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FULL PAPER

Carbanucleosides

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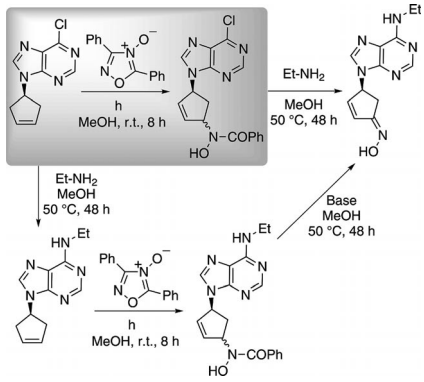

Keywords: Antiviral agents / Carbanucleosides / Ene reaction / Cycloaddition / Nitrosocarbonyls / Nitrile oxides

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Syntheses of New Carbanucleosides by Pericyclic Reactions

Luca Scagnelli,[a] Misal Giuseppe Memeo,[a] Serena Carosso,[a] Bruna Bovio,[a] and Paolo Quadrelli*[a]

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Introduction

Viral infections and malignancies are among the commonest causes of morbidity and mortality in our society, and this motivates strong interest in the design and synthesis of new molecules that can effectively counteract these diseases. The discovery of several new series of nucleoside analogues with antiviral activity has altered the classical way of thinking about the structures of nucleoside analogues as antiviral agents.[1] These derivatives play a fundamental role in viral chemotherapy, and modification of the sugar fragment, as well as its replacement with a carbocyclic moiety, have resulted in the synthesis of interesting nucleoside analogues that have shown remarkable activity towards a variety of viruses.[2] Methods for the synthesis of sugar and sugar-modified nucleosides, as well as carbocyclic nucleosides, have been studied extensively.[3] Various synthetic problems such as low yields and low stereoselectivity have frequently been encountered, however. These, as well as toxicity problems with the obtained compounds, are just some of the drawbacks that characterize these synthetic approaches.

Carbanucleosides serve as more metabolically stable structural analogues of both natural and unnatural antiviral, antifungal and antibacterial nucleosides.[4] Structurally, the typical feature of a nucleoside is the presence of a spacer between the nucleobase and a hydroxy functionality, where the spacer can be either an open-chain or a cyclic moiety (Figure 1). In the cyclic case, ring size modifications and the mono- or polycyclic nature of the spacer between the nucleobase and the hydroxy group affect both the conformational bias and the activities and functions of the nucleoside analogues, as has been detailed in various structural studies.[5] The carbocyclic moiety is normally represented by a cyclopentane ring, although four-, six- and seven-membered rings are often proposed as alternatives.[6]

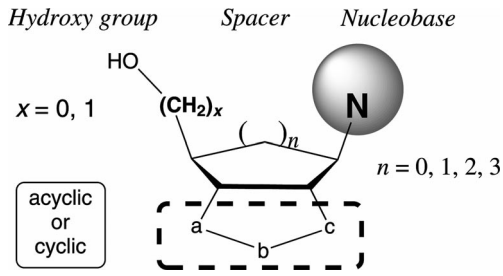

Figure 1. Structural features of carbocyclic nucleoside analogues.

A side arm carrying a hydroxy group is normally present. This feature discriminates between the two families of classical nucleoside analogues (x = 1), bearing hydroxymethylene moieties, and nor-nucleoside analogues (x = 0), in which the hydroxy groups are directly linked to the carbocyclic rings.[7] The nucleobases can be of the purine or pyrimidine type, and a variety of functionalized structures, suitable for various types of functionalization, have been reported.

Most effective antiviral agents act as prodrugs for their corresponding phosphorylated metabolites. The hydroxy group on the side arm is recognized and phosphorylated by the kinases that produce the corresponding nucleotides. Modifications in this part of the nucleoside structure are often required.[8] Recently, N–O chemistry, based on nitroso reagents, has been extensively applied for the synthesis of nucleosides as antivirals and antibiotics.[4,9] In some cases the N–O functionality serves as an isoster of the hydroxymethylene group [i.e., the hydroxylamine (HO–NH) moiety]. In others, the mild cleavage conditions usable for the N–O bond allow the stereoselective introduction of hydroxy and amino groups for further synthetic elaborations.

[a] Department of Chemistry, University of Pavia,
Viale Taramelli 12, 27100 Pavia, Italy
Fax: +39-0382-987323
E-mail: paolo.quadrelli@unipv.it
Homepage: http://chimica.unipv.eu/site/home/persone/scheda700003454.html

