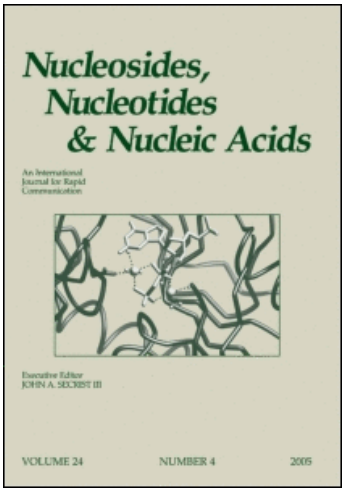

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Synthesis and Biological Evaluation of Novel 1′-Branched and Spironucleoside Analogues

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ABSTRACT

Novel anomeric spironucleosides and 1′-cyano-2′,3′-didehydro-2′,3′-dideoxyuridine, a structural analogue of known anti-HIV agents, were prepared by nucleophilic addition of organolithium reagents to 1′-cyano-2′-deoxy- and 1′-cyano-2′-deoxy-2′β-bromo-uridine derivatives, respectively. The yield and distribution of products depended on the reaction conditions, which were studied in detail. Although none of the compounds

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exhibited antiviral activity, two compounds displayed cytostatic activity against both murine leukemia and human T-lymphocyte cells.

Key Words: Nucleosides; Nucleophilic additions; Organolithium; Cyanides; Spiro compounds; Cytostatic activity.

INTRODUCTION

Branched nucleosides are attractive nucleoside derivatives due to their applications, for example as pharmacologically active psiconucleosides,[1–4] and as models for the study of radical-based nucleic acid damage.[5,6] Numerous cyano nucleosides have been synthesized and their pharmacological properties have been elucidated. 3′-*C*-cyano nucleosides are the most studied mainly due to their structural similarity to AZT and related drugs. 1′-Cyano, 2′-*C*-cyano, 4′-*C*-cyano and 5′-*C*-cyano nucleosides have been prepared and tested, in some cases demonstrating interesting biological activities.[7–9]

The cyano group may be used for further chemical manipulation due to its reactivity toward nucleophiles, and 1′-*t*-butylcarbonyl-2′-deoxyuridine **1** (Fig. 1) was prepared according to this strategy.[10] In a preliminarily report we have further shown that nucleophilic attack of organolithium reagents to the 1′-cyano group leads through an anionic cascade reaction sequence, to the formation of novel aza spironucleosides **2**.[11]

In view of the small number of reports utilizing nitriles in cascade reaction sequences[12,13] however, more work was needed to thorough explore this chemistry. We were especially interested in applying the nitrile reactivity,[14] through sequential nucleophilic additions, to the preparation of amino-spironucleosides.

Spironucleosides belong to an interesting class of conformationally restricted molecules with diverse biological activity. Pyrimidine nucleosides having a 3′-spiro moiety have recently been tested as anti-HIV agents.[15] In the case of anomeric spironucleosides, the parent compound (+)-hydantocidin and its analogues are known for their plant growth inhibitory activities.[16–18] However, other recently prepared anomeric spironucleosides[19,20] have not been examined for their antiviral or anti-cell proliferative activities.

In this paper we report a study of the anionic reactivity of the 1′-cyano function, and in particular the addition of organolithium reagents both to protected 1′-cyano-2′-deoxy-2′β-bromo uridine and 1′-cyano-2′-deoxy uridine derivatives as well as the evaluation of the antiviral and antitumoral activity of some of the spironucleosides and modified nucleosides prepared.

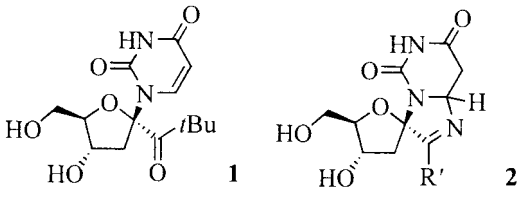
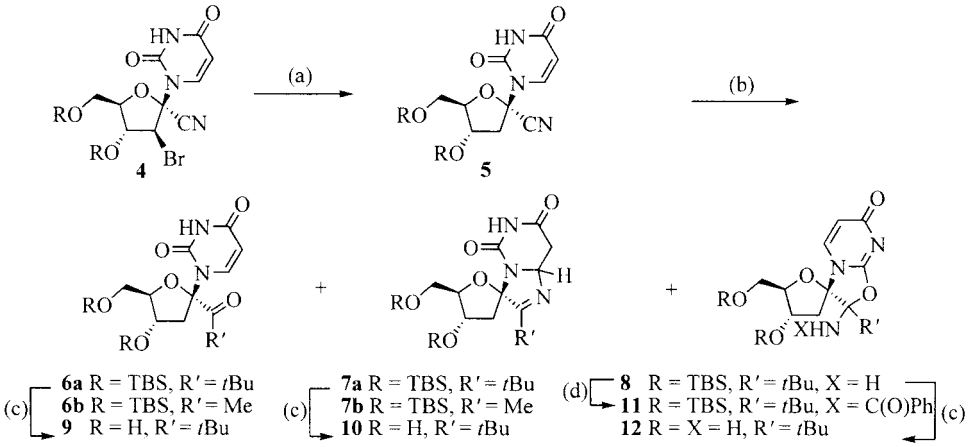


Figure 1. Cl′ modified nucleosides.



Scheme 1. Preparation of 1′-cyano-2′-deoxyuridine **5** and addition of organolithium reagents: (a) $(TMS)_3SiH$, AIBN, toluene, 80°C, 2 h, 94%. (b) *i*. R′Li, THF, −78°C, *ii*. quench. See Table 1 for conditions and yields. (c) NH_4F, CH_3OH, reflux, overnight. (d) PhCOCl, Et_3N, DMF, r.t., overnight, 82% (R = TBS).

RESULTS AND DISCUSSION

Synthesis

The Reaction of 1′-Cyano Uridine Derivative **5** with Organolithium Reagents

The synthetic transformations starting from 1′-cyano-2′-deoxy-2′β-bromo uridine **4**, prepared by a reported procedure[21] are given in Scheme 1.

