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# Simple and efficient routes for the preparation of isoxazolidinyl nucleosides containing cytosine and 5-methyl-cytosine as new potential anti-HIV drugs

Evelina Colacino, Antonella Converso, Angelo Liguori, Anna Napoli, Carlo Siciliano* and Giovanni Sindona

*Dipartimento di Chimica, Università della Calabria, Via Ponte P. Bucci, Cubo 15/C, I-87030 Arcavacata di Rende (CS), Italy*

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**Abstract**—A rapid and convenient large-scale strategy for the synthesis of some new isoxazolidinyl nucleosides, as potential antiviral drugs, is reported. In particular, a multistep methodology based either on the 1,3-dipolar cycloaddition approach or on a slight modification of the convertible nucleoside concept was exploited in the preparation of 4′-aza-2′,3′-dideoxynucleoside analogues containing cytosine and 5-methyl-cytosine. 

## 1. Introduction

A large number of nucleoside analogues have proved to be very effective against HIV-1, HBV and HSV-1 infections and they have also been employed in the treatment of some tumour forms.[1] Some of them, particularly those belonging to the 2′,3′-dideoxynucleoside family (such as ddT, ddI, ddG, ddA and ddC), have been found to inhibit the HIV reverse transcriptase after their anabolic activation to 5′-triphosphate derivatives by the cellular kinases.[2] Furthermore, it has been observed that chemical modification at 2′ and 3′ positions of the 2′,3′-dideoxyribofuranosyl moiety by introducing heteroatoms does not alter the recognition properties of modified nucleosides.[3] In particular, substitution of 3′ methylene carbon in the sugar ring by an oxygen or sulfur atom afforded dioxolanyl and oxathiolanyl analogues, respectively, whose antiviral activities have been tested in extensive clinical trials.[4] During the last years, this strategy has been successfully applied to the synthesis of hetero-modified 2′,3′-dideoxynucleosides which were more resistant to acid-promoted hydrolysis and, simultaneously, characterised by very low toxicity levels. Among these new drugs, dioxolane-T and BCH-189 have proved to possess a very potent antiretroviral effectiveness in vivo.[5]

At present, there is a growing interest in the preparation of modified 2′,3′-dideoxynucleoside analogues containing uracil and 5-methyl-cytosine as nucleobases, since some of them exhibited appreciable or excellent anti-HIV activity in vitro, without showing cytotoxicity up to very high intracellular concentrations.[6] All the previous evidence opened a very important research area in which the preparation of new hetero-substituted 2′,3′-dideoxynucleosides has become the main goal in the design and development of more potent and selectively active drugs.[7]

## 2. Results and discussion

Isoxazolidinyl nucleosides represent a relatively new challenging class of potential antiviral agents.[8] Our research group has recently proposed the 1,3-dipolar cycloaddition approach[9] as one of the most useful, robust and convenient tools for the totally convergent large-scale preparation of *N*,*O*-heterocyclic analogues of the natural nucleosides.[10] These compounds are generally obtained from the reaction between formyl nitrones bearing an acid-labile protecting group on the nitrogen atom, generated in situ,[11] and appropriately designed vinyl derivatives of the common pyrimidine and purine nucleobases, whose synthetic protocols were optimized in our laboratories and previously reported.[10b,c] The preparation of the 4′-aza analogue of the potent antiviral drug 2′,3′-dideoxythymidine, named AdT (**7**, Scheme 1), can be considered a representative example of the synthetic potentialities offered by the above cited preparative route.[10a] AdT showed a good anti-HIV 1 activity and reduced cytotoxicity in vitro, as demonstrated by testing this isoxazolidinyl nucleoside in parallel with AZT in CD4-lymphocytic cell lines sensitive to the cytopathic effect of human immunodeficiency virus.[12] The biological trials were also indicative of the possibility to employ AdT as a racemic mixture of the two α and β anomers. This evidence was a

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* Corresponding author. Tel.: +39-984-492042; fax: +39-984-492044; e-mail: carlo.siciliano@unical.it

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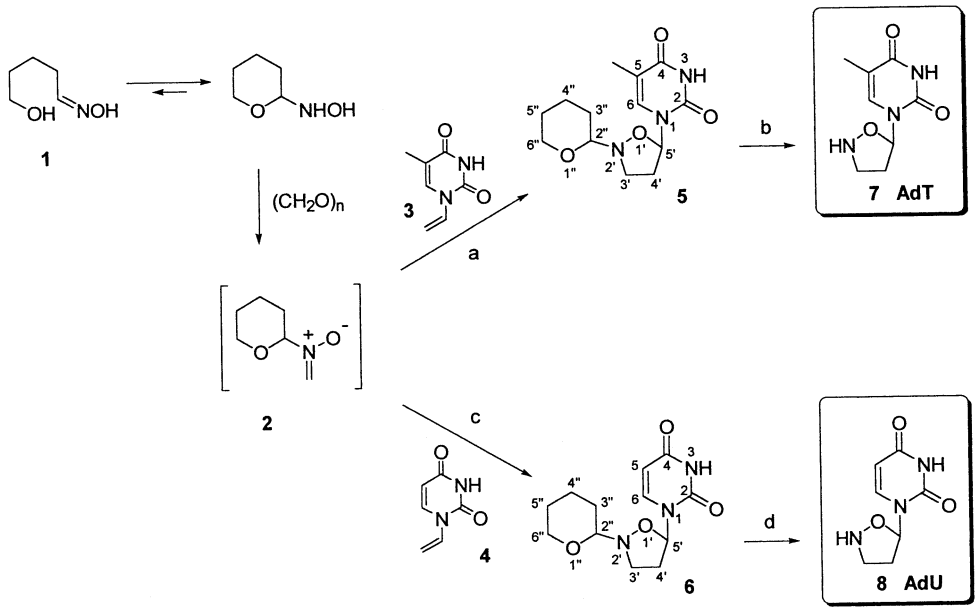



**Scheme 1.** *Reagents*: (a) and (b), see Ref. 10a; (c) **1**, paraformaldehyde, $CHCl_3$ (65% yield); (d) $HClO_4$ 60%, MeOH, $CHCl_3$ (91% yield).