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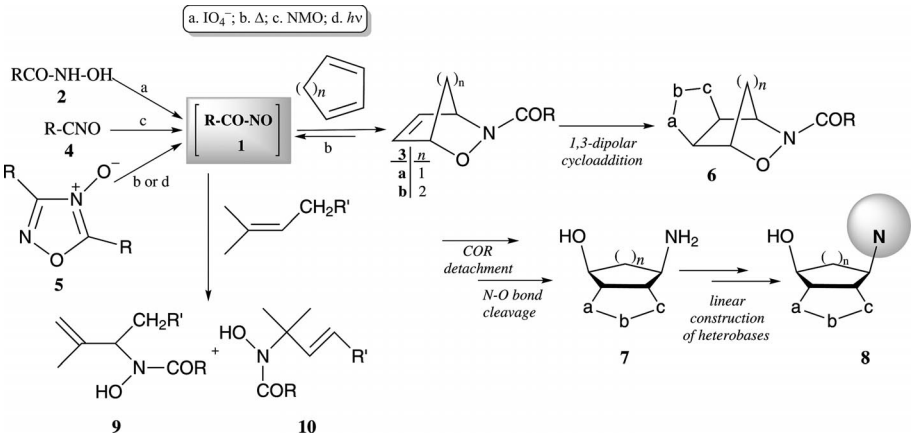


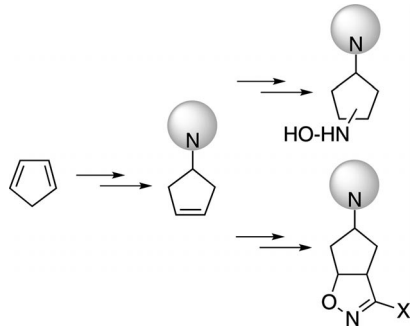
Scheme 1.

We have recently proposed the synthesis of a new class of carbocyclic nucleosides, starting from cyclopentadiene and based on nitrosocarbonyl intermediates (RCONO, **1**, Scheme 1) chemistry.[10] These intermediates, generated by the mild oxidation of nitrile oxides with tertiary amine *N*-oxides, are efficiently trapped by cyclic dienes to afford the hetero-Diels–Alder (HDA) cycloadducts **3**.

These are highly reactive dipolarophiles and have been employed to synthesize the conformationally restricted carbocyclic moiety aminols **7** through amide hydrolysis and N–O bond cleavage of the cycloadducts **6**.[11] Aminols **7** served for the linear construction of purine and pyrimidine nucleosides **8**.

Applications of nitrosocarbonyl compounds to the syntheses of biologically active molecules are well-known, essentially due firstly to the variety of generating methods available (oxidation of hydroxamic acids[12] and nitrile oxides,[13] as well as thermal[14] or photochemical[15] cycloreversions of 1,2,4-oxadiazole 4-oxides) and secondly to the exceptional reactivity of these intermediates in HDA reactions. Nitrocarbonyl intermediates are also powerful enophiles,[16] but no applications of ene reactions to the synthesis of nucleosides have so far been published.[17] Recently we have reported that nitrosocarbonyl intermediates, generated at room temp. by the mild oxidation of nitrile oxides, undergo clean ene reactions with trisubstituted olefins **9** (Scheme 1).[18]

The ene reactions of nitrosocarbonyl intermediates described above represent a valuable tool for the synthesis of nucleoside analogues in which the role normally attributed to the hydroxymethylene group is instead played by the hydroxyamino functionality. In continuation of our studies of ene reactions and their applications for nucleoside synthesis, we wish to report here the synthesis of carbanucleoside analogues through the application of pericyclic reactions. The nucleoside synthesis proceeds from the cheap cyclopentadiene, through insertion of commercially available heterobases, taking advantage of the chemistry of nitrosocarbonyl intermediates and nitrile oxides (Scheme 2).



Scheme 2.

Results

Convergent Synthesis of 6-Chloropurine-Substituted Cyclopentene

Freshly distilled cyclopentadiene was subjected to epoxidation with peracetic acid. The epoxide **11** (Scheme 3) was obtained in 40% yield as straw yellow oil at a good level of purity.[19]

Reduction of **11** with lithium aluminiumhydride in anhydrous diethyl ether at 0 °C overnight afforded cyclopent-3-en-1-ol (**12**) in 56% yield,[19] and this was converted into the corresponding mesyl derivative **13** (reddish oil, yield 61%).[20] Condensation with the sodium salt of 6-chloropurine (**14**) followed immediately, as the key step in the convergent synthesis of the nucleoside structures.[21]

6-Chloropurine (**14**, 1 equiv.) was suspended in anhydrous DMF, and a slight excess of NaH (95%) was added portionwise with vigorous stirring at 0 °C for a couple of hours to generate the corresponding sodium salt **15**. The cyclopentene mesylate **13** was added dropwise to the solution, and the reaction mixture was stirred at room temperature for 48 h. After this, the reaction was quenched by pouring the mixture into ice, and the products were extracted

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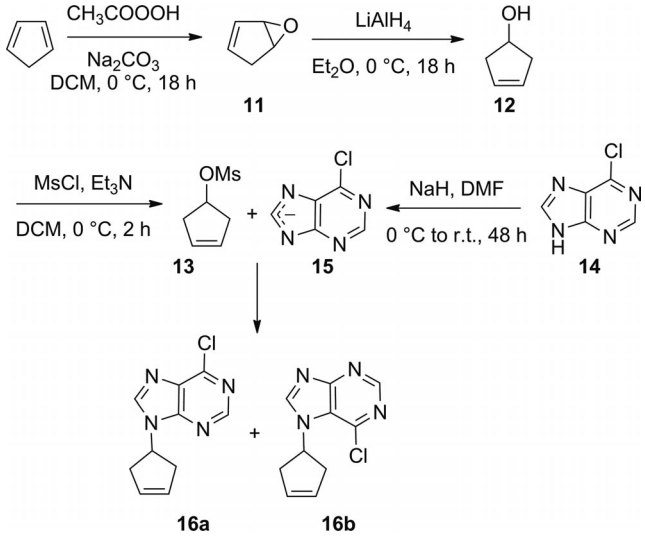

Scheme 3.