Reduction of **4** in the presence of $(TMS)_3SiH$[22] under standard free radical conditions, provided the crystalline cyanide **5** in 94% yield.[a] When a solution of **5** in dry THF (0.3 M) was treated with *t*-BuLi (3 equiv), added within 3 min, at −78°C, followed by quenching after 5 min reaction with an aqueous solution of H_2SO_4 (15%), a chromatographically inseparable, 9:1 mixture of two products resulted, in a 65% combined yield. By slow crystallization from diethyl ether, the major component was separated in a 45% yield. It was visible on TLC, under UV irradiation, and the structure of the expected 1′-alkylcarbonyl product **6a** was assigned based on its spectral characteristics.[10] The minor component was obtained by desilylation of the filtrate, followed by careful column chromatography (30% CH_2Cl_2 in CH_3CN). To this minor component, which did not give crystals satisfactory for X-ray analysis, the spironucleoside structure **10** was assigned,[11] based on the following considerations: 1) the compound was invisible on TLC by UV irradiation, 2) the ^1H NMR spectrum lacked the characteristic vinyl proton resonances of the uridine moiety, 3) a characteristic doublet of doublets centered at

[a]The reduction using Bu_3SnH was reported to give 77% yield, see Ref. [7].

Table 1. Organometallic reagent addition to **5**.[a]

Entry	Reaction time (min)	Initial conc. (M)	R′Li[b]	Quench[c]	Ratio (**6**:**7**:**8**)	Yield[d,e] %
1	5	0.3	*t*BuLi	A	90:10:0	75 (10)
2	5	0.3	MeLi	A	87:13:0	84 (12)
3	30	0.1	*t*BuLi	A	4:96:0	69
4	30	0.1	MeLi	A	6:94:0	78
5	5	0.3	*t*BuLi	B[f]	29[g]:6:65	95 (15)
6	5	0.3	*t*BuLi	B[h]	15[g]:6:79	98 (20)
7	720	0.3	*t*BuLi[i]	B	0:100:0	71 (50)

[a]All reactions were carried out at −78°C in dry THF except entry 6 (r.t.) and 7 (reflux).
[b]3 equivs used in all reactions.
[c]A: 15% aq. H_2SO_4. B: 5% aq. Li_2CO_3.
[d]Combined yield of **6**+**7**+**8** based on recovered starting material.
[e]Yield of recovered starting material in parentheses.
[f]Stirred for 1.5 h after quenching.
[g]Isolated after treatment with 1N HCl in $CHCl_3$.
[h]Stirred for 3 h after quenching.
[i]In the presence of 1.5 equiv. HMPA.

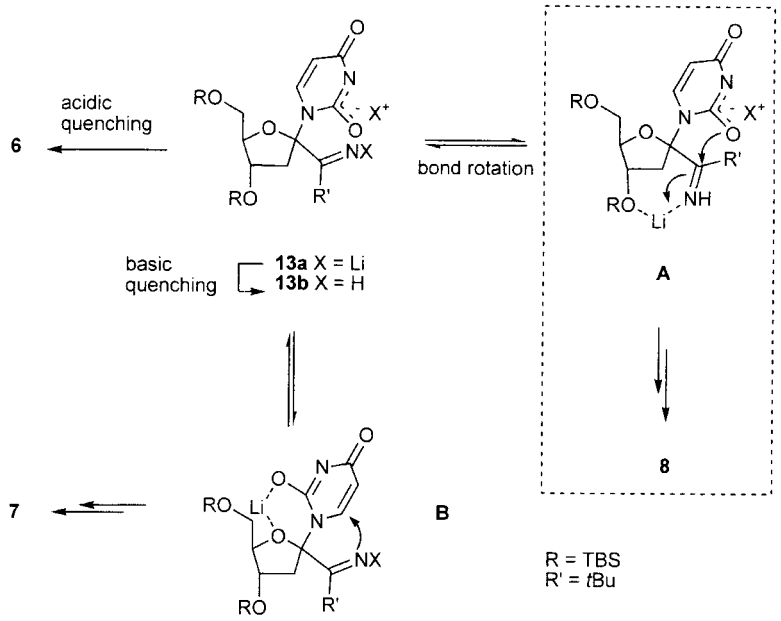
5.52 ppm could be assigned to the 6-H proton, which correlates with two doublets of doublets, centered at 2.34 and 2.96 ppm, corresponding to 5α and 5β-H, respectively, 4) a doublet centered on 2.82 ppm could be assigned to the 2′-Hs.

When MeLi was utilized under the same reaction conditions, the analogous products **6b** and **7b** were formed (entry 2, Table 1). The ^{1}H NMR spectrum of **7b** was analogous to that of **7a** with two major differences. The signal centered at 5.45 ppm corresponding to 6-H exhibited an additional small coupling with the methyl group protons and the 2′-Hs appeared now as well separated doublet of doublets ($\Delta\delta$ = 0.87 ppm), indicating a difference in the sugar conformation of the methyl analogue. On the other hand, there were no major differences in the coupling constants of the ^{1}H NMR signals between the protected **7a** and the corresponding desilylated **10**, indicating that no major conformation change had taken place upon deprotection of the spironucleoside.

Different reaction conditions influenced the yield of the products and are reported in Table 1.

Upon increasing the reaction time after addition of the organolithium reagent, a decrease in the yield of **6a** or **6b** with a concomitant increase in the yield of **7a** or **7b** was observed. In fact, the latter became the major products of the reaction when both lower concentrations of organolithium reagent and longer reaction times were applied (entries 3, 4, Table 1). Treatment of **5** with *t*-BuLi in the presence of HMPA led to the isolation of **7a** (21%) as the only product together with unreacted starting material (50%) even after prolonged reaction times (entry 7, Table 1).[23] Treatment of **5** with *t*-BuMgCl (4 equiv) and catalytic CuBr in refluxing THF, for 24 h,[24] gave no reaction (results not shown).

The quenching conditions also affected the distribution of the reaction products. Quenching with aqueous Li_2CO_3 solutions allowed for isolation of the unstable intermediate imine **13b** (Scheme 2, X = H) (entries 5, 6, Table 1). A $CHCl_3$ solution of the crude imine stirred in the presence of 1 N aqueous HCl, gave a smooth transformation to the corresponding ketone **6a**. It is worth pointing out that this quenching



Scheme 2. Proposed mechanistic scheme starting from the imine anion intermediate **13a**.

procedure proved to be a valid by-pass to the above described tedious chromatographic step needed to separate the 1′-alkylcarbonyl derivative **6** from the spironucleoside **7**.

