is the least sensitive cell line tested for this class of derivatives. The *ratio* between CC_{50} and IC_{50} on CEM cells of ***Z*-4** (2.5), ***Z*-5**

Table 2. Cytotoxic[a] and Cytostatic[b] Activities of Nucleoside Derivatives **1–11** (Concentrations Expressed as μM)

Compd	CEM CC_{50}	CEM IC_{50}	MRC5 IC_{50}	L1210 IC_{50}	NCI-H460 IC_{50}	HT29 IC_{50}	Caki-1 IC_{50}	HL-60 IC_{50}
1	>50	>50	>100	nd	39	>100	100	36
***Z*-2**	>50	18	>100	30	32	57	>100	31
***E*-3**	>50	41	>100	43	70	>100	>100	66
***E*-4**	0.62	0.47	3.1	0.43	1.7	3	>100	3.2
***E*-5**	0.18	0.16	3.1	0.21	2.3	2.9	28	3.2
***E*-6**	0.95	0.56	3	0.24	2.4	3.1	28.5	2.4
***E*-2**	27	14	49	24	30	37	>100	31
***Z*-3**	9.4	3.9	23	3.1	20	31	>100	21
***Z*-4**	0.42	0.17	3	0.47	1.7	3.1	32	3
***Z*-5**	0.18	0.04	1.8	0.12	0.43	2.7	25	0.3
***Z*-6**	0.58	0.15	4.4	1.8	2.2	9	29	4.4
(***Z*+*E*)-7**	>50	>50	>100	39	>100	>100	>100	>100
(***Z*+*E*)-8**	>50	>50	>100	35	>100	>100	>100	>100
(***Z*+*E*)-9**	14	5.2	32	9.1	30	34	>100	31
(***Z*+*E*)-10**	8.7	4.1	29	7.1	30	34	>100	22
(***Z*+*E*)-11**	23	16	33	23	35	49	>100	35

[a]CC_{50} or 50% cytotoxic concentration of the test compound.
[b]IC_{50} or 50% cytostatic concentration of the test compound.

Downloaded At: 11:44 26 January 2011

(4.5), and ***Z*-6** (3.8) reveals that these molecules are more cytostatic than cytotoxic. This is not true for the corresponding *E* isomers: ***E*-4** (1.3), ***E*-5** (1.1), and ***E*-6** (1.7). As the unprotected compounds (***Z*+*E*)-8**, (***Z*+*E*)-9**, (***Z*+*E*)-10**, and (***Z*+*E*)-11** are far less active against CEM and other cancer cell lines than their *O*-silylated analogues, it is evident that both the 3′-*O*-TBDMS and the cyanohalogenovinyl groups are required to confer pronounced antiproliferative activities and interesting selectivity indices for these compounds. It is clear from Table 2 that the major factor imparting cytotoxic or cytostatic activities to these modified nucleosides is the presence of a soft (electrofugal) halogen atom on the cyanovinyl group.

The nucleoside derivatives were also evaluated for their antiviral activity against HIV-1(III_B), HSV-1(KOS), and HSV-2(G) and vaccinia virus (VV). None exhibited EC_{50} significantly different from CC_{50} on CEM and E1SM cells in the HIV and HSV/VV determinations, respectively. Compounds **7–11** (as *E*/*Z* mixtures) were tested against vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus in Hela cell cultures, and against Parainfluenza-3 virus, Reovirus-1, Sindbis virus and Punta Toro virus in Vero cell cultures. None of these compounds exhibited any significant activity against any of these viruses. ***E*-2** and ***Z*-2** exhibited border line activity against CMV (AD169 and Davis strains, EC_{50} 10–20 μM, CC_{50} 50–100 μM in Hela cell cultures).

Downloaded At: 11:44 26 January 2011

EXPERIMENTAL

General Synthetic Methods (6)

300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR were measured on a VARIAN Gemini BB300 spectrometer.

Biological testing See reference (6).

1-(*Z*-3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxyhept-5-enofuranurononitrile)-thymine (*Z*-2). Cyanomethylenetriphenylphosphorane (2.12 g, 7.05 mmol) was dissolved in anhydrous benzene (25 mL). To this solution was added drop wise a solution of **1** in anhydrous benzene (15 mL). The mixture was stirred at 20 °C for 20 h then submitted to column chromatography (1:1 petroleum ether/ethyl acetate) to afford ***E*-2** (600 mg, 56%) and ***Z*-2** (60 mg, 6%) as white powders: mp 145.6–146.4 °C; R_F 0.3 (3:2 petroleum ether/ethyl acetate); $[\alpha]_D^{29}$+45.8° (*c* 0.4, MeOH). UV (MeOH) 208 (12,700), 264 (7500). IR (KBr) 3184 (NH), 3051 (=C-H), 2956, 2931, and 2858 (-C-H), 2225 (CN), 1700–1680 cm^{-1} (CO and C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.10 (bs, 1 H, NH), 7.01 (q, 1 H, $J_{Me\text{-}5,6}$ 1.2 Hz, H-6), 6.60 (*dd*, 1 H, $J_{5',6'}$ 11.3 Hz, $J_{4',5'}$ 8.2 Hz, H-5), 6.00 (*t*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 7 Hz, H-1′), 5.55 (*dd*, 1 H, $J_{4',6'}$ 0.9 Hz, H-6′), 4.70 (*ddd*, 1 H, $J_{3',4'}$ 4.9 Hz, H-4′), 4.40 (*dt*, 1 H, $J_{2'a,3'}$ 7 Hz, $J_{2'b,3'}$

4.9 Hz, H-3′), 2.55 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.2 Hz, Ha-2′), 2.35 (*ddd*, 1 H, Hb-2′), 1.95 (*d*, 3 H, Me-5), 0.9 (s, 9 H, CMe_3), 0.10 (s, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.90 and −4.80 ($SiMe_2$), 12.4 (Me-5), 17.8 (**C**Me_3), 25.6 (C**Me**$_3$), 40.0 (C-2′), 75.6 (C-3′), 84.8 and 88.2 (C-1′ and C-4′), 102.0 (C-6′), 111.1 (C-5), 115.0 (CN), 137.1 (C-6), 149.6 (C-5′), 150.2 (C-2), and 164.0 (C-4). EIMS *m/z* (%): 73 (100), 168 (73), 320 (94, $M^{\bullet+}$-*t*-Bu), and 362 (2, $M^{\bullet+}$-Me).

Anal. Calcd for $C_{18}H_{27}N_3O_4Si$ (377.52): C, 57.27; H, 7.21; N, 11.13. found: C, 57.33; H, 7.40; N, 10.67.