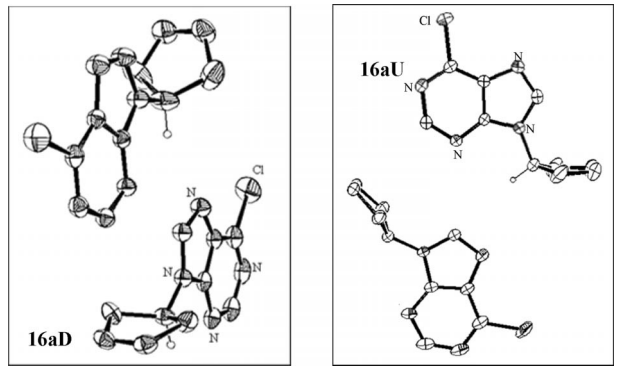

Figure 2. ORTEP plots of adduct **16a** with atom labelling (ellipsoids at 20% probability). Hydrogen atoms are omitted for clarity, with the exception of CH–N_{purine}. Labels **D** (downward) and **U** (upward) indicate the orientation of the methine carbon atom of the cyclopentene moiety.

from the water phase with dichloromethane. The residue obtained upon evaporation of the solvent was subjected to chromatographic separation to isolate the regioisomeric adducts **16a** and **16b** (36% and 23% yield, respectively).

The structure assignment is based on the corresponding analytical and spectroscopic data, as well as on X-ray analysis of the major compound **16a**. Regioisomer **16a** is a white, low-melting solid (m.p. 53–4 °C), the proton NMR spectrum of which (DMSO as solvent) clearly shows a 1:1 ratio between the cyclopentene and the 6-chloropurine moieties. The two allylic methylene units were found as double doublets at δ = 2.78 and 2.97 ppm, whereas the olefinic CH=CH system gave a singlet at δ = 5.90 ppm. The homoallylic proton adjacent to the nitrogen atom of the purine ring gave a multiplet at δ = 5.37 ppm. The purine ring was recognizable through the presence of the singlets corresponding to the CH=N protons at δ = 8.66 and 8.79 ppm. The ^{13}C NMR spectrum was consistent with a structure containing 10 carbon atoms.

The minor compound **16b** is a yellowish oil with a similar spectroscopic pattern. The two allylic methylene groups were found as double doublets at δ = 2.02 and 2.29 ppm, whereas the olefinic CH=CH system gave a singlet at δ = 5.14 ppm. A multiplet at δ = 4.99 ppm corresponded to the homoallylic proton, the singlets at δ = 7.87 and 7.99 ppm to the purine ring.

The evident symmetrical arrangement of adducts **16a** and **16b** resulted in some difficulties in the assignments of the regiochemistry, and NOESY experiments were unsuccessful from this point of view. A decisive contribution to solving the structural problem was provided by X-ray analyses of adduct **16a** conducted on single crystals, which allowed for the unequivocal attribution of both regioisomeric structures. ORTEP views of the adduct **16a** are shown in Figure 2; the corresponding crystallographic data are given in Table 4 and in the Supporting Information.

Curiously enough, the crystal structure of adduct **16a** showed the presence in the solid of both of the conformers associated with the cyclopentene moiety. In Figure 2 on the left (bottom), the structure **16aD** shows the purine ring lying in the orthogonal plane with respect to the average plane of the carbocyclic moiety attached to the methine carbon atom displaced downward (**D**). In the plot on the right (top), the purine ring is attached to the methine carbon atom displaced upward (**U**) with respect to the average plane of the cyclopentene moiety. These results suggested a flexible orientation of the heterobase ring linked to a cyclopentene system.

In the following sections the behaviour of the cyclopentene-purine **16a** as dipolarophile and ene partner is studied, together with the functionalization steps for the synthesis of new nucleoside analogues, thanks to the favourable positioning of the chlorine atom in the heterobase ring for further derivatization with suitable amines.

Adduct 16a as Dipolarophile and Synthesis of Adenine Derivatives

We have recently illustrated the synthetic potential of bromoisoxazoline moieties in nucleoside structures;[9b] moreover, these heterocyclic moieties are reported to be isosters of sugars and to be able to establish positive binding with amino acid residues in proteins.[22] A bromonitrile oxide BrCNO is easily generated in situ in AcOEt solution from the corresponding bromoxime shown in Scheme 4, the preparation of which is achieved by treatment of glyoxylic acid with hydroxylamine hydrochloride in the presence of bromine.[23]

With the cyclopentene-purine **16a** as dipolarophile in its racemic mixture form, the 1,3-dipolar cycloaddition reaction smoothly occurred to afford the single cycloadduct **17** (Scheme 4), in 98% yield and as a white solid compound (m.p. 223–5 °C) easily purified by column chromatography.