Nevertheless, in all cases a negative mass balance was observed which led us to investigate the presence of possible polar side products. Indeed, basic quenching of the reaction mixture and flash chromatography using a 20% ethanol in ethyl acetate elution allowed the isolation of a third product in yields up to 62%. The formation of this polar compound depended upon the reaction conditions, and increased when the reaction mixture was stirred for longer periods after quenching with Li_2CO_3, concomitantly with a decrease in the yield of the corresponding ketone **6a**. The structure of the new component was assigned to the nucleoside **8**, based on its spectroscopic data as well as its chemical transformation to the corresponding benzamide **11**. The ^1H NMR spectrum contained the resonances of the two vinyl hydrogens of the base moiety, and the ^{13}C NMR spectrum exhibited three quartenary carbon resonances at 106.2, 158.6 and 173.3 ppm, which could be attributed to the carbons bearing the amine group, the C-2 and the C-4, respectively. In addition, the presence of a free amino group was confirmed from the IR spectrum of **8** and through derivatization to the corresponding benzamide **11**, the mass spectrum of which was in agreement with its molecular weight. It is worth noting that the final benzamide **11** existed as a separable 4:1 mixture of diastereomers. Finally, compound **8** was also desilylated by treatment with ammonium fluoride in MeOH at 60°C, thus affording a quantitative yield of the unprotected amine **12**, with spectral features and mass analysis in agreement with the proposed structure.

On the basis of the above reported results, a mechanistic scheme (Scheme 2) can be proposed for the addition of organolithium reagents to the uridine nucleoside **5**. Initially, a fast addition of the organolithium reagent onto the nitrile generates an

intermediate imine anion (**13a**, X = Li) which can be quenched at low temperature, under acidic conditions, to generate the alkylcarbonyl nucleoside product **6**.

The addition can be favored by using a high concentration of starting material and an excess of the organometallic reagent in order to be virtually complete within a few minutes at −78°C. Quenching under basic conditions allows the imine (**13b**) to be isolated, which is then converted to the corresponding ketone **6**, as described above. It can also be seen in Scheme 2 that the ionic repulsion between the imine and amide enolate anions should lead to either 1′*C*–*CN* or 1′*C*–N bond rotation generating rotameric forms **A** or **B**. respectively. Rotamer **A** can be further stabilized by chelate formation with the contribution of 3′-oxygen,[14] whereas in rotamer **B** metal complexation with the ring oxygen could lead to another chelate.[11] Both rotamers are predisposed to undergo intramolecular cyclization leading to different spironucleoside structures. Therefore, the imine function in intermediate **A** can undergo nucleophilic attack by the amidic enolate oxygen anion, leading to the amino spironucleoside **8**. In the chelate **B**, the imine anion can undergo intramolecular 1,4-addition to the base enone, from which the spironucleoside **7** is formed. Lower concentration and longer reaction times under strongly basic conditions favor formation of **7**, whereas higher concentrations and stirring under mildly basic conditions favors formation of spironucleoside **8**. In both cases, chelation should block rotation and lead to good diastereoselectivities, and, in fact, the anomeric spironucleosides **7** and **8** were isolated as single diastereoisomers, although a minor isomer of **8** was detected only after derivatization to the benzamide **11**. The intramolecular addition of imine anions to double bonds is a known strategy for the synthesis of nitrogen heterocycles.[25] On the other hand, the intramolecular nucleophilic addition to an imine is well known in the intermolecular version,[26] whereas an intramolecular example has been recently described.[27] Moreover, it is worth noting that in our case the nucleophile is the uridine enolate anion, which attacks the imine double bond, thus forming an oxygen heterocyclic structure, that is stable enough to be characterized and derivatized.

Despite our efforts, neither of the two spironucleosides gave good crystals for X-ray analyses. An attempt to determine the configuration of the C-6 center in **7a** through 1-D NOE experiments led to characteristics NOEs between 6-H and 5β-H or 5α-H but none with the 2′-Hs, suggesting an *R* configuration for C-6 (Fig. 2). Indeed, a

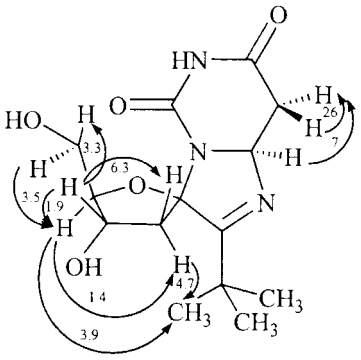
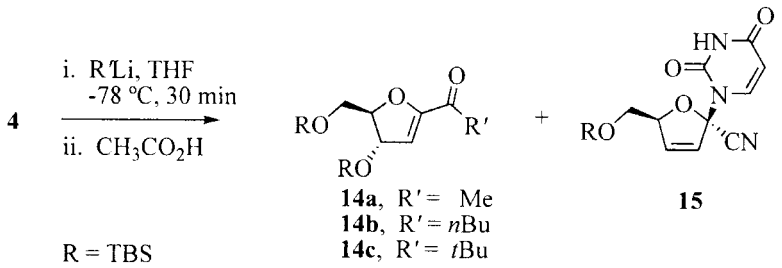


Figure 2. NOE connectivities for spironucleoside **10**.



Scheme 3. Addition of organolithium reagents to **4**.

theoretical conformational analysis of **7a**,**b** and **10** indicated that the distance between 6-H and 2′β-H in the rigid spironucleosides was consistently below 4 Å in the *S* isomer, whereas in the *R* isomer it was consistently above 4.5 Å.[b]

The calculations also predicted negligible conformational changes on going from the silyl-protected to the desilylated spironucleosides and also a substantial conformational change on going from *t*-butyl to methyl substitution with a concomitant increase in the distance between 6-H and 2′β-H, all in accord with the experimental ^{1}H NMR spectral data. For the spironucleoside **12**, the configuration of the chiral carbon atom bearing the amino group could be only inferred from a 2-D NOE experiment. Cross peaks indicated a correlation between the *t*-butyl group hydrogens and one of the 5′-H protons, thus suggesting the *S* configuration at this center.