1-(*E*-3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxyhept-5-enofuranurononitrile)-thymine (*E*-2). Cyanomethylenetriphenylphosphorane (2.12 g, 7.05 mmol) and benzoic acid (300 mg, 2.54 mmol) were dissolved in anhydrous benzene (25 mL). To this solution was added drop wise a solution of **1** (1 g, 2.82 mmol) in anhydrous benzene (15 mL). The mixture was stirred at 20 °C for 20 h. The reaction mixture was concentrated and the residue extracted with dichloromethane (30 mL). The organic phase washed with saturated aqueous sodium hydrogenocarbonate (50 mL) then with saturated aqueous sodium chloride (50 mL), dried (Na_2SO_4), concentrated, was submitted to column chromatography (1:1 petroleum ether/ethyl acetate) to afford ***E*-2** (680 mg, 64%) and ***Z*-2** (40 mg, 4%).: mp 100.5–105.2 °C; R_F 0.45 (3:2 petroleum ether/ethyl acetate); $[\alpha]_D^{31}$+45.2° (*c* 0.5, $CHCl_3$). UV (MeOH) 209 (21,100) and 264 (10,400). IR (KBr): 3190 (NH), 3056 (=C-H), 2955, 2930, and 2857 (-C-H) 2227 (CN), and 1700–1680 (CO and C=C). ^1H NMR ($CDCl_3$, 300 MHz): δ 8.40 (*bs*, 1 H, NH), 6.95 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz, H-6), 6.75 (*dd*, 1 H, $J_{5',6'}$ 16.5 Hz, $J_{4',5'}$ 4.7 Hz, H-5′), 6.15 (*t*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.6 Hz, H-1′), 5.60 (*dd*, 1 H, $J_{4',6'}$ 1.6 Hz, H-6′), 4.25 (*m*, 2 H, H-4′ and H-3′), 2.60 (*t*, 2 H, H-2′), 1.92 (*d*, 3 H, Me-5), 0.87 (*s*, 9 H, CMe_3), 0.06 (*s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.8 and −4.7 ($SiMe_2$). 12.6 (Me-5), 17.8 (**C**Me_3), 25.6 (C**Me**$_3$), 39.7 (C-2′), 74.7 (C-3′), 84.9 and 86.0 (C-1′ and C-4′), 101.0 (C-6′), 111.7 (C-5), 116.5 (CN), 135.5 (C-6), 149.8 (C-5′), 150.0 (C-2′), and 163.4 (C-4). EIMS *m/z* (%): 73 (100), 168 (55), 201 (30), and 320 (10, $M^{\bullet+}$-*t*-Bu).

Anal. Calcd for $C_{18}H_{27}N_3O_4Si$ (377.52): C, 57.27; H, 7.21; N, 11.13. Found: C, 57.13; H, 7.20; N, 11.11.

1-(*Z*-3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-fluorohept-5-enofuranurono-nitrile)thymine (*Z*-3). To a solution of triphenylphosphine (1,4g, 5.36 mmol) in anhydrous xylene (25 mL), (chlorocyanofluoromethyl)phenylmercury (9) (2,79 g, 7.61 mmol) was added. The mixture was heated to 120 °C and **1** (1 g, 2.82 mmol) was added under vigorous stirring and heated under refluxing for 24 h, cooled and poured into dichloromethane (100 mL). The organic phase was washed [saturated aqueous ammonium chloride (60 mL), saturated aqueous sodium hydrogenocarbonate (60 mL) then water

Downloaded At: 11:44 26 January 2011

(60 mL)], dried (Na_2SO_4), concentrated, and submitted to two successive column chromatographic separations (1:200 methanol/diethyl ether, then 1:1 petroleum ether/ethyl acetate) to give 200 mg (17.7%) of a mixture of ***E*-3** and ***Z*-3** as a white solid contaminated with phenylmercury chloride. To a solution of this solid (200 mg, 0.5 mmol) in tetrahydrofurane (1.5 mL), 80% V/V/acetic acid (3 mL) and 0.5 M hydrochloric acid (0.3 mL) were added. After 20 h at 25 °C, the solution was coevaporated with toluene (2×20 mL) and submitted to column chromatography (1:20 methanol/diethyl ether) to afford 105 mg (77%) of (***E*+*Z*)-8** which were 3′-*O*-*tert*-butyldimethyl-silylated (TBDMSOTf/pyridine) then submitted to column chromato-graphy (1:1 petroleum ether/ethyl acetate) to afford 25 mg of ***Z*-3**, 20 mg of (***E*+*Z*)-3** and 80 mg of ***E*-3** (total yield 80%): mp 159.4–161.7 °C; R_F 0.48 (1:1 petroleum ether/ethyl acetate); $[\alpha]_D^{28}$ +22.5° (*c* 0.2, MeOH). UV (MeOH) 208 (18,400) and 262 (9500). IR (KBr): 317.4 (NH), 3046 (=CH), 2956, 2932 and 2858 (-C-H), 2236 (CN), and 1693 (CO and C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.70 (*s*, 1 H, NH), 7.00 (*d*, 1 H, $J_{Me\text{-}5,6}$ 1.3 Hz, H-6), 6.05 (*dd*, 1 H, $J_{F,5'}$ 32.5 Hz, $J_{4',5'}$ 8.5 Hz, H-5′), 5.95 (*dd*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.6 Hz, H-1′), 4.80 (*ddd*, 1 H, $J_{3',4'}$ 4.0 Hz, $J_{F,4'}$ 1.3 Hz, H-4′), 4.40 (*dt*, 1 H, $J_{2'a,3'}$ 6.4 Hz, $J_{2'b,3'}$ 4.0 Hz, H-3′), 2.50 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, Ha-2′), 2.30 (*ddd*, 1 H, Hb-2′), 1.95 (*d*, 3 H, Me-5), 0.90 (*s*, 9 H, CMe_3), 0.1 (*s*, 6 H, $SiMe_2$). ^{13}C NMR $CDCl_3$, 75 MHz): δ −4.9 and −4.8 ($SiMe_2$), 12.5 (Me-5), 17.9 (**C**Me_3), 25.6 (C**Me**$_3$), 39.7 (C-2′), 76.0 (*d*, $^3J_{F,4'}$ 30 Hz, C-4′), 80.0 (C-3′), 88.7 (C-1′), 111.36 (C-5), 111.39 (*d*, $^2J_{F,CN}$ 69.0 Hz, CN), 123.8 (*d*, $^2J_{F,5'}$ 14.0 Hz, C-5′), 133.0 (*d*, $^1J_{F,6'}$ 375.0 Hz, C-6′), 136.9 (C-6), 149.9 (C-2), 163.5 (C-4). ^{19}F NMR ($CDCl_3$, 188 MHz): δ_{C6F6} 36.3 (*bd*, $J_{F,6'}$ 32.5 Hz). EIMS *m*/*z* (%): 73 (100), 127 (44), 201 (44), 270 (17), and 338 (29,$M^{\cdot+}$-CMe_3).

Anal. Calcd for $C_{18}H_{26}FN_3O_4Si$ (395.51): C, 54,66; H, 6.63; N, 10.62. Found: C, 54.78; H, 6.66; N, 10.57.