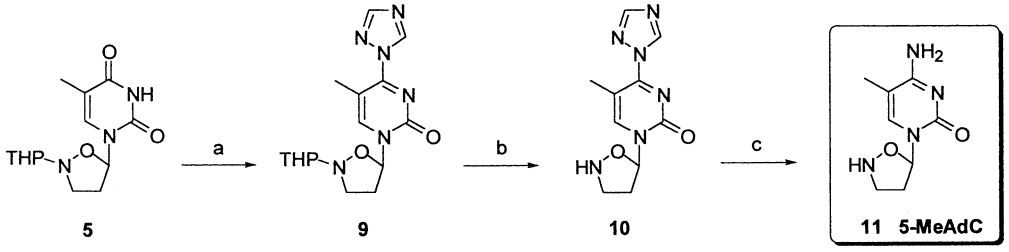
formidable and worthwhile characteristic of the new potential antiviral agent, the use of which could not be hampered by the need of a separation of each optically active form, a step perhaps of considerable importance to the greater number of the available pharmaceuticals. Moreover, diastereomeric mixtures of the hetero-substituted dideoxynucleoside analogues BCH-189 and dioxolane-T have previously been found to express potent antiviral activity against HBV and HIV viruses.[13] Consequently, it is of interest to plan the synthesis of new *N*,*O*-heterocyclic analogues to be monitored for their biological activity as potential antiviral drugs.

On the basis of the above mentioned promising results reported for AdT, some other isoxazolidinyl dideoxynucleoside analogues containing the common nucleobases (such as AdU, **8**; Scheme 1) have been prepared in excellent overall yields by applying an optimized and highly efficient version of the synthetic methodology firstly proposed for the preparation of AdT.[10a]

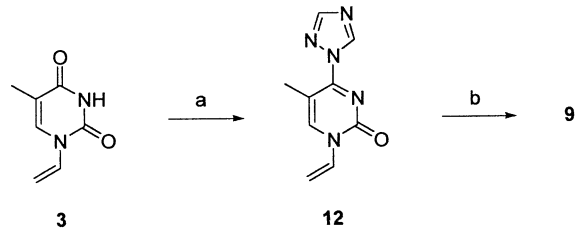
In a typical procedure, the synthesis of AdU was accomplished by refluxing a solution in chloroform of 1-vinyluracil (**4**) and *N*-tetrahydropyranylmethylene nitrone (**2**), the latter obtained in situ from the condensation of 5-hydroxypentanaloxime (**1**) with a molar excess of paraformaldehyde. Chromatographic purification of the crude mixture recovered from the cycloaddition reaction gave the protected cycloadduct **6** in good yield. The racemic isoxazolidinyl analogue **8** was finally obtained after removal of the protecting group from **6** by treating the latter compound with few drops of a $HClO_4$ dilute aqueous solution dissolved in a methanol/chloroform mixture (Scheme 1). It is remarkable to point out that, despite the reported preparation of AdT, the use of strong acidic conditions in this improved methodology allowed a straightforward recovery of compound **8** with an excellent purity grade.

Moreover, the acid-promoted hydrolysis did not effect the glycosidic linkage of the isoxazolidinyl nucleoside **8**, a fact that might prove to be useful in future 2′,3′-dideoxynucleoside analogue drugs design.

As an extension of our ongoing work on the synthesis of isoxazolidinyl analogues having potential antiretroviral effectiveness, we have now exploited the application of the convertible nucleoside approach[14] in order to prepare 4′-aza-2′,3′-dideoxyerythrofuranosyl nucleosides containing cytosine and 5-methyl-cytosine. To achieve this aim, the two *N*-tetrahydropyranyl derivatives **5** and **6** (Scheme 1) were selected as the most convenient precursors of the desired compounds.




**Scheme 2.** *Reagents*: (a) $POCl_3$, TEA, 1,2,4-(1*H*)-triazole, $CH_3CN$ (quantitative yield); (b) $HClO_4$ 60%, MeOH, $CHCl_3$ (92% yield); (c) $NH_3$, 1,4-dioxane (95% yield).



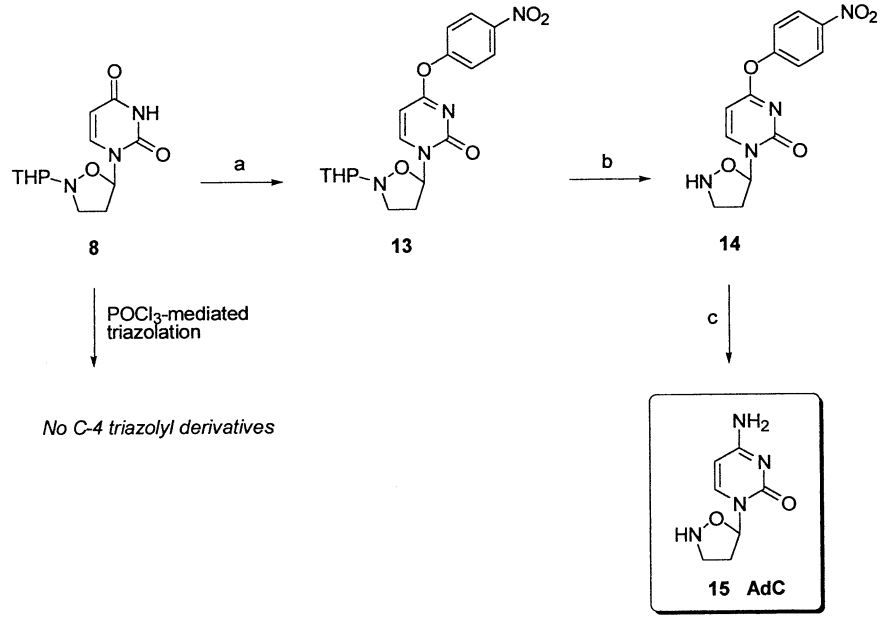

**Scheme 3.** *Reagents*: $POCl_3$, TEA, 1,2,4-(1*H*)-triazole, $CH_3CN$ (quantitative yield); (b) 5-hydroxypentanaloxime, paraformaldehyde, $CHCl_3$ (78% yield).

The synthesis of the 4′-aza analogue of 2′,3′-dideoxy-5-methyl-cytidine (5-MeAdC, **11**; Scheme 2) was performed by treating the diastereomeric mixture **5** dissolved in $CH_3CN$ with a molar excess of phosphorus oxychloride and 1,2,4-(1*H*)-triazole.[15] Under the adopted reaction conditions, the triazolation reaction afforded crude C-4 triazolyl derivative **9** in nearly quantitative yield. The stable intermediate **9** was isolated in form of a mixture of the possible diastereomers pure enough to be used in the next step, without recourse to chromatographic procedures. Compound **9** was successively deprotected under strong acidic conditions, which ensured very fast deblocking kinetics as observed, besides, in the above discussed preparation of AdU. The treatment afforded the C-4 substituted analogue **10** in very high isolated yield (92%). It is worth noting that the removal of the tetrahydropyranyl protecting group from **9** did not cause acid-promoted degradation of the free cyclic hydroxylaminic function, the glycosidic linkage exhibited by the nucleoside analogue **10** remaining also totally unaffected. The final nucleophilic displacement of the triazolyl moiety from the pyrimidine ring was accomplished using a saturated solution of gaseous ammonia in dry dioxane at room temperature. The ammonolysis was complete in 7 h and the racemic 4′-aza modified dideoxynucleoside containing 5-methyl-cytosine **11** was isolated in excellent yield by flash-chromatography.