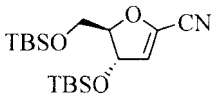
Addition of Organolithium Reagents to Protected 1′-Cyano-2′-deoxy-2′β-bromo Uridine **4**

The nucleophilic addition to 2′β-bromo-uridine derivative **4** is illustrated in Scheme 3. When **4** (0.1 M in dry THF) was treated with MeLi (3 equiv.) at −78°C for 30 min, followed by quenching with acetic acid (3 equiv.) at low temperature, standard workup and flash column chromatography of the crude product, two compounds were isolated in 42 and 38% yield and were assigned the structures of **14a** and **15**, respectively.

The same reaction was performed with *n*BuLi and *t*BuLi as the nucleophiles and yielded an ∼1:1 mixture of **14b**:**15** (73% combined yield) in the former case, whereas in the latter an ∼3:1 mixture of **14c**:**15** (85% total yield) was identified in the crude reaction mixture.

The distribution of the products was not altered by varying concentration or reaction temperature. The presence of the 2′β-bromo substituent drives the reaction to proceed first by a metal–halogen exchange. The subsequent elimination of either the 3′-TBSO substituent or the anomeric base provides the product **15** or an intermediate 2-deoxy-*D*-erythro-pent-1-enofuranosyl-cyanide (**16**) (Fig. 3).

[b]Energy minimization at the HF/6-31G level indicated a small ΔE (1.25 kcal/mole) between the *R* and *S* isomers at C-6 for **10** and a H6-H2′β distance of 3.35 Å in the *S* isomer compared with 4.65 Å in the *R* isomer.



16

Figure 3. Intermediate **16** for Scheme 3.

Whereas the former is a quaternary nitrile and therefore hindered under the reaction conditions, the latter undergoes a fast nucleophilic addition by the organolithium reagent to provide the observed abasic products **14a–c**. Indeed, the postulated intermediate cyanide **16**[c] was never observed in the crude reaction mixture, even when less than the stoichiometric amount of organolithium reagent was applied to the reaction mixture, indicating that it is more reactive than the starting material **4** towards the organolithium reagent. Elimination of the base is driven by the stabilization energy gained in the pentenofuranosyl intermediate **16** through conjugation with the 1′-cyano substituent. This could be the reason why *syn* elimination competes with the E_2-type elimination of the *anti* situated 3′-silyloxy substituent. The product of *syn* elimination becomes the major one when *t*-BuLi is used, indicating a possible interaction of this bulky organolithium with the *syn* situated nucleobase.

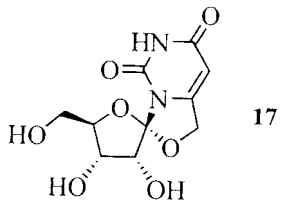
These results are interesting from a synthetic view point, since the standard conditions for the generation of the 2′,3′-didehydro-2′,3′-dideoxy- function are known to be either the reduction of 2′-bromo-3′-acetoxy-nucleosides via activated Zn/Cu couple in methanol, or an elimination process from suitable 2′,3′-disubstituted derivatives.[29–31] To our knowledge this is the first report of the utilization of organolithium reagents for the synthesis of 2′,3′-didehydro-2′,3′-dideoxy-nucleosides. Compound **15** is related to a thymine analogue, namely stavudine (d4T), approved as an anti-HIV agent and synthesized from thymidine by several methods.[32] Moreover, the antiviral activity against hepatitis B virus of an analogous compound, which does not possess the 1′-cyano function, has been previously documented.[33]

Biological Evaluation

The constant search for novel nucleoside structures that could be used as antiviral and anticancer agents,[34] combined with the interesting anti-HIV and/or anti-cell proliferative properties exhibited by known spironucleosides[15,35] prompted us to select some of the C-1′ spironucleosides obtained through our synthetic approach to be evaluated for their antiviral and antitumoral activities. In contrast to C-2′ and C-3′ spironucleosides, C-1′ spironucleosides are sterically constrained around the N-glycosidic bond and they contain the base moiety in a fixed (*syn* or *anti*) conformation. Thus, they may be considered potential tools for structure–activity relationship (SAR) studies within the same class of compounds.

In particular, we focussed on the two anomeric spironucleosides **10** and **12**, both belonging to the 2′-deoxyribo-series, in which the spiro-structure constrains the base in the *syn* and *anti* conformation, respectively.[36] The effects of a different spiro-structure

[c]Pentenofuranosyl cyanides have been reported. See Ref. [28].



17

Figure 4. Spironucleoside **17**.

and sugar residue (D-ribose) were also considered with our previously reported[20] anomeric spironucleoside **17** (Fig. 4).

Given the importance of *tert*-butyldimethylsilyl groups for the anti-HIV activity of TSAO derivatives and anti-cell proliferative properties of some *O*-silylated nucleoside compounds,[37,38] we also tested the 3′,5′-bis-*O*-silylated-amino derivative **8**.

Finally, the *O*-silylated 1′-cyano-2′,3′-didehydro-2′,3′-dideoxyuridine **15** (Scheme 3) was tested in view of the known inhibition of HIV and human hepatitis B virus (HBV)[33] by 2′,3′-dideoxy-2′,3′-didehydronucleosides.

Antiviral activity was determined against human immunodeficiency virus type 1 (HIV-1), HIV-2, Coxsackie B4 virus, respiratory syncytial virus (RSV), herpes simplex virus (HSV-1, HSV-2), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), vaccinia virus, vesicular stomatitis virus, reovirus-1, parainfluenza 3 virus, Sindbis virus and Punta Toro virus. No activity was noted against any of these viruses at subtoxic concentrations. The compounds are most likely not recognized by cellular (or viral) kinases to be converted to the potentially active metabolites.

The compounds were further evaluated for their inhibitory effects on the proliferation of murine leukemia (L1210) cells and human T-lymphocyte (Molt 4/C8 and CEM) cells. The results are shown in Table 2.

Marked cytostatic activity was noted with compound **8** bearing a *tert*-butyldimethylsilyl- substituent at both the C-3′ and C-5′ hydroxyl groups. The corresponding deprotected derivative **12** was not cytotoxic at 200 μg/mL, which clearly indicates that the toxicity observed for compound **8** could be attributed to the presence

Table 2. Anti-cell proliferative activity against murine leukemia cells (L1210) and human T-lymphocyte cells (Molt4/C8, CEM).