1-(*E*-3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-fluorohept-5-enofuranurononitrile)thymine (*E*-3). Prepared as described for ***Z*-3**; mp 179.0–180.0 °C; R_F 0.46 (1:1 petroleum ether/ethyl acetate); $[\alpha]_D^{29}$ +53.5° (*c* 0.35, MeOH). UV (MeOH): 207 (16,200), 264 (8600). IR (KBr): 3166 (NH), 3033 (=C-H), 2956, 2933, and 2856 (-C-H), 2222 (CN), 1699–1683 (CO and C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.70 (*bs*, 1 H, NH), 7.00 (*d*, 1 H, $J_{Me\text{-}5,6}$ 1.3 Hz, H-6), 6.35 (*dd*, 1 H, $J_{F,5'}$ 12.8 Hz, $J_{4',5'}$ 9.0 Hz, H-5′), 5.95 (*dd*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 7.3 Hz, H-1′), 4.45 (*m*, 2 H, H-4′ and H-3′), 2.50 (*m*, 1 H, $J_{2'a,2'b}$ 13.6 Hz, Ha-2′), 2.30 (*ddd*, 1 H, Hb-2′), 1.95 (*d*, 3 H, Me-5), 0.90 (*s*, 9 H, CMe_3), and 0.10 (*s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.7 and −4.8 ($SiMe_2$), 12.6 (Me-5), 18.0 (CMe_3), 25.8 (CMe_3), 40.0 (C-2′), 76.0 (C-3′), 82.2 (*d*, $^3J_{F,4'}$ 6.3 Hz, C-4′), 89.1 (C-1′), 110.5 (*d*, $^2J_{F,CN}$ 47.0 Hz, CN), 111.4 (C-5), 123.7 (*d*, $^2J_{F,5'}$ 17.7 Hz, C-5′),

Downloaded At: 11:44 26 January 2011

134.5 (*d*, $^{1}J_{F,6'}$ 248.2 Hz, C-6′), 137.6 (C-6), 150.3 (C-2), and 164.3 (C-4). ^{19}F NMR ($CDCl_3$, 188 MHz): δ_{C6F6} 51.9 (*bd*, $J_{F,6'}$ 13.5 Hz).

Anal. Calcd for $C_{18}H_{26}FN_3O_4Si$ (395.51): C, 54.66; H, 6.63; N, 10.62. Found: C, 54.54; H, 6.62; N, 10.56.

1-(*Z*-3-*O*-*tert*-Butyldimethylsilyl-6-chloro-2,5,6-trideoxyhept-5-enofuranurononitrile)thymine (*Z*-4). To a solution of chlorocyanomethylenetriphenylphosphorane (3.2 g, 9.62 mmol) in anhydrous benzene (20 mL) was added drop wise at 30 °C a solution of **1** (1.3 g, 3.7 mmol) in anhydrous benzene (15 mL). After 18 h stirring at 30 °C, the reaction mixture was concentrated, then submitted to column chromatography (1:1 petroleum ether/ethyl acetate) to afford a 13:27:3 mixture (1.2 g) of ***Z*-4**, ***E*-4** and ***E*-2** (^{1}H NMR). To a solution of this mixture (120 mg) in tetrahydrofurane (0.5 mL), 80% (V/V) aqueous acetic acid (1 mL) and 0.1 M aqueous HCl (0.5 mL) were added. After 24 h at 25 °C, the solvents were coevaporated with toluene (2 × 20 mL) and the concentrated reaction mixture submitted to column chromatography (1:20 MeOH/Et_2O) to give (***E*+*Z*)-9** (70 mg, 81%). To a solution of the (***E*+*Z*)-9** mixture (460 mg, 1.55 mmol) in anhydrous pyridine (14 mL) *tert*-butyldimethylsilyltriflate (1.06 mL, 4.65 mmol) was added and the reaction mixture stirred at 25 °C for 14 h, concentrated by coevaporation of the solvents with toluene (2 × 50 mL). The residue was dissolved in dichloromethane (50 mL) and the organic solution washed [water (50 mL) then saturated, aqueous sodium chloride (50 mL)], dried (Na_2SO_4), concentrated and submitted to column chromatography (1:1 petroleum ether/ethyl acetate) to give pure ***E*-9** and ***Z*-9** (total yield 450 mg, 70%) which were recrystallized from 1:9 petroleum ether/ethyl acetate: mp 203.2–205.2 °C; R_F 0.46 (1:1 petroleum ether/ethyl acetate); $[\alpha]_D^{33}$ +44.1 °(*c* 0.4, $CHCl_3$). UV (MeOH) 214 (16,700), 264 (9700). IR (KBr) 3179 (NH), 3041 (=C-H), 2956, 2930, and 2858 (-C-H), 2223 (CN), and 1699 – 1680 (CO and C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.40 (*bs*, 1 H, NH), 7.01 (*d*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz, H-6), 6.85 (*d*, 1 H, $J_{4',5'}$ 8.0 Hz, H-5′), 5.90 (*t*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.5 Hz, H-1′), 4.80 (*dd*, 1 H, $J_{3',4'}$ 4.0 Hz, H-4′), 4.60 (*dt*, 1H, $J_{2'a,3'}$ 6.6 Hz, $J_{2'b,3'}$ 4.0 Hz, H-3′), 2.55 (*dt*, 1 H, $J_{2'a,2'b}$ 13.5 Hz, Ha-2′), 2.35 (*ddd*, 1 H, Hb-2′), 1.90 (*d*, 1 H, Me-5), 0.90 (*s*, 9 H, CMe_3), and 0.10 (*s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ – 4.9 and – 4.8 ($SiMe_2$), 12.5 (Me-5), 17.9 (**C**Me_3), 25.6 (C**Me**$_3$), 39.8 (C-2′), 76.0 (C-3′), 83.2 and 89.5 (C-1′ and C-4′), 106.2 (C-6′), 111.3 (C-5), 114.4 (CN), 137.3 (C-6), 144.5 (C-5′), 150.0 (C-2), and 163.9 (C-4). EIMS *m*/*z* (%); 73 (100), 202 (44), 286 (7), 354 (7, $M^{\cdot +}$ -CMe_3).

Anal. Calcd for $C_{18}H_{26}ClN_3O_4Si$ (411.96): C, 52.48; H, 6.36; N, 10.20. Found: C, 52.35; H, 6.33; N, 10.20.

1-(*E*-3-*O*-*tert*-Butyldimethylsilyl-6-chloro-2,5,6-trideoxyhept-5-enofuranurononitrile)thymine (*E*-4). Prepared as described for ***Z*-4**: mp 221.0–

Downloaded At: 11:44 26 January 2011

222.2 °C; R_F 0.43 (1:1 petroleum ether/ethyl acetate); $[\alpha]_D^{30}$ +76.6° (*c* 0.4, $CHCl_3$). UV (MeOH): 212 (16,400), 264 (12,500). IR (KBR): 3179 (NH), 3047 (=C-H), 2955, 2930, and 2857 (-C-H), 2228 (CN), 1710 – 1680 (CO and C=C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.35 (*bs*, 1 H, NH), 7.01 (*s*, 1 H, H-6), 6.70 (*d*, 1 H, $J_{4',5'}$ 9.0 Hz, H-5′), 5.90 (*dd*, 1 H, $J_{1',2'a}$ 5.5 Hz, $J_{1',2'b}$ 7.4 Hz, H-1′), 4.54 (*dd*, 1 H, $J_{3',4'}$ 5.2 Hz, H-4′), 4.43 (*dt*, 1 H, $J_{2'a,3'}$ 7.1 Hz, $J_{2'b,3'}$ 5.2 Hz, H-3′), 2.55 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, Ha-2′), 2.35 (*ddd*, 1 H, Hb-2′), 1.90 (*s*, 3 H, Me-5), 0.90 (*s*, 9 H, CMe_3), and 0.10 (*s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.9 and −4.6 ($SiMe_2$), 12.4 (Me-5), 17.8 (**C**Me_3), 25.6 (C**Me**$_3$), 39.8 (C-2′), 75.6 (C-3′), 84.5 and 88.9 (C-1′ and C-4′), 106.1 (C-6′), 111.2 (C-5), 112.8 (CN), 137.6 (C-6), 144.8 (C-5′), 150.1 (C-2), 164.2 (C-4). EIMS *m*/*z* (%): 57 (100), 202 (35), 295 (4), and 354 (29, $M^{\bullet+}$ CMe_3).