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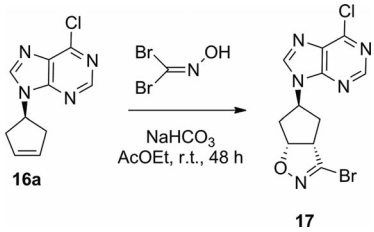
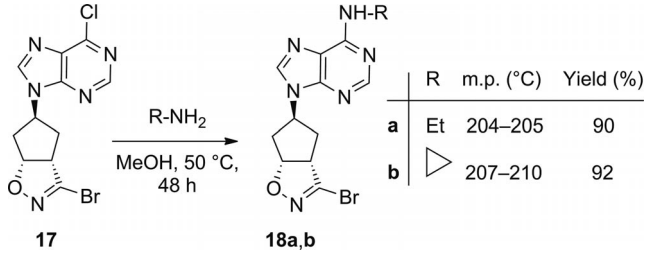


Scheme 4.

The structure of cycloadduct **17** was assigned on the basis of the relevant analytical and spectroscopic data. The ^{1}H NMR spectrum in DMSO showed complex multiplet signals centred at δ = 2.66 ppm, corresponding to the methylene protons of the cyclopentane ring. The isoxazoline protons gave two signals at δ = 5.35 ppm (dd, J = 9, 5 Hz, 5-H) and δ = 4.22 ppm (t, J = 9 Hz, 4-H), coupled between them and the adjacent methylenes. A signal at δ = 4.89 ppm is attributable to the methine proton; the singlets at δ = 8.79 and 8.81 ppm correspond to the CH=N system of the purine ring. The stereochemistry of the cycloadduct was determined through a NOESY experiment. The sole CH=N proton at δ = 8.79 ppm, corresponding to the imidazole ring of the 6-chloropurine, gave an NOE crosspeak only with the two deshielded protons located on the same plane of the cyclopentane ring at δ = 2.74 ppm and belonging to the methylene groups. The same protons correlate through NOE crosspeaks with the H4 and H5 protons of the isoxazoline ring that is located *anti* to them.

The chloro derivative **17** was functionalized with a couple of primary representative amines, linear and cyclic, as shown in Scheme 5, by the well-established protocol.[10,24] Upon heating of methanol solutions of **17** at moderate temperature with excesses either of ethylamine or of cyclopropylamine, the corresponding derivatives were isolated as solids in high yields by simple concentration of the solvent and were fully characterized. In its ^{1}H NMR spectrum (DMSO) the ethylamino derivative **18a** showed the presence of a triplet at δ = 1.19 ppm along with a broad singlet at δ = 3.59 ppm corresponding to the ethyl moiety, whereas the NH proton was found at δ = 7.54 ppm (broad singlet). The presence of the NH group in the structure was also confirmed by the IR spectrum, which showed a strong band at $\tilde{\nu}$ = 3270 cm^{-1}.



	R	m.p. (°C)	Yield (%)
a	Et	204–205	90
b	▷	207–210	92

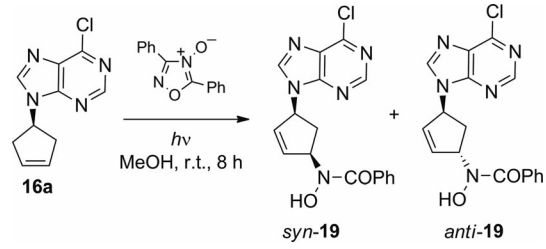
Scheme 5.

Similarly, the ^{1}H NMR spectrum (DMSO) of the cyclopropyl derivative **18b** showed the cyclopropyl moiety in the form of the presence of diagnostic signals at δ = 0.62 and 0.71 ppm, together with the CH–NH system at δ = 3.05 ppm. The NH group gave a doublet at δ = 7.88 ppm (J = 1 Hz) along with the IR band at $\tilde{\nu}$ = 3271 cm^{-1}.

Adduct 16a as Ene Partner and Synthesis of Hydroxylamine Derivatives

In previous works, as well as in the Introduction of this paper, we have detailed various methods for the generation of nitrosocarbonyl intermediates, along with the ability of these fleeting intermediates to behave as "super-enophiles" in ene reactions with a variety of olefins.[13–16,18] We have demonstrated, however, that the oxidation of nitrile oxides with NMO fails with less substituted ethylenes, because of competing 1,3-dipolar cycloaddition reactions between the 1,3-dipoles and the C=C double bonds in the olefins. This shortcoming can be avoided by using the mildest available method for the generation of nitrosocarbonyls: the photochemical fragmentation of the easily available 3,5-diaryl-1,2,4-oxadiazole 4-oxides.[25] When exposed to sunlight or 310 nm lamps (15 W), these heterocycles undergo cycloreversion into aromatic nitriles and nitrosocarbonyls, and in the absence of competing processes and in the presence of excess olefins these add to the C=C double bond to afford ene adducts in high yields.[26]

Ene reactions of nitrosocarbonyls constitute a valuable method for the introduction of a hydroxylamino functionality onto a carbocyclic spacer as an isoster of the hydroxymethylene group in a nucleoside structure.[4] We investigated the photochemical reaction between 3,5-diphenyl-1,2,4-oxadiazole-4 oxide and the cyclopentene-purine **16a** (Scheme 6) for the preparation of new nucleoside analogues.