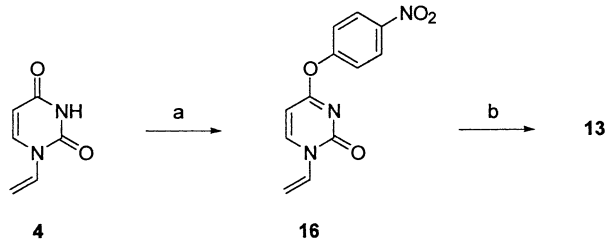
An alternative convergent large-scale approach to **9** was achieved by preparing the C-4 triazolyl derivative **12** of 1-vinyl-thymine[16] (Scheme 3). The appropriate dipolarophile was easily obtained in quantitative yield as a very stable crystalline compound, by reacting 1-vinyl-thymine under phosphorus oxychloride-promoted triazolation conditions. The 1,3-dipolar cycloaddition between **12** and 5-hydroxypentanaloxime was carried out with a fourfold molar excess of paraformaldehyde, and afforded with total regioselectivity the fully protected diastereomeric mixture of cycloadducts **9** in satisfactory yield, after flash-column chromatography.

The same triazolation protocol, successfully adopted in the preparation of **9**, failed in the case of the uracil derivative. This strategy, in fact, did not afford the analogous of **9** when the isoxazolidinyl nucleoside **8** was used, since the corresponding triazolyl derivative proved to be too unstable undergoing total hydrolysis to its precursor **8** under the adopted work up conditions. Nevertheless, the 4-nitrophenyl derivative **13** (Scheme 4) was considered a valuable alternative intermediate for the fulfilment of our purpose.

Compound **13** was prepared in very high isolated yield by treating 6 with a fourfold molar excess of phosphorus oxychloride in the presence of 4-nitrophenol.[17] Chromatography was necessary to obtain pure **13**, the 4′-aza function of which was unprotected under strong acidic conditions similar to those adopted for the acidolysis of products **6** and **9**. The unblocking of **13** was totally selective, affording pure **14** in very good overall yield. The new 4′-aza analogue of 2′,3′-dideoxycytidine **15**, named AdC, was then obtained by subjecting the precursor **14** to an ammonolysis




**Scheme 4.** *Reagents*: (a) $POCl_3$, 4-nitrophenol, *N*-methylpyrrolidine, 1,4-dioxane (90% yield); (b) $HClO_4$ 60%, MeOH, $CHCl_3$ (88% yield); (c) $NH_3$, 1,4-dioxane (93% yield).




**Scheme 5.** *Reagents*: (a) $POCl_3$, 4-nitrophenol, *N*-methylpyrrolidine, 1,4-dioxane (91% yield); (b) 5-hydroxypentanaloxime, paraformaldehyde, $CHCl_3$ (72% yield).

process performed in dioxane at room temperature. Under the selected conditions, the nucleophilic displacement of the 4-nitrophenyl moiety realised by ammonia took place, affording the desired racemic compound **15** in excellent yield as a stable white powder after flash-column chromatography of the crude reaction mixture.

The possibility to carry out the synthesis of **13** by a single 1,3-dipolar cycloaddition step was also investigated. In order to obtain the modified dipolarophile **16**, 1-vinyluracil[18] (**4**; Scheme 5) was treated with 4-nitrophenol under reaction conditions similar to those adopted for the conversion of **6** into **13**. The introduction of the aromatic moiety on the dipolarophile proved to be appropriate, since the fully protected isoxazolidinyl derivative **13** was obtained in very high overall yield from the 1,3-dipolar cycloaddition step performed in the presence of 5-hydroxypentanaloxime and paraformaldehyde. The removal of the tetrahydropyranyl group from compound **13** and the final ammonolysis step, both realized as usual, completed this alternative totally convergent approach to racemic AdC (**15**).

The studied 1,3-dipolar processes confirmed that no relevant limitations exist in the applicability of the proposed method when compounds **12** and **16** are used as dipolarophiles. These reactants, in fact, are very resistant to the adopted experimental conditions: in all cases, no thermal decomposition for these C-4 protected vinyl derivatives was observed at the temperatures selected for the preparation of *N*-tetrahydropyranyl analogues **9** and **13**, whose formation proceed with total regioselectivity. In the cycloaddition reaction mixtures, besides, were not detected traces of by-products generated from a possible nucleophilic attack performed by the dipole on the C-4 of the substituted pyrimidine rings, demonstrating that the chosen protection resulted to be more effective respect to other protecting groups susceptible of a nucleophilic displacement, as previously reported for dipolarophiles containing base labile or nucleophile sensitive protections.[10b]

Some other important points are in order here. First, the glycosidic linkage of each isoxazolidinyl nucleoside survives the strong acid conditions adopted for the removal of the tetrahydropyranyl protecting group. The presence of a free cyclic hydroxylamine function on the 4′-aza modified sugar moiety is to be considered a remarkable peculiarity of the designed isoxazolidinyl nucleosides **11** and **15**, as well as for AdT and AdU. In fact, the unprotected 4′-aza functionality in the glycone part of analogues **11** and **15** might act as preferred protonation site in solution, thereby enhancing the stability of the glycosidic bond. Secondly, the same functionality, in strict analogy to the alcoholic group in the 5′ position of natural nucleosides, could allow the anabolic activation of the synthesized analogues, generating recognizable phosphorylated chain terminators that can selectively inhibit the reverse transcriptases during the replicative cycle of the target viruses. Finally, modified nucleoside analogues **8**, **11** and **15** showed to be appreciably water soluble. All the above discussed characteristics might prove valuable in the employment of these easily available compounds as new potential antiviral drugs.