Anal. Calcd for $C_{18}H_{26}ClN_3O_4Si$ (411.96): C, 52.48; H, 6.36; N, 10.20. Found: C, 52.20; H, 6.30; N, 10.13.

1-(*Z*-6-Bromo-3-*O*-*tert*-butyldimethylsilyl-2,5,6-trideoxy-5-enofuranurononitrile)thymine (*Z*-5). To a solution of bromocyanomethylenetriphenylphosphorane (2.16 g, 5.64 mmol) in anhydrous benzene (25 mL) was added drop wise at 30 °C a solution of **1** (1 g, 2.82 mmol) in anhydrous benzene (15 mL). After 24 h stirring at 30 °C and column chromatography (1:1 petroleum ether/ethyl acetate) a mixture (950 mg) of **5** (18:32 *E*/*Z*, 70%) and ***E*-2** (5%, NMR) was obtained. The mixture (680 mg) was de-*O*-silylated as described for ***Z*-4** to give (***E*+*Z*)-10** (350 mg, 69%) which was directly 3′-*O*-*tert*-butyldimethylsilylated as described for ***Z*-4** to give ***E*-5** and ***Z*-5** (total yield 88%) which were recrystallized from 1:9 petroleum ether/ethyl acetate: mp 218.2–218.9 °C; R_F 0.50 (3:2 petroleum ether/ethyl acetate); $[\alpha]_D^{28}$ +24.6° (*c* 0.4, MeOH). UV (MeOH): 214 (16,600) and 262 (12,500). IR (KBr): 3178 (NH), 3032 (=C-H), 2954, 2930, and 2856 (-C-H), 2228 (CN), 1700 – 1680 (CO and C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.10 (*bs*, 1 H, NH), 7.08 (*d*, 1 H, $J_{4',5'}$ 8.2 Hz, H-5′), 7.02 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.2 Hz, H-6), 6.00 (*t*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.5 Hz, H-1′), 4.70 (*dd*, 1 H, $J_{3',4'}$ 3.9 Hz, H-4′), 4.50 (*dt*, 1 H, $J_{2'a,3'}$ 6.7 Hz, $J_{2'b,3'}$ 3.9 Hz, H-3′), 2.55 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.4 Hz, Ha-2′), 2.35 (*ddd*, 1 H, Hb-2′), 1.95 (*d*, 3 H, Me-5), 0.90 (*s*, 9 H, CMe_3), 0.10 (*s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.80 ($SiMe_2$), 12.4 (Me-5), 17.0 (**C**Me_3), 25.6 (C**Me**$_3$), 39.8 (C-2′), 75.8 (C-3′), 85.2 and 89.0 (C-1′ and C-4′), 93.0 (C-6′), 112.0 (C-5), 116.0 (CN), 137.2 (C-6), 147.6 (C-5′), 151.0 (C-2), and 164.0 (C-4). EIMS *m*/*z* (%): 73 (100, 246 and 248 (32), 295 (10), 398 and 400 (7, $M^{\bullet+}$ -CMe_3).

Anal. Calcd for $C_{18}H_{26}BrN_3O_4Si$ (456.42): C, 47.37; H, 5.74; N, 9.21. Found: C, 47.65; H, 5.79; N, 9.04.

1-(*E*-6-Bromo-3-*O*-*tert*-butyldimethylsilyl-2,5,6-trideoxyhept-5-enofuranurononotrile)thymine (*E*-5). Prepared as described for ***Z*-5**: mp 221.0–223.0 °C; R_F 0.50 3:2 petroleum ether/ethyl acetate); $[\alpha]_D^{31}$ +67° (*c* 0.4,

Downloaded At: 11:44 26 January 2011

$CHCl_3$). UV (MeOH): 213 (13,900) and 260 (11,250). IR (KBr): 3177 (NH), 3048 (=C-H), 2955, 2930, and 2857 (-C-H), 2222 (CN), 1691–1680 (CO and C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.00 (*bs*, 1 H, NH), 7.05 (*s*, 1 H, H-6), 6.90 (*d*, 1 H, $J_{4',5'}$ 8.8 Hz, H-5′), 5.90 (*dd*, 1 H, $J_{1',2'a}$ 5.8 Hz, $J_{1',2'b}$ 7.1 Hz, H-1′), 4.60 (*dd*, 1 H, $J_{3',4'}$ 5.0 Hz, H-4′), 4.50 (*td*, 1 H, $J_{2'a,3'}$ 7.1 Hz, $J_{2'b,3'}$ 5.2 Hz, H-3′), 2.55 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.5 Hz, Ha-2′), 2.35 (*ddd*, 1 H, Hb-2′), 1.95 (*s*, 3 H, Me-5), 0.90 (*s*, 9H, CMe_3), 0.07 and 0.06 (2 *s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.9 and −4.8 ($SiMe_2$), 12.4 (Me-5), 17.8 (**C**Me_3), 25.6 (C**Me**$_3$), 39.8 (C-2′), 75.3 (C-3′), 85.5 and 88.8 (C-1′ and C-4′), 90.2 (C-6′), 111.1 (C-5), 113.5 (CN), 137.6 (C-6), 148.9 (C-5′), 150.1 (C-2), 164.3 (C-4). EIMS *m/z* (%): 73 (100), 246 and 248 (22), 295 (9), 398 and 400 (22, $M^{\cdot+}$ -CMe_3).

Anal. Calcd for $C_{18}H_{26}BrN_3O_4Si$ (456.42): C, 47.37; H, 5.74; N, 9.21. Found: C, 47.12; H, 5.76; N, 9.18.

1-(*Z*-3-*O*-*tert*-Butyldimethylsilyl-1,5,6-trideoxy-6-iodohept-5-enofuranurononitrile)thymine (*Z*-6). To a solution of cyanoiodomethylenetriphenylphosphorane (1.2 g, 2.82 mmol) in anhydrous benzene (15 mL) was drop wise added at 50°C a solution of **1** (500 mg, 1.41 mmol). After 1 h of stirring at 50°C, then 14 h stirring at 30°C, the concentrated reaction mixture was submitted to column chromatography (1:1 petroleum ether/ethyl acetate) to afford a mixture (500 mg) of **6** (59:41 *Z*/*E*, 80%) and ***E*-2** (5%, NMR). This mixture was de-*O*-silylated as described for ***Z*-4** to give (***E*+*Z*)-11** (417 mg, 94%) which was directly 3′-*O*-*tert*-butyldimethylsilylated as described for ***Z*-4** to give ***Z*-6** and ***Z*-6** (total yield 72%) which were crystallized from 1:9 petroleum ether/ethyl acetate: mp 226.6–227.5°C; R_F 0.42 (3:2 petroleum ether/ethyl acetate); $[\alpha]_D^{32}$ +51.0° (*c* 0.4, $CHCl_3$). UV (MeOH) 209 (13,900), 261 (14,400); IR (KBr) 3180 (NH), 3037 (=C-H), 2954, 2930, and 2856 (-C-H), 2220 (CN), and 1690 (CO and C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.30 (*bs*, 1 H, NH), 7.05 (*d*, 1 H, $J_{4',5'}$ 8.0 Hz, H-5′), 7.03 (*s*, 1 H, H-6), 5.90 (*t*, 1 H, $J_{1',2'a} = J_{1'2'b}$ 6.6 Hz, H-1′), 4.50 (*dd*, 1 H, $J_{4',5'}$ 8.0 Hz, $J_{3',4'}$ 3.8 Hz, H-4′), 4.40 (*ddd*, 1 H, $J_{2'a,3'}$ 6.3 Hz, $J_{2'b,3'}$ 4.1 Hz, $J_{3',4'}$ 3.8 Hz, H-3′), 2.50 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.5 Hz, Ha-2′), 2.30 (*ddd*, 1 H, Hb-2′), 1.90 (*s*, 3 H, Me-5), 0.90 (*s*, 9 H, CMe_3), 0.08 and 0.06 (2 *s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ −4.70 ($SiMe_2$), 12.5 (Me-5), 17.9 (CMe_3), 25.7 (CMe_3), 40.0 (C-2′), 61.8 (C-6′), 75.8 (C-3′), 89.0 and 89.1 (C-4′ and C-1′), 111.3 (C-5), 117.2 (CN), 137.1 (C-6), 150.2 (C-2), 153.7 (C-5′), and 164.2 (C-4). EIMS *m/z* (%): 73 (100), 75 (98), 201 (15), 294 (27), 320 (14), 378 (8), and 446 (11, $M^{\cdot+}$ -CMe_3).