Scheme 6.

3,5-Diphenyl-1,2,4-oxadiazole 4-oxide (1.5 equiv.) was added portionwise with stirring to a methanol solution of **16a** over a period of a couple of hours under irradiation with 2 × 15 W lamps centred at 310 nm (Scheme 6). This ensures an excess of the cyclopentene derivative during the generation of the nitrosocarbonyl benzene through the fragmentation of the parent heterocycle. After evaporation of the solvent, the residue was subjected to chromatographic separation to purify the ene diastereoisomeric adducts *syn*-**19** and *anti*-**19**, obtained in moderate yields (26%) as an inseparable mixture.

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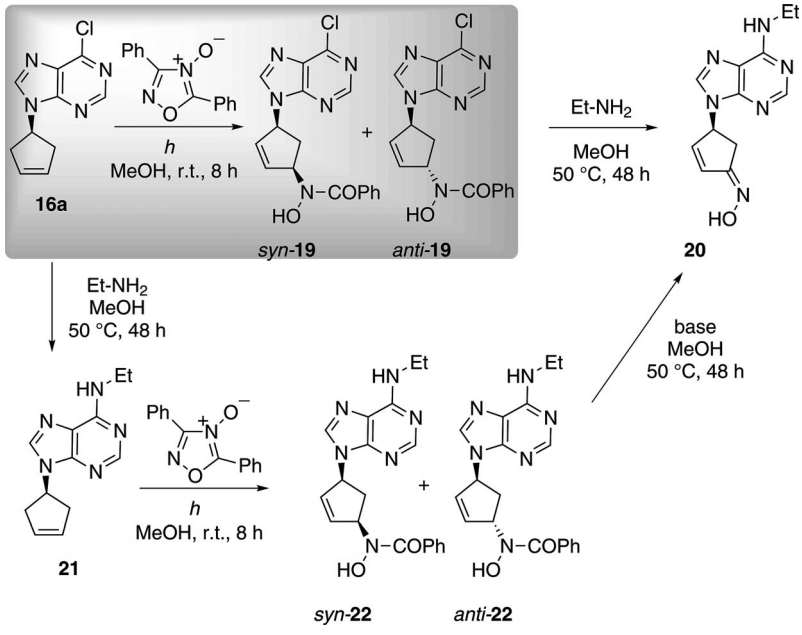
The structure assignment of the solid ene adducts (m.p. 176–9 °C) is based on the corresponding analytical and spectroscopic data. In the IR spectrum the presence of a broad band around 3200 cm^{-1} indicated the introduction of a hydroxy group (in the hydroxylamine moiety), and a band found at 1634 cm^{-1} was in the expected range for a conjugated carbonyl group. The ^1H NMR spectrum in DMSO showed two pairs of multiplets corresponding to the methylene system of the cyclopentene ring at δ = 2.10 and 3.02 ppm and at δ = 2.44 and 2.63 ppm coupled with the two methine protons at δ = 5.67 and δ = 6.28 ppm. The CH=CH protons gave a cumulative signal for both diastereoisomers at δ = 5.95 ppm. Other than the aromatic protons of the phenyl groups, the CH=N signals of the purine rings of *syn*-**19** and *anti*-**19** are nicely separated and found at δ = 8.69 and 8.77 ppm and at δ = 8.46 and 8.82 ppm. The presence of highly deshielded singlets at δ = 9.73 and 9.82 ppm indicated the presence of the acid protons of the hydroxylamine moieties originating from the ene addition of the nitrosocarbonylbenzene. By comparison of the intensities of these two signals we were able to determine the diastereoisomeric ratio in the mixture (2.4:1). To complete the structural assignment, we investigated the possibility of attributing the relative positions of the purine ring and the hydroxylamine group through NOESY experiments. Unfortunately, as a result of the high symmetry of the ene adducts and the unfortunate coincidence of chemical shifts in the NMR spectra, we were unable to attribute the stereochemical configurations of products **19** beyond any reasonable doubt even with a change in the solvent.

To functionalize the ene adducts **19** with amines to test their biochemical behaviour, we performed the reactions by the standard protocol already described.[10,24] A sample of compound **19** as a mixture of diastereoisomers was dissolved in methanol, and the solution was saturated with ethylamine. The sealed vial was heated at 50 °C for 48 h (Scheme 7).

After this, and removal of the solvent, compound **20** was obtained as a solid in 91% yield. The structural assignment was based on the relevant analytical and spectroscopic data. The IR spectrum showed the C=C double bond band at $\tilde{\nu}$ = 1585 cm^{-1}, along with another band at $\tilde{\nu}$ = 1622 cm^{-1} in the range typical of a C=N group. At higher frequencies, a broad band centred at $\tilde{\nu}$ = 3000 cm^{-1} and a sharper one at $\tilde{\nu}$ = 3306 cm^{-1}, corresponding to a hydroxy group and a NH group, respectively, were found.