## 3. Conclusions

The convertible nucleoside approach has been adopted to accomplish a facile and highly selective modification of the pyrimidine rings of some racemic isoxazolidinyl nucleoside analogues. This strategy appears to be particularly convenient for the preparation of 4′-aza-2′,3′-dideoxyerythrofuranosyl derivatives containing cytosine and 5-methylcytosine residues. These compounds can also be obtained by applying a totally convergent synthetic methodology based on the 1,3-dipolar cycloaddition approach. The biological activities of the water soluble racemic drugs **8**, **11** and **15**, against retroviral infections, as well as their introduction in antisense oligonucleotide strands, are currently under investigation.

## 4. Experimental

### 4.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. $^1H$ and $^{13}C$ NMR spectra were measured at 300 MHz on a Bruker AC300 spectrometer as dilute solutions in DMSO-$d_6$ or $CDCl_3$. The chemical shift values ($\delta$) are expressed in ppm and downfield relative to TMS. Mass spectra were recorded on a Fisons Vacuum Generators ZAB-2F spectrometer by fast atom bombardment (FAB), using 3-nitrobenzyl alcohol (NBA) or glycerol (Gly) as matrices, with a neutral xenon beam operated at 8 KeV and a total ion current of 10 μA. IR spectra were recorded on a Perkin Elmer Paragon 1000 PC FT-IR spectrometer. Reaction mixtures and purity of all compounds were monitored by thin layer chromatography (TLC) using Merck silica gel 60-$F_{254}$ precoated aluminium plates. Flash-chromatography was performed on Merck Kieselgel 60H without gypsum, by using as eluent mixtures the same solvent systems adopted for TLC analysis. Elemental analyses were performed using a Perkin Elmer Elemental Analyzer instrument. All reagents and solvents were of highest grade commercially available. Solvents were dried and freshly distilled prior to use. All reagents were supplied by Aldrich Chemical and used without further purification. Dipolarophiles **3** and **4** were prepared according to the literature procedures.[16,18] All the 1,3-dipolar cycloadditions were carried out under inert atmosphere, using traces of *tert*-butylcathecol as polymerisation inhibitor. Removal of the solvents was performed on a Heidolph rotary evaporator under reduced pressure conditions.

**4.1.1. (2″RS,5′RS)-1-[2′-(Tetrahydropyran-2″-yl)-1′,2′-isoxaxolidin-5′-yl]-uracil (6).** *Method A*. Paraformaldehyde (1.91 g, 63.6 mmol), 1-vinyl-uracil (**4**; 2.0 g, 15.9 mmol) and 5-hydroxypentanaloxime (**1**; 4.67 g, 39.8 mmol) were suspended in dry $CHCl_3$ (30 mL). The resulting mixture was refluxed for 38 h until complete conversion of the dipolarophile (TLC: $Et_2O$/MeOH 95:5, v/v). The solution was then cooled at room temperature and evaporated to dryness in vacuo. Purification of the recovered crude material by flash-chromatography afforded the *title compound* **6** (2.47 g, 65%) as a white powder; [Found: C, 54.01; H, 6.48; N, 15.69. $C_{12}H_{17}N_3O_4$ requires C, 53.92; H, 6.41; N, 15.72]; $\nu_{max}$ (KBr) 3600–3180 (br), 3169, 3046, 2931, 1728 (C═O), 1718 (C═O), 1457, 1265, 1220, 1118, 1034 $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.85 (1H, d, $J$=8.1 Hz, 6-CH), 7.81 (1H, d, $J$=8.1 Hz, 6-CH), 6.18 (1H, dd, $J$=7.6, 5.1 Hz, 5′-CH), 6.16 (1H, dd, $J$=7.5, 5.2 Hz, 5′-CH), 5.63 (1H, d, $J$=8.1 Hz, 5-CH), 5.59 (1H, d, $J$=8.1 Hz, 5-CH), 4.39 (1H, dd, $J$=10.6, 2.2 Hz, 2″-CH), 4.16 (1H, dd, $J$=10.0, 2.5 Hz, 2″-CH), 4.09–4.01 (2H, m, 6″-C$H_aH_b$), 3.67–3.59 (2H, m, 3′-C$H_aH_b$), 3.52–3.45 (2H, m, 6″-C$H_aH_b$), 3.29–3.22 (2H, m, 4′-C$H_aH_b$), 3.19–3.12 (2H, m, 3′-CH$_a$$H_b$), 2.91–2.82 (2H, m, 4′-CH$_a$$H_b$), 1.91–1.80 (4H, m, 5″-$CH_2$), 1.68–1.49 (8H, m, 3″-$CH_2$ and 4″-$CH_2$); $m/z$ (+, NBA) 290 (29, $MNa^+$), 268 (78, $MH^+$), 184 (38), 156 (45), 141 (41), 139 (28), 112 (13), 85 (100%).

**4.1.2. (±)-1-(2′-H-1′,2′-Isoxazolidin-5′-yl)-uracil (8, AdU).** To a magnetically stirred solution of **6** (267.3 mg, 1 mmol), in MeOH/$CHCl_3$ (5 mL, 7:3 v/v), was added dropwise 60% aqueous $HClO_4$, at room temperature, until complete conversion of the starting protected cycloadducts (TLC: $Et_2O$/MeOH 90:10 v/v). The pH value of the reaction mixture was then adjusted to neutrality by adding solid $NaHCO_3$. The resulting suspension was filtered through a CELITE 545® short pad (MeOH as eluent), and the mother liquor was evaporated to dryness in vacuo. Purification by flash-chromatography of the recovered crude material afforded the *title compound* **8** (166.7 mg, 91%) as a white powder, mp 177–179°C; [Found: C, 45.82; H, 5.01; N, 22.88. $C_7H_9N_3O_3$ requires C, 45.90; H, 4.95; N, 22.94]; $\nu_{max}$ (KBr) 3586–3165 (br), 3026, 1726 (C═O), 1718 (C═O), 1521, 1463, 1332, 1277, 1196, 1014 $cm^{-1}$; $\delta_H$ (DMSO-$d_6$) 9.32 (1H, s, 3-NH), 7.68 (1H, br s, 2′-NH), 7.12 (1H, d, $J$=8.1 Hz, 6-CH), 6.34 (1H, dd, $J$=7.0, 5.3 Hz, 5′-CH), 5.63 (1H, d, $J$=8.1 Hz, 5-CH), 3.30–3.26 (1H, m, 3′-C$H_aH_b$), 3.06–3.01 (1H, m, 3′-CH$_a$$H_b$), 2.58–2.53 (1H, m, 4′-C$H_aH_b$), 2.31–2.26 (1H, m, 4′-CH$_a$$H_b$); $\delta_C$ (DMSO-$d_6$) 164.4, 157.8, 142.6, 100.8, 93.8, 49.5, 42.3; $m/z$ (+, Gly) 206 (39, $MNa^+$), 184 (68, $MH^+$), 151 (37), 141 (45), 139 (45), 112 (20), 72 (100%).