Anal. Calcd for $C_{18}H_{26}IN_3O_4Si$ (503.42): C, 42.95; H, 5.21; N, 8.35. Found: C, 42.74; H, 5.18; N, 8.29.

1-(*E*-3-*O*-*tert*-Butyldimethylsilyl-2,5,6-trideoxy-6-iodohept-5-enofuranurononitrile)thymine(*E*-6). Prepared as described for ***Z*-6**: mp 213.2–214.0°C; R_F

Downloaded At: 11:44 26 January 2011

0.42 (3:2 petroleum ether/ethyl acetate; $[\alpha]_D^{32}$ +61.0° (*c* 0.5, $CHCl_3$). UV (MeOH): 204 (17800) and 261 (18800). IR (KBr): 3184 (NH), 3044 (=C-H), 2954, 2926, and 2857 (-C-H), 2212 (CN), 1691 (CO and C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.50 (*bs*, 1 H, NH), 7.15 (*d*, 1 H, $J_{4',5'}$ 8.8 Hz, H-5′), 7.00 (*s*, 1 H, H-6) 5.90 (*dd*, 1 H, $J_{1',2'a}$ 5.6 Hz, $J_{1',2'b}$ 7.4 Hz, H-1′), 4.50 (*dd*, 1 H $J_{3',4'}$ 5.3 Hz, H-4′), 4.40 (*dt* 1 H, $J_{2'a,3'}$ 7.0 Hz, $J_{2'b,3'}$ 5.3 Hz, H-3′), 2.50 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.1 Hz, Ha-2′), 2.30 (*ddd*, 1 H, Hb-2′), 1.90 (*s*, 3 H, Me-5), 0.90 (*s*, 9 H, CMe_3), 0.08 and 0.06 (2 *s*, 6 H, $SiMe_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ − 4.8 (Si Me_2), 12.4 (Me-5), 17.6 (CMe_3), 25.6 (C**Me**$_3$), 39.8 (C-2′), 55.8 (C-6′), 75.1 (C-3′), 86.8 (C-4′), 88.5 (C-1′), 111.1 (C-5), 115.5 (CN), 137.4 (C-6), 150.0 (C-2), 157.0 (C-5′), and 164.2 (C-4). EIMS *m*/*z* (%): 73 (100), 294 (32), 320 (14), 378 (7), and 446 (55, $M^{\cdot+}$-CMe_3).

Anal. Calcd for $C_{18}H_{26}IN_3O_4Si$ (503.42): C, 42.95; H, 5.21; N, 8.35. Found: C, 42.76; H, 5.34; N, 8.31.

De-*O*-silylation of compounds 2–6. To a solution of one of the compounds **2–6** (100–120 mg) in tetrahydrofurane (0.5 mL), 80% aqueous acetic acid (1 mL) and 0.5 M aqueous hydrochloric acid (0.1 mL) soultions were added. After 20 h stirring at 25°C, the reaction mixture was concentrated by coevaporation with toluene (2 × 30 mL), then submitted to column chromatography (1:20 methanol/diethyl ether) to give one of the compounds **7–11**.

1-(*Z*-2,5,6-Trideozyhept-5-enofuranurononitrile)thymine (*Z*-7). Obtained by de-*O*-silylation of ***Z*-2** (110 mg) as 60 mg (78%) of a white solid: mp 178.0–178.7°C; R_F (0.1 (1:20 MeOH/Et_2O);$[\alpha]_D^{31}$ +134° (*c* 0.4, MeOH). UV (MeOH): 204(16200) and 265 (9280). IR(KBr): 3288 (OH), 3204 (NH), 3064 (=C-H), 2938 (-C-H), 2230 (CN), 1699-1665 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): δ 10.0 (*bs*, 1 H, NH), 7.47 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz, H-6), 6.77 (*dd*, 1 H, $J_{5',6'}$ 11.3 Hz, $J_{4',5'}$ 8.8 Hz, H-5′), 6.30 (*t*, 1 H, $J_{1',2'a}$ = $J_{1',2'b}$ 6.9 Hz, H-1′), 5.76 (*dd*, 1 H, $J_{4',6'}$ 1.1 Hz, H-6′), 4.88 (*bs*, 1 H, HO-3′), 4.65 (*ddd*, 1 H, $J_{3',4'}$ 4.4 Hz, H-4′), 4.46 (*ddd*, 1 H, $J_{2'a,3'}$ 6.9 Hz, $J_{2'b,3'}$ 4.7 Hz, H-3′), 2.48 (*dt*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, Ha-2′), 2.37 (*ddd*, 1 H, Hb-2′), and 1.80 (*d*, 3 H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.3 (Me-5), 39.7 (C-2′), 75.5 (C-3′), 85.9 and 86.5 (C-1′ and C-4′), 102.4 (C-6′), 111.1 (C-5), 116.0 (CN), 137.2 (C-6), 151.2 (C-3), 151.4 (C-5′), and 164.2 (C-4). EIMS (***E*+*Z*** mixture) *m*/*z* (%): 126(100), 138 (38), 181 (4), and 263 (5, $M^{\cdot+}$).

Anal. Calcd for $C_{12}H_{13}N_3O_4$ (263.26) (***E*+*Z*** mixture): C, 54.75; H, 4.98; N, 15.96. Found: C, 54.49; H, 4.96; N, 15.73.

1-(*E*-2,5,6-Trideoxyhept-5-enofuranurononitrile)thymine (*E*-7). Obtained by de-*O*-silylation of **E-2** (110 mg) as 50 mg (65%) of a white solid: mp 210.0–211.1°C; R_F 0.20 (1:20 MeOH/Et_2O); $[\alpha]_D^{32}$ +7.2° (*c* 0.4, $CHCL_3$). UV (MeOH): 209 (20500), and 264 (9800). IR(KBr): 3435 (OH), 3210

Downloaded At: 11:44 26 January 2011

(NH), 3058 (=C-H), 2931 (-C-H), 2226 (CN), 1711–1654 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): δ 9.80 (*bs*, 1 H, NH), 7.45 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz, H-6), 7.00 (*dd*, 1 H, $J_{5',6'}$ 16.5 Hz, $J_{4',5'}$ 5.2 Hz, H-5′), 6.35 (*t*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.6 Hz, H-1′), 5.83 (*dd*, 1 H, $J_{4',6'}$ 1.6 Hz, H-6′), 4.80 (*d*, 1 H, $J_{3',OH}$ 4.1 Hz, HO-3′), 4.43 (*m*, 1 H, H-3′), 4.37 (*ddd*, 1 H, $J_{4',5'}$ 5.2 Hz, $J_{3',4'}$ 4.1 Hz, H-4′), 2.40 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, $J_{2'a,3'}$ 7.1 Hz, Ha-2′), 2.25 (*ddd*, 1 H, $J_{2'b,3'}$ 4.1 H, Hb-2′), and 1.81 (*d*, 3H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.4 (Me-5), 39.1 (C-2′), 74.8 (C-3′), 85.8 and 85.9 (C-1′ and C-4′), 101.2 (C-6′), 111.4 (C-5), 117.8 (CN), 136.8 (C-6), 151.3 (C-5′), 152.4 (C-2), and 164.1 (C-4). EIMS and *Anal*. (cf ***Z*-7**).