The presence of the ethylamino group was clearly shown in the ^1H NMR spectrum (DMSO) by the triplet at δ = 1.17 ppm (CH_3) and the broad singlet at δ = 3.52 ppm (CH_2), along with the singlet for NH at δ = 7.71 ppm. The remaining parts of the proton spectrum are fully consistent with the cyclopentene-purine structure, with the following features. In the aromatic range of the spectrum, the phenyl system of the benzoyl group was missing, and a sharp and highly deshielded singlet appeared at δ = 10.90 ppm, indicating the presence of an acidic hydroxy group, typical of oximes. This result can be easily explained in terms of substitution of the chlorine atom with the ethylamino group and concomitant base-catalysed benzoyl elimination, favoured by the formation of the conjugated C=N double bond. Clearly, the change in hybridization of the hydroxylamine carbon atom on transformation into an oxime carbon atom erases the diastereoisomeric mixture, so a single compound is obtained.

A diversion can be planned if the conservation of the *N*-benzoyl-hydroxylamine functionality is desired or required.



Scheme 7.

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The cyclopentene-purine **16a** is easily convertible into the ethylamino derivative **21** (Scheme 7), isolatable as a solid compound (m.p. 105–7 °C) in 90% yield on purification by column chromatography. The reported structure was confirmed by the analytical and spectroscopic data. The main features, relative to the starting compound, were the presence of the ethylamino group [^{1}H NMR (δ, DMSO): 1.30 (t, CH_3), 3.72 (br. s, CH_2), 5.75 ppm (br. s, NH)]. Compound **21** was allowed to react with nitrosocarbonylbenzene, photochemically generated through the fragmentation of 3,5-diphenyl-1,2,4-oxadiazole 4-oxide under the same experimental conditions as applied previously. Upon evaporation of the solvent a residue was obtained and subjected to chromatographic separation to isolate an inseparable diastereoisomeric mixture of ene adducts **22syn** and **22anti** as an oil in 36% yield. The ^{1}H NMR spectrum in DMSO showed two pairs of multiplets corresponding to the methylene system in the cyclopentene ring, at δ = 2.00 and 3.00 ppm and at δ = 2.32 and 2.51 ppm, together with the methine protons at δ = 5.59 and δ = 5.78 ppm. The CH=CH protons gave a cumulative signal for both diastereoisomers at δ = 6.18 ppm. In addition to the aromatic protons of the phenyl groups, the CH=N signals of the purine rings of **22syn** and **22anti** are nicely separated and found at δ = 7.95 and 8.09 ppm and at δ = 8.18 and 8.22 ppm. The NH signals are combined in a broad singlet at δ = 7.74 ppm, and the two highly deshielded singlets at δ = 9.77 and 9.82 ppm indicate the presence of the acid protons of the hydroxylamine moieties. The ethylamino group is found in the expected range. The diastereoisomeric ratio was calculated to be close to 1:1. Any attempt to attribute the stereochemistry to the two sets of signals in the mixture through NMR experiments unfortunately failed, again because of the highly symmetric nature of these type of adducts.

To verify the ability of organic bases to promote the benzoyl group elimination in compound **22**, we dissolved a sample of **22** in methanol and the solution was saturated with ethylamine gas. The sealed vial was heated at 50 °C for 48 h and the debenzoylated compound **20** was isolated in 80% yield.

Antiviral Tests

We submitted samples of some representative compounds (**18a**, **18b**, **19** and **21** to the NIH/NIAID (USA) for primary antiviral evaluation. Compounds **19** and **21** were tested for their inhibitory activity against Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Varicella-Zoster virus (VZV), Vaccinia virus (VV), Punta Toro virus (PTV), hepatitis B virus (HBV) and hepatitis C virus (HCV). The antiviral activities of the above compounds were tested in vitro in cell line HFF (strain E-377 for HSV-1, strain G for HSV-2, strain Ellen for VZV and strain Copenhagen for VV), the Vero 76 cell line (strain Adames) for PTV, cell line 2.2.15 (strain ayw) for HBV and the Huh-Luc/Neo ET cell line (strain CON-1) for HCV. They were also tested against respiratory virus influenza A H1N1 cell line MDCK (strain California 7/2009) and neuramidase (NA) cell line HFF (strain NA). Table 1 and Table 2 report the primary antiviral activities of the tested compounds and control experiments on specified drugs.

Table 1. Primary antiviral activities of compound **19**.