	IC_{50} (μM)[a]		
Compd	L1210	Molt 4/C8	CEM
8	5.95	6.30	9.46
10	>1606	>1606	>1606
12	>611	>611	>611
15	120.2	63	48.6
17	1741	1741	1741

[a]Compound concentration required to inhibit cell growth by 50%. Results are means of three independent experiments.

of the silyl groups. This notion was also supported by the cytostatic activity shown by the *O*-silylated 1′-cyano-2′,3′-didehydro-2′,3′-dideoxyuridine **15**.

EXPERIMENTAL SECTION

General Procedures. Commercially available chemicals were purchased from Aldrich and Sigma and used without further purification. Solvents were purchased from Merck (HPLC grade) and dried prior to use. Reaction courses and product mixtures were routinely monitored by TLC on 0.25 mm silica gel plates (Merck 60 F_{254} and Merck RP-18 W/UV254). Products were isolated either by preparative TLC on 2 mm RP silica gel plates (Macherey-Nagel RP 18 W/UV 254) or by chromatography on silica gel 60 (Merck, 400-230 mesh). Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian VXR (^{1}H 400 MHz, ^{13}C 100.6 MHz) spectrometer, using the solvents indicated in parentheses also as the reference peak. IR spectra were recorded on a Perkin Elmer BX FT-IR System. EI MS spectrum was recorded on a Finnigan MAT GCQ instrument equipped with a direct insertion probe DIP™. ESI MS spectra were recorded on a Micromass ZMD instrument with electrospray ionisation and a ZQ mass detector.

Preparation of 1′-cyano-2′-deoxyuridine 5. To a stirred solution of **4**[19] (6.45 g, 11.5 mmol) in toluene (25 mL) in a 50-mL round-bottomed flask equipped with a condenser, tris(trimethylsilyl)silane (5.2 mL, 17.25 mmol) and α,α′-azobis(isobutyronitrile) (200 mg, 1.22 mmol) were added. The resulting mixture was deaerated and then was stirred at 80°C for 1.5 h under a N_2 atmosphere. It was then allowed to cool at r.t., diluted to 50 mL with pentane, cooled at 0°C and filtered to yield 5.19 g (94%) of **5** as a white crystalline solid. M.p. (hexane) 181–183°C (lit.[7] 194–195°C). Spectral characteristics were identical to those previously reported.[7]

Representative Procedure for the Addition of Organolithium Reagents to 5 (Table 1, entry 6). To a magnetically stirred solution of **5** (150 mg, 0.31 mmol) in dry THF (0.5 mL), kept under argon at −78°C (solid CO_2/acetone bath), *tert*-butyllithium (0.93 mmol. 0.6 mL of a 1.6 M solution in *n*-hexane) was slowly added via a syringe. The reaction was stirred for 5 min, then quenched by adding a 5% aqueous Li_2CO_3 solution at low temperature and stirred for 3 h allowing the temperature to be raised to 20°C. The resulting solution was then partitioned with ethyl acetate and water, the organic phase was dried over anhydrous sodium sulfate and evaporated to afford an oil which was purified by silica gel chromatography (*n*-hexane with increasing amounts of ethyl acetate and then up to 20% ethyl alcohol). Three products were separated, which gave a 98% yield based on the recovery of the starting material: the spironucleoside **7a** (ethyl acetate/*n*-hexane 80/20 as eluent, 8 mg, 0.015 mmol, 5% yield) with spectral characteristics identical to those previously reported,[11] the starting material **5** (30 mg, 0.062 mmol, 20%), the unstable imine **13b** (ethyl acetate/*n*-hexane 70/30 as eluent, 20 mg, 0.04 mmol, 12%), and the spironucleoside **8** (20% ethyl alcohol in ethyl acetate as eluent, 109 mg, 0.2 mmol, 63% yield). −^{1}H NMR (acetone-d_6): δ 0.08, 0.10, 0.15, 0.16 (s, each 3H, SiMe), 0.90, 0.91 (s, each 9H, *t*BuSi), 1.25 (s, 9H, *t*Bu), 2.70 (dd, 1H, *J* = 15.0, 7.9 Hz, 2′α-H), 2.96 (dd, 1H, *J* = 15.0, 9.5 Hz, 2′β-H), 3.98 (dd, 1H, *J* = 3.3, 11.8 Hz, 5′-H), 4.05 (m, 1H, 3′-H), 4.89 (q, 1H, *J* = 8 Hz, 4′-H), 5.70 (d, 1H,

J = 7.6 Hz, 5-H), 6.62 (bs, 2H, NH_2), 7.58 (d, 1H, J = 7.6 Hz, 6-H), 9.01 (bs, 1H, NH). – ^{13}C NMR (acetone-d_6): δ −5.2, −5.0, −4.7, −4.3 (each SiMe), 18.4, 19.1 (each C), 26.1, 26.4, 26.8 (*t*Bu), 39.7 (C), 43.1 (CH_2), 62.1 (CH_2), 70.0 (CH), 88.9 (CH), 97.4 (C), 106.2 (C), 107.9 (CH), 137.3 (CH), 158.6, 173.3 (C). – IR (nujol): cm^{-1} 3197 (NH_2), 2931 (N–H), 2854 (C–H), 1662 (C = O), 1640 (C = C), 1555 (N = C), 1462, 1377, 1252, 1106, 1056, 837. – EI MS *m/z* 482 (M^+–*t*Bu), 427, 370, 238, 164, 89, 75 (100), 57, 41. ESI MS *m/z* 562 $[M + Na]^+$, 540 $[M + H]^+$ – $C_{26}H_{49}N_3O_5Si_2$ (539.86): Calcd. C, 57.84; H, 9.15; N, 7.78; found. C, 57.99; H, 9.18; N, 7.75.