1-[(*E*+*Z*)-2,5,6-Trideoxy-6-fluorohept-5-enofuranurononitrile)thymine (*E*+*Z*)-8. Obtained by de-*O*-silylation of (***E*+*Z*)-3** (200 mg) as 10 mg ***Z*-8**, 70 mg of a mixture of ***E*-8** and ***Z*-8**, and 25 mg of ***E*-8** (total yield 77%): R_F 0.1 (1:20 MeOH/Et_2O). IR (KBr): 3353 (OH), 3199 (NH), 3044 (=C-H), 2930 (-C-H), 2238 (CN), 1721–1652 (CO and C-C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): ***Z*-8** δ 10.20 (*bs*, 1 H, NH), 7.48 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz, H-6), 6.44 (*dd*, 1H, $J_{F,5'}$ 33.8 Hz, $J_{4',5'}$ 8.8 Hz; H-5′), 6.30 (*t*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.9 Hz; H-1′), 4.90 (*bs*,1H,HO-s′),4.77 (*ddd*, 1 H, $J_{3',4'}$ 3.6 Hz, $J_{F,4'}$ 1.1 Hz, H-4′), 4.50 (*dt*, 1 H, $J_{2'a,3'}$ 3.6 Hz, $J_{2'b,3'}$ 6.0 Hz, H-3′), 2.48 (*ddd*, 1 H, $J_{2'a,2'b}$ 12.9 Hz, Ha-2′), 2.37 (*ddd*, 1H, Hb-2′), and 1.80 (*d*, 3 H, Me-5); ***E*-8** δ 10.05 (*bs*, 1 H, NH), 7.50 (*q*, 1 H, $J_{Me\text{-}5,6}$ 0.6 Hz, H-6), 6.68 (*dd*, 1 H, $J_{F,5'}$ 14.4 Hz, $J_{4',5'}$ 9.3 Hz, H-5′), 6.30 (*dd*, 1 H, $J_{1',2'a}$ 6.6 Hz, $J_{1',2'b}$ 6.9 Hz, H-1′), 4.90 (*bs*, 1 H, HO-3′), 4.53 (*dt*, 1 H, $J_{2'a,3'}$ 6.6 Hz, $J_{2'b,3'} = J_{3',4'}$ 4.5 Hz, H-3′), 4.45 (*ddd*, 1 H, $J_{F,4'}$ 1.2 Hz, H-4′), 2.50 (*dd*, 1 H, $J_{2'a,2'b}$ 13.5 Hz, Ha-2′), 2.37 (*ddd*, 1 H, Hb-2′), and 1.80 (*d*, 3 H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): ***Z*-8** δ 11.7 (Me-5), 38.6 (C-2′), 75.0 (C-3′), 79.4 (C-4′), 86.1 (C-1′), 110.6 (C-5), 112.2 (*d*, $^2J_{F,7'}$ 47.0 Hz, CN), 125.3 (*d*, $^2J_{F,5'}$ 8.8 Hz, C-5′), 133.1 (*d*, $^1J_{F,6'}$ 247.0 Hz, C-6′), 133.0 (C-6), 150.6 (C-2), 163.2 (C-4); ***E*-8** δ 11.7 (Me-5), 38.6 (C-2′), 74.8 (C-3′), 81.5 (*d*, $^3J_{F,4'}$ 6.5 Hz, C-4′), 86.1 (C-1′), 110.5 (C-5), 110.6 (*d*, $^2J_{F,7'}$ 47.0 Hz, CN), 125.0 (*d*, $^2J_{F,5'}$ 17.1 Hz, C-5′), 133.1 (*d*, $^1J_{F,6'}$ 242.5 Hz, C-6′), 136.7 (C-6), 150.6 (C-2), 163.7 (C-4). ^{19}F NMR ($(CD_3)_2CO$, 188 MHz): ***Z*-8** δ_{C6F6} 45.1 (*dd*, $J_{F,5'}$ 33.8 Hz, $J_{F,4'}$ 1.1 Hz); ***E*-8** δ_{C6F6} 47.7 (*dd*, $J_{F,5'}$ 14.4 Hz, $J_{F,4'}$ 1.2 Hz). EIMS *m*/*z* (%): 126 (100), 138 (38), 181 (4), and 263 (5, $M^{\cdot+}$).

Anal. Calcd for $C_{12}H_{12}FN_3O_4$ (281.24): C, 51.25; H, 4.30; N, 14,94. Found: C, 51.04; H, 4.42; N, 14.69.

1-(*Z*-6-Chloro-2,5,6-trideoxyhept-5-enofuranurononitrile)thymine (*Z*-9). De-*O*-silylation of ***Z*-4**(100 mg) afforded 50 mg (70%) of ***Z*-9**: mp 188.4–189.5°C; R_F 0.3 (1:20 MeOH/Et_2O); $[\alpha]_D^{33}$+31.4° (*c* 0.4, $CHCL_3$). UV (MeOH): 214 (17400) and 264 (10450). IR (KBr): 3356 (OH), 3190 (NH), 3046 (=C-H), 2955(-C-H), 2230 (CN), 1723–1652 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): δ 10.20 (*bs*, 1 H, NH), 7.50 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz,

Downloaded At: 11:44 26 January 2011

H-6), 7.20 (*d*, 1 H, $J_{4',5'}$ 8.0 Hz, H-5′), 6.30 (*dd*, 1 H, $J_{1',2'a}$ 7.7 Hz, $J_{1',2'b}$ 6.3 Hz, H-1′), 5.00 (*d*, 1 H, $J_{HO\text{-}3',3'}$ 4.4 Hz, HO-3′), 4.80 (*dd*, 1 H, $J_{3',4'}$ 3.0 Hz, H-4′), 4.50 (*m*, 1 H, H-3′), 2.55 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, $J_{2'a,3'}$ 6.0 Hz, Ha-2′), 2.35 (*ddd*, 1 H, $J_{2'b,3'}$ 3.3 Hz, Hb-2′), and 1.80 (*d*, 3 H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.3 (Me-5), 39.5 (C-2′), 75.8 (C-3′), 83.6 and 87.3 (C-1′ and C-4′), 105.2 (C-6′), 111.3 (C-5), 115.4 (CN), 137.3 (C-6), 147.2 (C-5′), 151.3 (C-2), and 164.4 (C-4). EIMS *m/z* (%): 126 (100), 153 (44), 172 (24), and 297 (6, $M^{\cdot+}$).