Virus	Cell line	EC_{50}	EC_{90}	CC_{50}	SI_{50}[#]
HSV-1[a]	HFF	>60	>60	295	<5
Acyclovir		*1.75*	*3.09*	*>100*	*>57*
HSV-2[a]	HFF	>60	>60	228	<4
Acyclovir		*3.34*	*>100*	*>100*	*>30*
VZV[a]	HFF	>60	>60	130	<2
Acyclovir		*0.69*	*5.76*	*>100*	*>145*
VV[a]	HFF	>300	>300	>300	n.a.
Cidofovir		*4.75*	*8.22*	*>300*	*>63*
PTV[b]	Vero 76	18		100	5.6
Ribavirin		*8*		*>1000*	*>120*
HBV[c]	2.2.15	>100	>100	>100	n.a.
Lamivudine		0.048	0.135	2322	48375
HCV[d]	HL/Neo ET	>20	>20	>20	n.a.
IFNα-2b		>2	>2	0.14	>14
Flu-A H1N1[e]	MDCK	33.65	>100	>100	>3
Ribavirin		*5.85*	*13.28*	*>100*	*>17*
NA[f]	HFF			58.70	
Cidofovir				*>300*	

[a] Drug conc. range 0.096–300 μM; control conc. range 0.032–100 μM. [b] Drug conc. range 0.1–100 μM; control conc. range 1–1000 μM. [c] Drug conc. range 0.1–100 μM; control conc. range 0.3–100 μM. [d] Drug conc. range 0.063–20 μM; control conc. range 0.006–2 IU mL^{-1}. [e] Drug conc. range 0.096–300 μM; control conc. range 0.096–300 μM. [f] Drug conc. range 0.8–100 μM; control conc. range 0.8–100 μM. [#] n.a.: not applicable.

Table 2. Primary antiviral activities of compound **21**.

Virus	Cell line	EC_{50}	EC_{90}	CC_{50}	SI_{50}[g]
HSV-1[a]	HFF	>300	>300	>300	n.a.
Acyclovir		*1.75*	*3.09*	*>100*	*>57*
HSV-2[a]	HFF	>300	>300	>300	n.a.
Acyclovir		*3.34*	*>100*	*>100*	*>30*
VZV[a]	HFF	>300	>300	>300	n.a.
Acyclovir		*0.69*	*5.76*	*>100*	*>145*
VV[a]	HFF	>300	>300	>300	n.a.
Cidofovir		*4.75*	*8.22*	*>300*	*>63*
PTV[b]	Vero 76	>81		81	<1
Ribavirin		*8*		*>1000*	*>120*
HBV[c]	2.2.15	>100	>100	>100	n.a.
Lamivudine		0.048	0.135	2322	48375
HCV[d]	HL/Neo ET	>20	>20	>20	n.a.
IFNα-2b		>2	>2	0.14	>14
Flu-A H1N1[e]	MDCK	67.78	>100	>100	>1
Ribavirin		*5.85*	*13.28*	*>100*	*>17*
n.a.[f]	HFF			298.69	
Cidofovir				*>300*	

[a] Drug conc. range 0.096–300 μM; control conc. range 0.032–100 μM. [b] Drug conc. range 0.1–100 μM; control conc. range 1–1000 μM. [c] Drug conc. range 0.1–100 μM; control conc. range 0.3–100 μM. [d] Drug conc. range 0.063–20 μM; control conc. range 0.006–2 IU mL^{-1}. [e] Drug conc. range 0.096–300 μM; control conc. range 0.096–300 μM. [f] Drug conc. range 0.8–100 μM; control conc. range 0.8–100 μM. [g] n.a.: not applicable.

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Both the hydroxylamine derivative **19** and the adenine cyclopentene **21** were found to be mainly inactive in the reported Herpes-type viruses experiments. The same lack of activity was also observed in the cases of PTV and HBV. Insufficient activity was found for compounds **19** and **21** in the case of Flu-A H1N1 virus and NA. Some differences between the two cyclopentenyl derivatives can be observed. Compound **19** seems to be capable of inhibiting the Flu-A H1N1 virus, whereas compound **21** showed a CC_{50} value very close to the limit value expressed by the reference Cidofovir.

The bromoisoxazoline derivatives **18a** and **18b** were also tested, but only against the respiratory virus influenza A H1N1 cell line MDCK (strain California 7/2009), in view of the results previously obtained for compounds **19** and **21**. Table 3 reports the primary antiviral activities of the tested compounds and control experiments with specified drugs.

Table 3. Primary antiviral activities of compounds **18a** and **18b**.

Entry		Virus[a]	EC_{50}	EC_{90}	CC_{50}	SI_{50}
1	**18a**	Flu-A H1N1	0.80	>100	>100	>125
2	**18b**		>100	>100	>100	<1
3	*Ribavirin*		*5.85*	*13.28*	*>100*	*>17*

[a] Drug conc. range 0.8–100 μM; control conc. range 0.8–100 μM.