**4.1.3. (2″RS,5′RS)-1-[2′-(Tetrahydropyran-2″-yl)-1′,2′-isoxaxolidin-5′-yl]-4-[1,2,4-(1*H*)-triazol-1-yl]-5-methyl-pyrimidin-2-(1*H*)-one (9).** *Method A*. To a magnetically stirred solution of **5** (2.18 g, 10 mmol) in dry $CH_3CN$ (40 mL) was added freshly distilled $POCl_3$ (1.9 mL, 20 mmol), dry triethylamine (3.22 mL, 25 mmol) and 1,2,4-(1*H*)-triazole (1.38 g, 20 mmol). The reaction mixture was maintained at room temperature for 45 min (TLC: $Et_2O$/MeOH 95:5, v/v). Triethylamine (7 mL) and distilled water (3 mL) was then added and the resulting solution was stirred at room temperature for an additional 10 min. Removal of the volatiles in vacuo afforded an aqueous residue which was diluted with distilled water (12 mL) and extracted twice with $CHCl_3$ (30 mL). The organic layers were dried ($Na_2SO_4$) and evaporated to dryness in vacuo to give the *title compound* **9** (3.32 mg, 100%) which was obtained as a white powder and pure enough to be used in next step without need of purification. [Found: C, 54.26; H, 6.10; N, 25.28. $C_{15}H_{20}N_6O_3$ requires C, 54.21; H, 6.07; N, 25.29]; $\nu_{max}$ (KBr) 3160, 3042, 2950, 1725 (C═O), 1649, 1530, 1462, 1379, 1273, 1122, 1055 $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.12 (2H, s, 3-CH triazole), 7.95 (2H, s, 5-CH triazole), 7.05 (2H, s, 6-CH), 6.16 (1H, dd, $J$=7.2, 4.8 Hz, 5′-CH), 6.14 (1H, dd, $J$=7.3, 4.7 Hz, 5′-CH), 4.40 (1H, dd, $J$=11.0, 2.4 Hz, 2″-CH), 4.23 (1H, dd, $J$=10.5, 2.8 Hz, 2″-CH), 4.18–4.12 (2H, m, 3′-C$H_aH_b$), 4.09–4.02 (2H, m, 6″-C$H_aH_b$), 3.49–3.43 (2H, m, 6″-CH$_a$$H_b$), 3.41–3.34 (2H, m, 3′-CH$_a$$H_b$), 3.15–3.10 (2H, m, 4′-C$H_aH_b$), 2.96–2.89 (2H, m, 4′-CH$_a$$H_b$), 1.85–1.76 (4H, m, 5″-$CH_2$), 1.62–1.49 (8H, m, 3″-$CH_2$ and 4″-$CH_2$); $m/z$ (+, NBA) 333 (38, $MH^+$), 248 (24), 239 (22), 178 (45), 156 (22), 152 (29), 85 (100%).

**4.1.4. (±)-1-(2′-H-1′,2′-Isoxazolidin-5′-yl)-4-[1,2,4-(1*H*)-triazol-1-yl]-5-methyl-pyrimidin-2-(1*H*)-one (10).** A magnetically stirred solution of **9** (332.3 mg, 1 mmol) in MeOH/$CHCl_3$ (5 mL, 7:3 v/v) was treated with 60% aqueous $HClO_4$. The acid was added dropwise at room temperature until complete conversion of the starting protected cycloadducts (TLC: $Et_2O$/MeOH 90:10, v/v). The pH value of the reaction mixture was then adjusted to neutrality by adding solid $NaHCO_3$. The resulting suspension was filtered through a CELITE 545® short pad (MeOH as eluent) and the mother liquor was evaporated to dryness in vacuo. Purification by flash-chromatography of the recovered crude material afforded **10** (228.4 mg, 92%) as a white powder, mp 197–200°C; [Found: C, 48.33; H, 4.80; N, 33.92. $C_{10}H_{12}N_6O_2$ requires C, 48.39; H, 4.84; N, 33.87]; $\nu_{max}$ (KBr) 3580–3118 (br), 3022, 1721 (C═O), 1648, 1532, 1460, 1379, 1180, 1141, 1010 $cm^{-1}$; $\delta_H$ (DMSO-$d_6$) 8.12 (1H, s, 3-CH triazole), 8.05 (1H, s, 5-CH triazole), 7.77 (1H, br s, 2′-NH), 6.28 (1H, dd, $J$=8.9, 6.8 Hz, 5′-CH), 6.19 (1H, s, 6-CH), 3.28–3.24 (1H, m, 3′-C$H_aH_b$), 2.79–2.75 (1H, m, 3′-CH$_a$$H_b$), 2.30–2.26 (1H, m, 4′-C$H_aH_b$), 2.25–2.21 (1H, m, 4′-CH$_a$$H_b$), 1.97 (3H, s, Me); $\delta_C$ (DMSO-$d_6$) 164.2, 158.1, 127.9, 113.7, 98.9, 52.2, 40.8, 11.8; $m/z$ (+, NBA) 249 (34, $MH^+$), 207 (22), 205 (19), 178 (43), 72 (100%).

**4.1.5. (±)-1-(2′-H-1′,2′-Isoxazolidin-5′-yl)-5-methyl-pyrimidin-2-(1*H*)-one (11; 5-MeAdC).** A solution of **10** (248.3 mg, 1 mmol) in dry 1,4-dioxane (10 mL) was treated with gaseous $NH_3$, under magnetic stirring at room temperature for 7 h (TLC: $Et_2O$/MeOH 85:15, v/v). Removal of the solvent in vacuo gave crude **11** which was purified by flash-chromatography. Compound **11** was recovered as a white powder (186.4 mg, 95%), mp 192–194°C; [Found: C, 48.95; H, 6.08; N, 28.52. $C_8H_{12}N_4O_2$ requires C, 48.98; H, 6.12; N, 28.57]; $\nu_{max}$ (KBr) 3590–3130 (br), 3015, 1720 (C═O), 1530, 1461, 1328, 1282, 1270, 1190, 1084 $cm^{-1}$; $\delta_H$ (DMSO-$d_6$) 7.85 (1H, br s, 2′-NH), 7.21 (2H, br s, 4-$NH_2$), 6.33 (1H, dd, $J$=7.9, 6.1 Hz, 5′-CH), 6.00 (1H, s, 6-CH), 3.36–3.32 (1H, m, 3′-C$H_aH_b$), 2.94–2.89 (1H, m, 3′-CH$_a$$H_b$), 2.41–2.36 (1H, m, 4′-C$H_aH_b$), 2.28–2.23 (1H, m, 4′-CH$_a$$H_b$); $\delta_C$ (DMSO-$d_6$) 164.8, 158.9, 124.5, 107.8,

98.9, 49.5, 40.8; *m/z* (+, Gly) 197 (65, $MH^+$), 155 (38), 153 (44), 126 (45), 72 (100%).