Anal. Calcd for $C_{12}H_{12}ClN_3O_4$ (297.70) (*E* + *Z* mixture): C, 48.42; H, 4.06; N, 14.11. Found: C, 48.32; H, 4.10; N, 13.97.

1-(*E*-6-Chloro-2,5,6-trideoxyhept-5-enofuranurononitrile)thymine (*E*-9). De-*O*-silylation of ***E*-4** (100 mg) afforded 50 mg (70%) of ***E*-9**: mp 203.5–206.0°C; R_F 0.3 (1:20 MeOH/$Et_2$0); $[\alpha]_D^{33}$+102.2° (*c* 0.4, $CHCl_3$). UV (MeOH): 214 (18700) and 267 (12500). IR (KBr): 3326 (OH), 3176 (NH), 3054 (=C-H), 2925 (-C-H), 2236 (CN), 1716-1661 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): δ 10.10 (*bs*, 1 H, NH), 7.50 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.4 Hz, H-6), 7.06 (*d*, 1 H, $J_{4',5'}$ 8.8 Hz, H-5′), 6.30 (*dd*, 1 H, $J_{1',2'a}$ 6.6 Hz, $J_{1',2'b}$ 6.9 Hz, H-1′), 4.90 (*d*, 1 H, $J_{HO\text{-}3',3'}$ 3.3 Hz, HO-3′), 4.57 (*m*, 2 H, H-3′ and H-4′), 2.55 (*dt*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, $J_{1',2'a} = J_{2'a,3'}$ 6.6 Hz, Ha-2′), 2.35 (*ddd*, 1 H, $J_{2'b,3'}$ 4.7 Hz, Hb-2′), and 1.80 (*d*, 3 H, $J_{Me\text{-}5,6}$ 1.4 Hz, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.3 (Me-5), 39.4 (C-2′), 75.3 (C-3′), 84.9 and 87.0 (C-1′ and C-4′), 105.2 (C-6′), 111.2 (C-5), 113.8 (CN), 137.5 (C-6), 147.3 (C-5′), 151.2 (C-2), and 164.3 (C-4). EIMS and *Anal*. (cf. ***Z*-9**).

1-[*Z*-6-Bromo-2,5,6-trideoxyhept-5-enofuranurononitrile)thymine (*Z*-10). De-*O*-silylation of ***Z*-5** (120 mg) afforded 70 mg (80%) of ***Z*-10**: mp 176.3–176.5°C; R_F 0.3 (1:20 MeOH/Et_2O); $[\alpha]_D^{30}$+31.6° (*c* 0.5, $CHCl_3$). UV (MeOH): 213 (16400) and 264 (13650). IR (KBr): 3357 (OH), 3186 (NH), 3041 (=C-H), 2950 (-C-H), 2226 (CN), 1723–1651 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): δ 10.0 (*bs*, 1 H, NH), 7.50 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.1 Hz, H-6), 7.45 (*d*, 1 H, $J_{4',5'}$ 8.0 Hz, H-5′), 6.30 (*dd*, 1 H, $J_{1',2'a}$ 8.0 Hz, $J_{1',2'b}$ 6.3 Hz, H-1′), 4.98 (*d*, 1 H, $J_{HO\text{-}3',3'}$ 4.4 Hz, HO-3′), 4.70 (*dd*, 1 H, $J_{3',4'}$ 3.0 Hz, H-4′), 4.55 (*m*, 1 H, H-3′), 2.48 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.7 Hz, $J_{2'a,3'}$ 6.0 Hz, Ha-2′), 2.31 (*ddd*, 1 H, $J_{2'b,3'}$ 6.3 Hz, Hb-2′), and 1.80 (*d*, 3 H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.3 (Me-5), 39.6 (C-2′), 75.8 (C-3′), 85.8 and 87.5 (C-1′ and C-4′), 91.3 (C-6′), 111.3 (C-5), 116.2 (CN), 137.2 (C-6), 150.4 (C-5′), 151.3 (C-2), and 164.1 (C-4). EIMS *m/z* (%): 126 (100), 144 and 146 (33), 172 and 174 (33), 197 and 199 (21), 216 and 218 (17), and 341 and 343 (2.5, $M^{\cdot+}$).

Anal. Calcd for $C_{12}H_{12}BrN_3O_4$ (342.15) (*E* + *Z* mixture): C, 42.13;H, 3.54; N, 12.28. Found: C, 42.41; H, 3.80; N, 12.01.

Downloaded At: 11:44 26 January 2011

1-(*E*-6-Bromo-2,5,6-trideoxyhept-5-enofuranurononitrile)thymine (*E*-10). De-*O*-silylation of ***E*-5** (100 mg) afforded 60 mg (80%) of ***E*-10**: mp 201.5–201.7°C; R_F 0.2 (1:20 MeOH/Et_2O); $[\alpha]_D^{31}$ +82° (*c* 0.4, $CHCl_3$). UV (MeOH): 213 (12800) and 264 (14000). IR (KBr): 3326 (OH), 3174 (NH), 3050 (=C-H), 2925 (-C-H), 2230 (CN), 1715–1660 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): δ 10.0 (*bs*, 1 H, NH), 7.50 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1.4 Hz, H-6), 7.25 (*d*, 1 H, $J_{4',5'}$ 8.8 Hz, H-5′), 6.30 (*dd*, 1 H, $J_{1',2'a}$ 6 Hz, $J_{1',2'b}$ 6.9 Hz, H-1′), 4.95 (*d*, 1 H, $J_{HO\text{-}3',3'}$ 3.8 Hz, HO-3′), 4.57 (*m*, 2 H, H-3′ and H-4′), 2.55 (*ddd*, 1H, $J_{2'a,2'b}$ 13.7 Hz, $J_{2'a,3'}$ 6.9 Hz, Ha-2′), 2.35 (*ddd*, 1 H, $J_{2'b,3'}$ 4.7 Hb-2′), and 1.80 (*d*, 3 H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.3 (Me-5), 39.4 (C-2′), 75.1 (C-3′), 86.0 and 86.9 (C-1′ and C-4′), 89.5 (C-6′), 111.2 (C-5), 114.6 (CN), 137.4 (C-6), 151.2 (C-2), 151.4 (C-5′), and 164.1 (C-4). EIMS and *Anal*. (cf ***Z*-10**).

1-(*Z*-2,5,6-Trideoxy-6-iodohept-5-enofuranurononitrile)thymine (*Z*-11). De-*O*-silylation of ***Z*-6** (100 mg) afforded 75 mg (97%) of ***Z*-11**: mp 217.6–218.0°C; R_F 0.2 (1:20 MeOH/Et_2O); $[\alpha]_D^{31}$ +57° (*c* 0.4, MeOH). UV (MeOH): 206 (11000) and 257 (11300). IR (KBr): 3359 (OH), 3180 (NH), 3060 (=C-H), 2890 (-C-H), 2227 (CN), 1693–1656 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 200 MHz): δ 10.0 (*bs*, 1 H, NH), 7.50 (*s*, 1 H, H-6), 7.45 (*d*, 1 H, $J_{4',5'}$ 7.7 Hz, H-5′), 6.35 (*dd*, 1 H, $J_{1',2'a} = J_{1',2'b}$ 6.8 Hz, H-1′), 4.95 (*d*, 1 H, $J_{HO\text{-}3',3'}$ 4.1 Hz, HO-3′), 4.55 (*m*, 2 H, H-3′ and H-4′), 2.50 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.6 Hz, $J_{2'a,3'}$ 5.9 Hz, Ha-2′), 2.35 (*ddd*, 1 H, $J_{2'b,3'}$ 3.2 Hz, Hb-2′), and 1.80 (*s*, 1 H, Me-5). ^{13}C NMR ((CD3)2CO, 75 MHz): δ 12.3 (Me-5), 39.7 (C-2′), 61.4 (C-6′). 75.7 (C-3′), 87.3 and 89.7 (C-1′ and C-4′), 111.3 (C-5), 118.7 (CN), 137.1 (C-6, 151.3 (C-2), 156.2 (C-5′), and 164.1 (C-4). EIMS ($E + Z$ mixture) m/z (%): 127 (100), 192 (70), 220 (76), 245 (44), and 390 (5, $M^{\cdot+}$).