Conversion of the Imine 13b to Ketone 6a. To a magnetically stirred solution of **13b** (85 mg, 0.16 mmol) in chlorofom (3 mL), aqueous HCl (1 N, 1 mL) was added and the biphasic reaction mixture was left for 45 minutes, until TLC showed the disappearance of the starting material. The reaction was transferred to a separatory funnel and the organic phase was separated, desiccated over anhydrous sodium sulfate and evaporated under vacuum, affording the expected 1′-*t*-butylketo derivative **6a** (82 mg, 0.15 mmol, 95% yield). The product crystallized by slow evaporation of an initially clear, diluted ether solution. The isolated crystals were hygroscopic and transformed through an oil to a white powder. M.p. (ether): 179–181°C. R_f (EtOAc): 0.93. –Spectral characteristics were identical to those previously reported.[10]

Typical Procedure for the Desilylation of Modified Uridines. To a solution of **6a** (1.26 g, 2.38 mmol) in MeOH (25 mL) NH_4F (1.5 g, 40 mmol) was added, and the resulting mixture was refluxed overnight. Evaporation of the solvent and flash column chromatography yielded 670 mg (90%) of **9** as a white powder. R_f (EtOAc): 0.21. It crystallizes when clean by condensing an acetonitrile solution and vigorously shaking the dense solution in the presence of ether drops. Spectral characteristics were identical to those previously reported.[5,6]

Spironucleoside 7b. Following the above reported procedure for the addition of MeLi to the cyano uridine **5** (150 mg, 0.31 mmol) and after acidic work-up, a mixture of ketone **6b** and spironucleoside **7b** was obtained (132 mg, 0.26 mmol, 84% yield), which were deprotected and characterized as already reported.[11]

Spironucleoside 10 from 7a. Desilylation of **7a** (50 mg, 0.092 mmol) following the above reported general procedure gave a crude mixture which was flash-chromatographed with 70% CH_3CN in CH_2Cl_2 thus giving pure **10** (26 mg, 0.085 mmol, 92% yield). M.p. (CH_3CN): 142–143°C. – ^{1}H NMR (acetone-d_6/D_2O: 98/2): δ 1.37 (9 H, s, *t*Bu), 2.34 (dd, 1H, J = 16.0, 12.9 Hz, 5α-H), 2.82 (d, 2H, J = 8.1 Hz, 2′-Hs), 2.96 (1 H, dd, J = 16.0, 3.9 Hz, 5β-H), 3.74 (d, 2H, J = 4.9, 5′-Hs), 4.06 (dt, 1H, J = 6.6, 4.9 Hz, 4′-H), 4.89 (td, 1H, J = 8.1, 6.6 Hz, 3′-H), 5.52 (dd, 1H, J = 12.9, 3.9 Hz, 6-H). – ^{13}C NMR (acetone-d_6): δ 29.9 (*t*Bu), 36.5 (C), 38.4, 43.7, 63.8 (each CH_2), 72.8, 78.7, 89.6 (each CH), 102.4 (C), 149.8, 170.0, 180.6 (each C). ESI MS 310, $[M–H]^-$, 333 $[M–H + Na]^-$. – $C_{14}H_{21}N_3O_5$ (311.33): Calcd. C, 54.01; H, 6.80; N, 13.50; found. C, 54.20; H, 6.81; N, 13.47.

Benzoylation of Spironucleoside 8. To a magnetically stirred solution of the spironucleoside **8** (220 mg, 0.40 mmol) in anhydrous DMF (4 mL) kept under argon, equimolar amounts of triethylamine and benzoyl chloride were added consecutively and the reaction mixture was left at room temperature overnight. TLC (ethyl acetate) indicated

that the starting material was not totally consumed. Water was added and the product was extracted with ethyl acetate (3 × 10 mL), then the combined organic extracts were desiccated with anhydrous sodium sulfate and evaporated to dryness. The crude product was chromatographed with ethyl acetate with increasing amount of methanol up to 5%. Two diastereomers were isolated as white powders, in a 4:1 ratio and 82% total yield (based on recovery of starting material) denominated major (**11a**) and minor (**11b**) and eluted in the following order: Major **11a** (88 mg, 0.136 mmol, 66%): ^{1}H NMR (acetone-d_6): δ 0.09, 0.10, 0.15, 0.16, (s, each 3H, SiMe), 0.92 (s, 18H, 2 ×*t*BuSi), 1.25 (s, 9H, *t*Bu), 2.67 (dd, 1H, *J* = 7.6, 16 Hz, 2′α-H), 2.97 (dd, 1H, *J* = 9.6, 16 Hz, 2′β-H), 3.84 (dd, 1H, *J* = 3.6, 12 Hz, 5′α-H), 3.96 (dd, 1H, *J* = 2.4, 12 Hz, 5′β-H), 4.06 (m, 1H, 4′-H), 4.87 (q, 1H, *J* = 16 Hz, 3′-H), 5.67 (d, 1H, *J* = 8.0 Hz, H-5), 6.38 (bs, 1H, NH), 7.52 (m, 2H, Ar-H), 7.56 (d, 1H, *J* = 8 Hz, 6-H), 7.63 (m, 1H, Ar-H), 8.03 (dd, 2H, *J* = 1.6, 6 Hz, Ar-H). −^{13}C NMR (acetone-d_6): δ −5.7, −5.4, −5.1 (each SiMe), 17.7 (C), 25.4, 25.7 (*t*Bu), 39.0 (C), 42.4 (CH_2), 62.1 (CH_2), 69.2 (CH), 88.2 (CH), 93.9 (C), 105.3 (C), 107.3 (C), 128.6, 128.8, 129.6, 129.7, 133.0 (each CH), 136.4 (CH), 157.9 (C = O), 165.8 (C = O), 172.2 (C). − $C_{33}H_{53}N_3O_6Si_2$ (643.96): Calcd. C, 61.55; H, 8.30; N, 6.53; found. C, 61.45; H, 8.32; N, 6.52. Minor **11b** (22 mg, 0.034 mmol, 16%): ^{1}H NMR (acetone-d_6): δ 0.09, 0.10, 0.17, 0.18, (s, each 3H, SiMe), 0.92 (s, 18H, 2 ×*t*BuSi), 1.28 (s, 9H, *t*Bu), 2.77 (dd, 1H, *J* = 7.6, 16 Hz, 2′α-H), 3.03 (dd, 1H, *J* = 9.6, 16 Hz, 2′β-H), 4.39 (m, 1H, 4′-H), 4.50 (dd, 1H, *J* = 4.8, 12.4 Hz, 5′-H), 4.70 (dd, 1H, *J* = 2.4, 12.4 Hz, 5′-H), 5.00 (q, 1H, *J* = 15.5 Hz, 3′-H), 5.49 (d, 1H, *J* = 8.0 Hz, 5-H), 6.38 (bs, 1H, NH), 7.52 (m, 2H, Ar-H), 7.56 (d, 1H, *J* = 8 Hz, 6-H), 7.63 (m, 1H, Ar-H), 8.03 (dd, 2H, *J* = 1.6, 6 Hz, Ar-H). −^{13}C NMR (acetone-d_6): δ −5.8, −5.4, −5.1 (each SiMe), 18.4 (C), 25.3, 25.8 (*t*Bu), 38.9 (C), 41.9 (CH_2), 63.3 (CH_2), 70.5 (CH), 85.3 (CH), 93.9 (C), 105.3 (C), 107.6 (C), 128.6, 128.8, 129.6, 129.7,133.5 (each CH), 137.1 (CH), 157.9 (C = O), 165.8 (C = O), 172.2 (C). − EI MS, *m/z* 627 (M^+−16), 483, 446, 147, 129, 111, 101, 83. − $C_{33}H_{53}N_3O_6Si_2$ (643.96): Calcd. C, 61.55; H, 8.30; N, 6.53; found. C, 61.40; H, 8.29; N, 6.50.