**4.1.6. 1-Vinyl-4-[1,2,4-(1*H*)-triazol-1-yl]-5-methyl-pyrimidin-2-(1*H*)-one (12).** A magnetically stirred solution of 1-vinyl-thymine (**3**; 1.52 g, 10 mmol), dry triethylamine (3.22 mL, 25 mmol) and 1,2,4-(1*H*)-triazole (1.38 g, 20 mmol) in dry 1,4-dioxane (35 mL), was treated with freshly distilled $POCl_3$ (1.9 mL, 20 mmol). After 10 min at 0°C, the stirred mixture was maintained at room temperature for 30 min (TLC: $Et_2O$/MeOH 95:5, v/v). Triethylamine (7 mL) and distilled water (3 mL) was then added and the resulting solution was kept at room temperature for an additional 10 min. Removal of the volatiles in vacuo gave an aqueous residue which was diluted with distilled water (12 mL) and extracted twice with $CHCl_3$ (30 mL). The organic layers were dried ($Na_2SO_4$) and evaporated to dryness in vacuo to afford pure **12** (203.2 mg, 100%) as a white powder, mp 183–186°C. The *title compound* **12** can be crystallized from boiling toluene/ethanol and recovered as colorless crystals, mp 187–189°C; [Found: C, 53.16; H, 4.50; N, 34.41. $C_9H_9N_5O$ requires C, 53.20; H, 4.43; N, 34.48]; $\nu_{max}$ (KBr) 3426, 3185, 3064, 1716 (C=O), 1657, 1528, 1490, 1371, 1275, 1180, 1120 $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.14 (1H, s, 3-CH triazole), 8.02 (1H, s, 5-CH triazole), 7.33 (1H, dd, *J*=13.8, 8.8 Hz, C*H*=$CH_aH_b$), 7.05 (1H, s, 6-CH), 4.68 (1H, dd, *J*=13.8, 1.2 Hz, CH=C*H*$_aH_b$), 4.53 (1H, dd, *J*=8.8, 1.2 Hz, CH=$CH_aH_b$), 1.93 (3H, s, Me); $\delta_C$ ($CDCl_3$) 164.7, 151.8, 148.2, 133.7, 117.9, 114.2, 93.9, 70.8, 12.6; *m/z* (+, NBA) 204 (45, $MH^+$), 178 (100), 161 (52%).

**4.1.7. (2″RS, 5′RS)-1-[2′-(Tetrahydropyran-2″-yl)-1′,2′-isoxaxolidin-5′-yl]-4-[1,2,4-(1*H*)-triazol-1-yl]-5-methyl-pyrimidin-2-(1*H*)-one (9).** *Method B*. Paraformaldehyde (1.91 g, 63.6 mmol), the vinyl derivative **12** (3.23 g, 15.9 mmol) and 5-hydroxypentanaloxime (**1**; 4.57 g, 39.8 mmol) were suspended in dry $CHCl_3$ (30 mL). The resulting mixture was refluxed for 42 h under magnetic stirring until complete conversion of the dipolarophile **12** (TLC: $Et_2O$/MeOH 95:5, v/v). The solution was then cooled at room temperature and evaporated to dryness in vacuo. Purification of the recovered crude material by flash-chromatography afforded the *title compound* **9** (4.12 g, 78%).

**4.1.8. (2″RS,5′RS)-1-[2′-(Tetrahydropyran-2″-yl)-1′,2′-isoxaxolidin-5′-yl]-4-(4-nitrophenoxy)-pyrimidin-2-(1*H*)-one (13).** *Method A*. A solution of **6** (341.8 mg, 1.28 mmol) and *N*-methylpyrrolidine (0.19 mL, 17.84 mmol) in dry $CH_3CN$ (30 mL) was maintained under magnetic stirring at room temperature for 10 min. Freshly distilled $POCl_3$ (0.24 mL, 2.54 mmol) was then added. After 30 min, 4-nitrophenol (620.3 mg, 4.46 mmol) was added portionwise and the resulting yellow solution was allowed to react for 6 h (TLC: $Et_2O$/MeOH 98:2, v/v). The reaction mixture was filtered and the mother liquor concentrated in vacuo. The recovered crude yellow solid was dissolved in cold distilled water (10 mL) and brine (10 mL), then extracted twice with $CH_2Cl_2$ (30 mL). The collected organic layers were washed once with distilled water (10 mL), dried ($Na_2SO_4$) and evaporated to dryness in vacuo. Purification by flash-chromatography of the crude material afforded pure **13** (448 mg, 90%) as a pale yellow solid; [Found: C, 55.60; H, 5.23; N, 14.38. $C_{18}H_{20}N_4O_6$ requires C, 55.67; H, 5.19; N, 14.43]; $\nu_{max}$ (KBr) 3198, 3102, 1723 (C=O), 1650, 1610, 1587, 1528, 1470, 1325, 1271, 1108, 1047 $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.25–8.21 (4H, m, ArH), 7.42–7.38 (4H, m, ArH), 7.14 (2H, d, *J*=8.4 Hz, 6-CH), 6.35 (1H, d, *J*=8.4 Hz, 5-CH), 6.32 (1H, d, *J*=8.4 Hz, 5-CH), 6.18 (1H, dd, *J*=7.7, 5.3 Hz, 5′-CH), 6.12 (1H, dd, *J*=7.8, 5.2 Hz, 5′-CH), 4.48 (1H, dd, *J*=10.1, 2.1 Hz, 2″-CH), 4.45 (1H, dd, *J*=9.9, 2.2 Hz, 2″-CH), 4.18–4.13 (2H, m, 6″-C*H*$_aH_b$), 3.57–3.51 (2H, m, 6″-$CH_aH_b$), 3.35–3.28 (2H, m, 3′-C*H*$_aH_b$) 3.21–3.14 (2H, m, 3′-$CH_aH_b$), 3.00–2.92 (2H, m, 4′-C*H*$_aH_b$), 2.37–2.28 (2H, m, 4′-$CH_aH_b$), 1.88–1.80 (4H, m, 5″-$CH_2$), 1.61–1.48 (8H, m, 3″-$CH_2$ and 4″-$CH_2$); *m/z* (+, NBA) 389 (32, $MH^+$), 303 (13), 241 (27), 239 (12), 233 (25), 226 (10), 165 (6), 140 (8), 124 (15), 115 (7), 107 (54), 85 (100%).