Anal. Calcd for $C_{12}H_{12}IN_3O_4$ (389.15 ($E + Z$ mixture): C, 37.04; H, 3.11; N, 10.80. Found: C, 37.49; H, 3.49; N, 10.47.

1-(*E*-2,5,6-Trideoxy-6-iodohept-5-enofuranurononitrile)thymine (*E*-11). De-*O*-silylation of ***E*-6** (100 mg) afforded 70 mg (90%) of ***E*-11**: mp 202.7–203.8°C; R_F 0.2 (1:20 MeOH/Et_2O); $[\alpha]_D^{28}$ +61° (*c* 0.4, MeOH). UV (MeOH): 206 (13600) and 261 (14000). IR (KBr): 3330 (OH), 3172 (NH), 3046 (=C-H), 2923 (-C-H), 2222 (CN), 1724-1661 (CO and C=C). ^{1}H NMR ($(CD_3)_2CO$, 200 MHz): δ 10.0 (*bs*, 1 H, NH), 7.50 (*q*, 1 H, $J_{Me\text{-}5,6}$ 0.9 Hz, H-6), 7.45 (*d*, 1 H, $J_{4',5'}$8.6 Hz, H-5′), 6.30 (*dd*, 1H, $J_{1',2'a} = J_{1',2'b}$ 6.8 Hz, H-1′), 4.95 (*d*, 1 H, $H_{o\text{-}3',3'}$ 4.5 Hz, HO-3′), 4.55 (*m*, 1 H, H-3′ and H-4′), 2.55 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.6 Hz, $J_{2'a,3'}$ 6.3 Hz, Ha-2′), 2.35 (*ddd*, 1 H, $J_{2'b,3'}$ 4.5 Hz, Hb-2′), and 1.80 (*d*, 3 H, Me-5). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): δ 12.3 (Me-5), 39.4 (C-2′), 56.0 (C-6′), 74.9 (C-3′), 86.7 and 87.5 (C-1′ and C-4′), 111.2 (C-5), 116.8 (CN), 137.3 (C-6), 151.2 (C-2), 159.1 (C-5′), and 164.1 (C-4). EIMS and *Anal*. (cf. ***Z*-11**).

Downloaded At: 11:44 26 January 2011

Downloaded At: 11:44 26 January 2011

ACKNOWLEDGMENTS

The authors thank Dr Eder for the elemental analyses and Prof. F. Gulaçar for the mass spectra. The excellent technical asistance in the virological experiments by Mrs. Ann Absillis, Mrs. Frieda De Meyer, Mrs. Anita Camps, Mrs. Lies Vandenheurck and Mrs. Anita van Lierde and in the antiproliferative experiments by Mrs Céline Joulin and Mr Pascal Aumond was highly appreciated. This work was generally supported by the Swiss National Research Foundation (grant # 20.50827-97), by the European Commission, by FORTIS/ISEP, and by the Geconcerteerde Onderzoeksacties Vlaanderen, Krediet Nr 00/12.

REFERENCES

1. Tronchet, J.M.J.; Zerelli, S.; Dolatshahi, N.; Türler, H. Influence of the Structure of the Sugar Moiety on the Cytotoxic and Antviral Properties of Sugar Electrophiles. Chem. Pharm. Bull. **1988**, *36*, 3722–3725.
2. Tronchet, J.M.J.; Pallie, K.D.; Graf-Poncet, J.; Tronchet, J.F. Antiviral and Cytotoxic Activities of α-Methyleneglycurononitriles and Nucleosides Thereof. Eur. J. Med. Chem. **1986**, *21*, 111–118.
3. Ricca, A.; Tronchet, J.M.J.; Weber, J. Structure-Activity Relationship between the 3D Distribution of the Electrophilicity of Sugar Derivatives and their Cytotoxic and Antiviral Properties. J. Comput.-Aided Mol. Design **1992**, *6*, 541–552.
4. Tronchet, J.M.J.; Grivet, C.; Grand, E.; Seman, M.; Dilda, P. Synthesis and Antiproliferative Activity of *O*-Silylated Nucleoside Triazene-*N*-Oxide Derivatives. Carbohydr. Lett. **2000**, *4*, 5–12.
5. Camarasa, M.-J.; De las Heras, F.G.; Frederico, G.; Perez-Perez, M.J. Aldol Reaction of Nucloside 5′-Carboxyaldehydes with Acetone. Synthesis of 5′-*C*-Chain Extended Thymidine Derivatives. Nucleosides Nucleotides **1990**, *9*, 533–546.
6. Tronchet, J.M.J.; Kovacs, I.; Dilda, P.; Seman, M.; Andrei, G.; Snack, R.; De Clercq, E.; Balzarini, J. Synthesis and Anti-HIV Activity of Thymidine Analogues Bearing a 4′-Cyanovinyl Group and Some Derivatives Thereof. Nucleosides Nucleotides Nucleic Ac., in press.
7. Schimenz, G.P.; Gunter, P.; Engelhard, H. Trimethoxyphenyl Derivatives. IV. The Synthesis of Cyanomethylenetriphenylphosphorane and its Reaction with Aromatic Aldehydes. Chem. Ber **1961**, *94*, 578–585.
8. Trippett, S.; Walker, D.M. Reaction of Wittig Reagents with Phenyl Isocyanate. J. Chem. Soc. **1959**, 3874–3876.
9. Tronchet, J.M.J.; Martin, O.R. Cyanohalogenomethylenetriphenylphosphoranes. Helv. Chim. Acta **1979**, *62*, 1401–1405.
10. Crich, D.; Mo, X.-S. One Post Selective 5′-Oxidation/Olefination of 2′-Deoxynucleosides. Synlett. **1999**, *1*, 67–68.
11. Metter, V.E.; Pascual, C.; Pretsch, E.; Pross, A.; Simon, W.; Sternhell, S. Estimation of the Chemical Shifts of Olefinic Protons using Additive Increments. II

The Compilation of Additive Increments for 43 Functional Groups. Tetrahedron **1069**, *25*, 691–697.
12. Savitsky, G.B.; Ellis, P.D.; Namikawa, K.; Mariel, G.E. Effect of *Cis-Trans*-Isomerism on the Carbon-13 Chemical Shifts of Some Unsymmetrically Substituted Ethylenes. J. Chem. Phys. **1968**, *49*, 2395–2404.
13. Keah, H.H.; Rae, I.D. Vicinal Carbon-Hydrogen Coupling as an Aid to Stereochemical Assignments is Substituted Propenoic Acids. Aust. J. Chem. **1993**, *46*, 1413–1419.

Received September 5, 2001
Accepted December 26, 2001

Downloaded At: 11:44 26 January 2011