Desilylation of 8. Following the above reported procedure the spironucleoside **8** (58 mg, 0.10 mmol) was deprotected. After evaporation of the solvent, the crude was separated by PLC (chloroform: methanol 9:1 as the eluent, R_f = 0.12), thus affording pure **12** (30 mg, 0.098 mmol, 98% yield). ^{1}H NMR (DMSO-d_6): δ 0.58 (s, 9H, *t*Bu), 2.09 (m, 2H, 2′-Hs), 2.97 (d, 1H, *J* = 12 Hz, 5′-H), 3.04 (d, 1H, *J* = 12 Hz, 5′-H), 3.25 (broad s, 1H, 4′-H), 4.44 (broad s, 1H, OH), 4.98 (broad s, 1H, OH), 5.06 (d, 1H, *J* = 7.2 Hz, 5-H), 6.93 (d, 1H, *J* = 7.2 Hz, 6-H), 8.51 (bs, 2H, NH_2). −^{13}C NMR (DMSO-d_6): δ 18.8 (C), 26.4 (*t*Bu), 40.9, 63.3 (each CH_2), 68.2, 87.4, (each CH), 96.1, 103.5 (each C), 106.9 135.9 (each CH), 156.5, 172.0 (each C) − ESI MS, *m/z* 310 [M−H]$^-$, 324 [M−2H + Na]$^-$. − $C_{21}H_{25}N_3O_6$ (415.44): Calcd. C, 60.71; H, 6.07; N, 10.11; found. C, 60.79; H, 6.07; N, 10.09.

Typical Procedure for the Addition of Organolithium Reagents to 1′-cyano-2′deoxy-2′-bromo Uridine 4. To a magnetically stirred solution of **4** (100 mg, 0.18 mmol) in dry THF (5 mL), kept under argon at −78°C, *n*BuLi (0.39 mmol, 0.2 mL of a 1.96 M solution in *n*-hexane) was slowly added via syringe. The reaction was stirred for 30 min at −78°C, then quenched by adding methanol (0.5 mL), followed by sat. aqueous $NaHCO_3$ (10 mL), and ether (10 mL). The organic phase was separated, washed with H_2O (10 mL) and brine (10 mL) and was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a foam which was separated by silica gel

chromatography (5% ethyl acetate in cyclohexane with increasing amounts of ethyl acetate up to 40%). Two products were isolated in 73% total yield and eluted in the following order: compound **14b** (oil, 5% ethyl acetate in cyclohexane, 24.6 mg, 0.06 mmol, 34% yield). $-^1$H NMR ($CDCl_3$): δ 0.02, 0.05 (s, 3H each, MeSi), 0.09 (s, 6H, 2 × MeSi) 0.86, 0.88 (s, 9H each, tBuSi), 0.90 (t, 3H, J = 7.1 Hz, CH_3), 1.24–1.42 (m, 2H, CH_2), 1.49–1.67 (m, 2H, CH_2), 2.63 (t, 2H, J = 7.1 Hz, CH_2), 3.58 (dd, 1H, J = 11.8, 6.2 Hz, 5′α-H), 3.76 (dd, 1H, J = 11.8, 4.5 Hz, 5′β-H), 4.38 (m, 1H, 4′-H), 5.05 (dd, 1H, J = 3.2, 2.8 Hz, 3′-H), 5.78 (d, 1H, J = 2.8 Hz, 2′-H). $-^{13}$C NMR ($CDCl_3$): δ − 5.4, −4.5, −4.3 (each CH_3), 13.8 (CH_2), 18.1, 18.3 (each C), 22.3 (CH_2), 25.8 (2 (tBu)), 25.9 (CH_2), 38.8 (CH_2), 62.4 (CH_2), 76.2 (CH), 89.5 (CH), 110.3 (CH), 156.2, 194.3 (each C). − $C_{22}H_{44}O_4Si_2$ (428.75): Calcd. C, 61.63; H, 10.34; found. C, 61.55; H, 10.31.

Compound **15** (white powder, 40% ethyl acetate in cyclohexane, 24.8 mg, 0.07 mmol, 39% yield). $-^1$H NMR ($CDCl_3$): δ − 0.12, 0.16 (s, 3H each, MeSi), 0.81 (s, 9H, *t*BuSi), 3.78 (dd, 1H, J = 11.9, 2.7 Hz, 5′α-H), 3.95 (dd, 1H, J = 11.9, 3.1 Hz, 5′β-H), 5.31 (m, 1H, 4′-H), 5.71 (dd, 1H, J = 8.3, 1.7, 5-H), 6.30 (dd, 1H, J = 6.0, 1.4 Hz, 3′-H), 6.56 (dd, 1H, J = 6.0, 2.2 Hz, 2′-H), 7.73 (d, 1H, J = 8.3 Hz, 6-H), 8.94 (bs, 1H, NH). $-^{13}$C NMR ($CDCl_3$): δ − 5.5, − 5.4 (each CH_3), 18.3 (C), 25.7 (*t*Bu), 63.0 (CH_2), 90.7 (CH), 91.6 (C), 102.2 (CH), 114.5 (CN), 126.5, 134.8, 138.1 (each CH), 149.3, 162.9 ppm (each C). ESI MS *m/z* 348 $[M-H]^-$. $C_{16}H_{23}N_3O_4Si$ (349.15): Calcd. C, 54.99; H, 6.63; N, 12.02; found. C, 54.98; H, 6.62; N, 12.04.