**4.1.9. (±)-1-(2′-H-1′,2′-Isoxazolidin-5′-yl)-4-(4-nitrophenoxy)-pyrimidin-2-(1*H*)-one (14).** A magnetically stirred solution of **13** (338.4 mg, 1 mmol) in MeOH/$CHCl_3$ (5 mL, 7:3 v/v) was treated with 60% aqueous $HClO_4$. The acid was added dropwise at room temperature until complete conversion of the starting protected cycloadducts (TLC: $Et_2O$/MeOH 90:10, v/v). The pH value of the reaction mixture was then adjusted to neutrality by adding solid $NaHCO_3$. The resulting suspension was filtered trough a CELITE 545® short pad (MeOH as eluent) and the mother liquor was evaporated to dryness in vacuo. Purification by flash-chromatography of the recovered crude material afforded **14** (267.8 mg, 88%) as a white powder, mp 191–193°C; [Found: C, 51.28; H, 3.98; N, 18.39. $C_{13}H_{12}N_4O_5$ requires C, 51.32; H, 3.94; N, 18.42]; $\nu_{max}$ (KBr) 3568–3119 (br), 3021, 1722 (C=O), 1613, 1589, 1538, 1494, 1458, 1330, 1264, 1137, 1010 $cm^{-1}$; $\delta_H$ (DMSO-$d_6$) 8.36–8.32 (2H, m, ArH), 8.25 (1H, d, *J*=8.5 Hz, 6-CH), 7.47–7.43 (2H, m, ArH), 7.18 (1H, br s, 2′-NH), 7.03 (1H, d, *J*=8.5 Hz, 5-CH), 6.11 (1H, dd, *J*=7.3, 5.5 Hz, 5′-CH), 3.38–3.32 (1H, m, 3′-C*H*$_aH_b$), 3.12–3.06 (1H, m, 3′-$CH_aH_b$), 2.68–2.63 (1H, m, 4′-C*H*$_aH_b$), 2.37–2.32 (1H, m, 4′-$CH_aH_b$); $\delta_C$ (DMSO-$d_6$) 164.4, 162.9, 158.5, 142.3, 132.2, 125.8, 116.7, 98.5, 49.5, 40.8; *m/z* (+, Gly) 305 (50, $MH^+$), 272 (28), 261 (38), 259 (28), 233 (100), 72 (100%).

**4.1.10. (±)-1-(2′-H-1′,2′-Isoxazolidin-5′-yl)-cytosine (15; AdC).** A magnetically stirred solution of **14** (304.3 mg, 1 mmol) in dry 1,4-dioxane (10 mL) was treated with gaseous $NH_3$ at room temperature for 8 h (TLC: $Et_2O$/MeOH 85:15, v/v). Removal of the solvent in vacuo gave a solid residue which was purified by flash-chromatography to afford pure **15** (169.4 mg, 93%) as a white powder, mp 188–190°C; [Found: C, 46.11; H, 5.43; N, 30.81. $C_7H_{10}N_4O_2$ requires C, 46.15; H, 5.49; N, 30.77]; $\nu_{max}$ (KBr) 3578–3127 (br), 3018, 1719 (C=O), 1528, 1461, 1388, 1325, 1288, 1259, 1175, 1071 $cm^{-1}$; $\delta_H$ (DMSO-$d_6$) 8.37 (1H, d, *J*=8.2 Hz, 6-CH), 7.52 (1H, d, *J*=8.2 Hz, 5-CH), 7.23 (2H, br s, 4-$NH_2$), 7.00 (1H, br s, 2′-NH), 6.28 (1H, dd, *J*=7.4, 5.8 Hz, 5′-CH), 3.29–3.23 (1H, m, 3′-C*H*$_aH_b$), 3.00–2.95 (1H, m, 3′-$CH_aH_b$), 2.44–2.39 (1H, m, 4′-C*H*$_aH_b$), 2.33–2.28 (1H, m, 4′-$CH_aH_b$); $\delta_C$ (DMSO-$d_6$) 164.2, 158.7, 132.6, 98.8, 93.6, 49.5, 41.8; *m/z* (+, Gly) 183 (79, $MH^+$), 148 (32), 138 (38), 111 (45), 72 (100%).

**4.1.11. 1-Vinyl-4-(4-nitrophenoxy)-pyrimidin-2-(1*H*)-one (16).** A solution of 1-vinyl-uracil (**4**; 177.6 mg; 1.28 mmol) and *N*-methylpyrrolidine (0.19 mL, 17.84 mmol) in dry 1,4-dioxane (30 mL) was maintained under magnetic stirring at room temperature in the presence of *tert*-butyl-catechol (10 mg, 0.06 mmol). Freshly distilled $POCl_3$ (0.24 mL, 2.54 mmol) was then added. After 35 min, 4-nitrophenol (620.3 mg, 4.46 mmol) was added portion-wise and the mixture was allowed to react for 8 h (TLC: $Et_2O$/MeOH 99:1, v/v). The solid residue was filtered off and the solvent was evaporated to dryness in vacuo. The recovered pale yellow crude solid was dissolved in cold distilled water (10 mL), dried ($Na_2SO_4$), then evaporated to dryness in vacuo. After precipitation from $Et_2O$/*n*-hexane and chilling at −10°C, compound **16** was obtained pure (301.9 mg, 91%) as a pale yellow powder, mp 185–188°C; [Found: C, 55.57; H, 3.50; N, 16.26. $C_{12}H_9N_3O_4$ requires C, 55.60; H, 3.47; N, 16.22]; $\nu_{max}$ (KBr) 3085, 1967, 1885, 1723 (C═O), 1698, 1681, 1642, 1555, 1503, 1345, 1135, 1028 $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.11–8.07 (2H, m, ArH), 7.32 (1H, dd, *J*=8.3 Hz, 6-CH), 7.18 (1H, dd, *J*=12.7, 7.4 Hz, C*H*═$CH_aH_b$), 7.12–7.08 (2H, m, ArH), 5.17 (1H, d, *J*=8.3 Hz, 5-CH), 4.75 (1H, dd, *J*=12.7, 1.1 Hz, CH═C$H_a$H$_b$), 4.59 (1H, dd, *J*=8.8, 1.2 Hz, CH═C$H_a$*H*$_b$); $\delta_C$ ($CDCl_3$) 164.7, 163.4, 158.3, 141.2, 134.8, 133.7, 125.0, 116.6, 93.6, 93.1; *m*/*z* (+, NBA) 260 (49, $MH^+$), 235 (100), 138 (25%).