1-[(4*R*,5*R*)-4-[*tert*-butyl(dimethyl)silyl]oxy-5-([*tert*-butyl(dimethyl)silyl]oxymethyl)-4,5-dihydro-2-furanyl]-2,2-dimethyl-1-propanone (14c). Oil. − 64% yield $-^1$H NMR ($CDCl_3$): δ 0.04, 0.06 (s, 3H each, MeSi), 0.08 (s, 6H, 2 × MeSi) 0.87, 0.88 (s, each 9H, *t*BuSi), 1.23 (s, 3H, *t*Bu), 3.60 (dd, 1H, J = 10.7, 5.9 Hz, 5′α-H), 3.74 (dd, 1H, J = 10.7, 4.9 Hz, 5′β-H), 4.43 (m, 1H, 4′-H), 4.96 (dd, 1H, J = 3.5, 2.8 Hz, 3′-H), 5.76 (d, 1H, J = 2.8 Hz, 2′-H). $-^1$H NMR (C_6D_6): δ 0.02 (s, 6H, 2 × MeSi), 0.06, 0.08 (s, 3H each, MeSi), 0.94 (s, 18H, 2 ×*t*BuSi), 1.26 (s, 3H, *t*Bu), 3.51 (dd, 1H, J = 10.7, 5.9 Hz, 5′α-H), 3.64 (dd, 1H, J = 10.7, 4.9 Hz, 5′β-H), 4.59 (m, 1H, 4′-H), 5.00 (dd, 1H, J = 3.5, 2.8 Hz, 3′-H), 6.03 (d, 1H, J = 2.8 Hz, 2′-H). $-^{13}$C NMR (C_6D_6): δ −5.5, −4.4, −4.2 (each CH_3), 18.1, 18.3 (each C), 25.8 (*t*Bu), 25.9 (2 × *t*Bu), 43.5 (C), 62.8 (CH_2), 75.7 (CH), 90.9 (CH), 110.4 (CH), 157.8, 198.3 ppm (each C). − $C_{22}H_{44}O_4Si_2$ (428.75): Calcd. C, 61.63; H, 10.34; found. C, 61.59; H, 10.35.

1-[(4*R*,5*R*)-4-[*tert*-butyl(dimethyl)silyl]oxy-5-([*tert*-butyl(dimethyl)silyl]oxymethyl)-4,5-dihydro-2-furanyl]-1-ethanone (14a). Oil. − 42% yield $-^1$H NMR ($CDCl_3$): δ 0.03, 0.06 (s, 3H each, MeSi), 0.10 (s, 6H, 2 × MeSi) 0.87, 0.89 (s, each 9H, *t*BuSi), 2.32 (s, 3H, CH_3), 3.59 (dd, 1H, J = 10.8, 6.2 Hz, 5′α-H), 3.78 (dd, 1H, J = 10.8, 4.5 Hz, 5′β-H), 4.40 (m, 1H, 4′-H), 5.07 (dd, 1H, J = 3.2, 2.9 Hz, 3′-H), 5.80 (d, 1H, J = 2.9 Hz, 2′-H). $-^{13}$C NMR ($CDCl_3$): δ −5.6, −4.6, −4.5 (each CH_3), 17.9, 18.2 (each C), 25.7 (2 ×*t*Bu), 26.5 (CH_3), 62.3 (CH_2), 76.1 (CH), 89.6 (CH), 110.9 (CH), 156.1, 191.3 (each C). − $C_{19}H_{38}O_4Si_2$ (386.67): Calcd. C, 59.02; H, 9.91; found. C, 59.21; H, 9.89.

Cytostatic Activity Assay. All assays were performed in 96-well microtiter plates (Falcon 3072; Becton Dickinson, Paramus, NJ).[39] To each well were added ca. 6 × 10^4 murine leukemia L1210, or human lymphocyte Molt4/C8 and CEM cells (100 μl) and a given amount of the test compound (100 μl). The cells were allowed to proliferate for 48 to 72 h at 37°C in a humidified CO_2-controlled atmosphere. At the end of the incubation

period, the cells were counted in a Coulter counter (model ZB; Coulter Electronics Ltd., Harpenden, Hertfordshire, England). The 50% cytostatic concentration (CC_{50}) was defined as the concentration of compound that inhibited cell proliferation by 50%.

Antiviral Activity of Test Compounds in Cell Cultures. CEM cells were suspended at ca. 300,000 cells per ml of culture medium and infected with ca. 100 times the 50% cell-culture-infective doses of HIV-1(III_B) or HIV-2(ROD).[40] Then, 100 μl of the infected cell suspensions were added to 200-μl microtiter plate well containing 100 μl of appropriate serial (five-fold) dilutions of the test compounds. The inhibitory effect of the test compounds on HIV-induced syncytium formation in CEM cells was examined microscopically on day 4 or 5 post infection. The EC_{50} was defined as the compound concentration that inhibits syncytium formation in the HIV-infected cell cultures by 50%.

The procedures of the anti-CMV and -VZV assays were as follows:[41] confluent HEL cells grown in 96-well microtiter plates were inoculated with CMV or VZV at an input of 100 PFU per well. After a 1 to 2-h incubation period, residual virus was removed, and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 2 mM L-glutamine and 0.3% $NaHCO_3$) containing varying concentrations of the compounds. Antiviral activity was expressed as EC_{50} (50% effective concentration), or compound concentration required to reduce virus-induced cytopathicity after 7 days (CMV) or 5 days (VZV) by 50% compared to the untreated control.

The procedures of the antiviral evaluations of the test compounds against herpes simplex virus type 1 [HSV-1 (KOS)] and type 2 [HSV-2 (G)], vaccinia virus and vesicular stomatitis virus in HEL cell cultures, Coxsackie B4 virus and respiratory syncytial virus (RSV) in HeLa cell cultures and parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus in Vero cell cultures were as follows:[41] confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After 1–2 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... μg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

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