**4.1.12. (2″RS,5′RS)-1-[2′-(Tetrahydropyran-2″-yl)-1′,2′-isoxaxolidin-5′-yl]-4-(4-nitrophenoxy)-pyrimidin-2-(1*H*)-one (13).** *Method B*. A suspension of paraformaldehyde (1.91 g, 63.6 mmol), the vinyl derivative **16** (4.12 g, 15.9 mmol) and 5-hydroxypentanaloxime (**1**; 4.57 g, 39.8 mmol) in dry $CHCl_3$ (30 mL) was refluxed under magnetic stirring for 41 h, until complete conversion of **16** (TLC: $Et_2O$/MeOH 95:5, v/v). The solution was then cooled at room temperature and evaporated to dryness in vacuo. Purification of the recovered crude material by flash-chromatography afforded the *title compound* **13** (4.45 g, 72%) as a pale yellow solid.

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## References

1. For excellent reviews, see: (a) Huryn, D. M.; Okabe, M. *Chem Rev.* **1992**, *92*, 1745. (b) De Clercq, E. *AIDS Res. Human Retrovir.* **1992**, *8*, 119. (c) Périgaud, C.; Gosselin, G.; Imbach, J.-L. *Nucleosides Nucleotides* **1992**, *11*, 903. (d) De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491.
2. De Clercq, E. *Nucleosides Nucleotides* **1994**, *13*, 1271.
3. Kim, H. O.; Schinazi, R. F.; Shanmuganathan, K.; Jeong, L. S.; Beach, J. W.; Nampalli, S.; Cannon, D. L.; Chu, C. K. *J. Med. Chem.* **1993**, *36*, 519.
4. (a) Jeong, L. S.; Schinazi, R. F.; Beach, J. W.; Kim, H. O.; Nampalli, S.; Shanmuganathan, K.; Alves, A. J.; McMillan, A.; Chu, C. K.; Mathis, R. J. *Med. Chem.* **1993**, *36*, 181. (b) Jeong, L. S.; Schinazi, R. F.; Beach, J. W.; Kim, H. O.; Shanmuganathan, K.; Nampalli, S.; Chun, M. W.; Chung, W.-K.; Cho, B. G.; Chu, C. K. *J. Med. Chem.* **1993**, *36*, 2627.
5. (a) Norbeck, D. W.; Spanton, S.; Broder, S.; Mitsuya, H. *Tetrahedron Lett.* **1989**, *30*, 6263. (b) Choi, W.-B.; Wilson, L. J.; Yeola, S.; Liotta, D. C.; Schinazi, R. F. *J. Am. Chem. Soc.* **1991**, *113*, 9377.
6. Van Draanen, N. A.; Koszalka, G. W. *Nucleosides Nucleotides* **1994**, *13*, 1679.
7. Converso, A.; Siciliano, C.; Sindona, G. Synthesis of Modified 2′,3′-Dideoxynucleosides as Potential Antiviral Drugs. *Targets in Heterocyclic Systems — Chemistry and Properties*; Attanasi, O. A., Spinelli, D., Eds.; Italian Society of Chemistry: Parma, 1998; Vol. 2, p. 17.
8. For recent reports, see: (a) Pan, S.; Amankulor, N. M.; Zhao, K. *Tetrahedron* **1998**, *54*, 6587 and references cited therein. See also: (b) Chiacchio, U.; Rescifina, A.; Iannazzo, D.; Romeo, G. *J. Org. Chem.* **1999**, *64*, 28. (c) Chiacchio, U.; Corsaro, A.; Gumina, G.; Rescifina, A.; Romeo, G.; Piperno, A.; Romeo, R. *J. Org. Chem.* **1999**, *64*, 9321. (d) Merino, P.; del Alamo, E. M.; Bona, M.; Franco, S.; Merchan, F. L.; Tejero, T.; Vieceli, O. *Tetrahedron Lett.* **2000**, *41*, 9239 and references cited therein.
9. (a) Padwa, A. In *1,3-Dipolar Cycloaddition Chemistry*; Taylor, E. C., Weissberger, A., Eds.; Wiley-Interscience: New York, 1984. (b) Carruthers, W. *Cycloaddition Reactions in Organic Synthesis*; Pergamon: Oxford, 1990.
10. (a) Leggio, A.; Liguori, A.; Procopio, A.; Siciliano, C.; Sindona, G. *Tetrahedron Lett.* **1996**, *37*, 1277. (b) Giglio, G.; Leggio, A.; Liguori, A.; Napoli, A.; Procopio, A.; Siciliano, C.; Sindona, G. *Synth. Commun.* **1996**, *26*, 4211. (c) Leggio, A.; Liguori, A.; Procopio, A.; Siciliano, C.; Sindona, G. *Nucleosides Nucleotides* **1997**, *16*, 1515. (d) Leggio, A.; Liguori, A.; Maiuolo, L.; Napoli, A.; Procopio, A.; Siciliano, C.; Sindona, G. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3097.
11. Mzengeza, S.; Whitney, A. R. *J. Chem. Soc., Chem. Commun.* **1984**, 606.
12. Perno, C. F.; Sindona, G., results to be published.
13. Soudeyns, H.; Yao, Q.; Gao, Q.; Belleau, B.; Kraus, J.-L.; Nguyen-Ga, N.; Spira, B.; Wainberg, M. A. *Antimicrob. Agents Chemother.* **1991**, *35*, 1386.
14. Allerson, C. R.; Chen, S. L.; Verdine, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 7423.
15. (a) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171. (b) Sung, W. L. *J. Org. Chem.* **1982**, *47*, 3623. (c) Reese, C. B.; Skone, P. A. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1263.
16. Ueda, N.; Kondo, K.; Kono, M.; Takemoto, K.; Imoto, M. *Makromol. Chem.* **1968**, *120*, 13.
17. Miah, A.; Reese, C. B.; Song, Q. *Nucleosides Nucleotides* **1997**, *16*, 53.
18. (a) Kaye, H. *J. Polym. Sci., Part B* **1969**, *7*, 1. (b) Kaye, H.; Chang, S.-H. *Tetrahedron* **1970**, *26*, 1369.