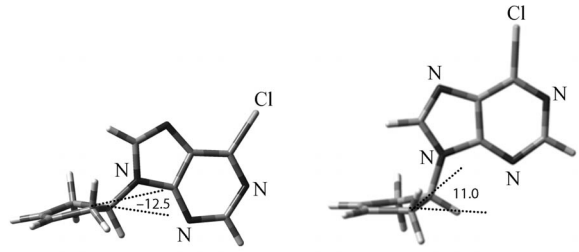
These primary antiviral activity data might suggest potential for the development of docking experiments with the selected protein responsible for the replication of the influenza type A virus of type H1N1. The outbreak of the human influenza A pandemic (H1N1) caused considerable public concern. Recent studies focused attention on the understanding of this new virus by analysing the relationship between its molecular characteristics and its pathogenic properties.[27] Results of this analysis indicated that the human pandemic influenza A (H1N1) virus was a new, reassorted virus combining genetic materials from the avian flu (H1N1) virus, the classical swine flu (H1N1) virus, the human flu (H3N2) virus and the Eurasian swine flu (H1N1) virus. Analysis of the sequences for receptor-binding and cleavage sites of hemagglutinin (HA) allowed it to be determined that replication could be inhibited by molecules such as Oseltamivir (Tamiflu), a diamine derivative of shikimic acid,[28] and Zanamivir (Relenza), a derivative of 3,4-diamino-2-(hydroxymethyl)-3,4-dihydro-2*H*-pyran-6-carboxylic acid.[29]

The Influenza A virus is an orthomyxovirus, and its receptor binding complex consists of two primary structural proteins: hemagglutinin (HA) and neuraminidase (NA). It has been determined that hemagglutinin is the primary protein responsible for binding to receptor sites on the cell membrane, allowing the virion to enter the cell.[30]

Being aware of the structural differences between our compounds and the active compounds cited above, we are planning firstly to perform a secondary screening of the same molecules to complete these in vitro preliminary results, with the possibility also to introduce minor structural modifications to upgrade the antiviral activities of our nucleoside analogues. Docking and protein binding studies to shine some light on the structure–activity relationships of these nucleoside analogues will follow.

Discussion

The introduction of a heterobase, in this case 6-chloropurine, onto a cyclopentene spacer generates a fairly symmetrical substrate in which the heterobase does not influence or restrict the conformational mobility of the cyclopentene ring. X-ray analysis in fact showed the presence in the crystal structure of the two conformers in which the methylene bridge is displaced upward and downward with respect to the average plane of the unsaturated moiety. The conformational space of product **16a** was explored through optimization by the DFT approach at the B3LYP/6–31G(d) [31] level. Figure 3 shows the calculated structures for the two conformers **16aD** and **16aU**.



16aD (ΔG = 1.05 kcal/mol) **16aU** ($\Delta G \equiv$ 0.0 kcal/mol)

Figure 3. Calculated structures of the conformers **16aD** and **16aU** and relative energies. Deviations from planarities are reported in degrees.

The difference in energy between the two conformers is small, and the deviation from the average plane defined by the unsaturated system is just over 10°. Nevertheless, an interesting difference can be observed in the consequent position of the purine ring and in particular of the H–C=N system belonging to the imidazole moiety.

This is the moment to comment on the X-ray structures shown in Figure 2. The purine ring is in the special position *x*, 1/2, *z*, whereas the cyclopentene rings have a mirror plane. In the purine moiety, the sum angles at N9 [360.0(3)°] and N9′ [360.0(3)°] are consistent with sp^2 hybridization of N9 and N9′, through which the 2*pz* lone pair takes part in π-bonding within the heterocyclic system. The cyclopentene rings are tilted by 90.0(2)° with respect to the purine ring. This excludes any conjugation between the cyclopentene and purine systems; indeed, the N9–C10 [1.470(4) Å] and N9′–C10′ [1.473(5) Å] distances are longer than the corresponding single bonds [1.426(12) Å] in aromatic compounds. Interestingly, there is exact planarity in the purine moieties, because of the *x*, 0.2500, *z* coordinates. In the pur-

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ine five-membered ring the N7–C8 [1.301(5) Å] and N7′–C8′ [1.316(5) Å] distances in molecules **16aD** and **16aU** correspond to double bonds, whereas the remaining four distances correspond to values intermediate between single and double bonds. In the purine six-membered rings, N3–C4 [1.323(5) Å] and N5–C6 [1.311(5) Å] in molecule **16aD** and N3′–C4′ [1.326(6) Å] and N5′–C6′ [1.312(6) Å] in molecule **16aU** correspond to double bonds, whereas the remaining distances are longer, thus suggesting π delocalization over the purine system.

The cyclopentene ring in molecule **16aD** is slightly puckered, with the maximum deviations from the least-squares plane of 0.075(4) Å below this plane for C11 and of 0.090(3) Å above it for C10. The cyclopentene ring in molecule **16aU** is more puckered, with the maximum deviations from the least-squares plane of 0.1699(5) Å below the plane (for C10′) and 0.139(4) Å above it. The puckering parameters of the cyclopentene moieties for molecules **16aD** and **16aU** calculated as described by Cremer and Pople[32] are $Q = 0.1460$, $\phi = 0°$ and $Q = 0.2704$, $\phi = 0°$, respectively. The cyclopentene rings each show an envelope (*E*) conformation (ideal value $\phi = 0°$). If the pseudorotation concept[33] is applied to this ring system in the two independent molecules **16aD** and **16aU**, the *phase angle* (***P***) is 180° or 0°; a pseudorotation over $\boldsymbol{P} = 180°$ gives the mirror image of the rings with all signs of torsion angles θ inverted. The ***P*** value is calculated from a formula:

$$\tan P = (\theta_2 + \theta_4) - (\theta_1 + \theta_3)/2\theta_0(\sin 36° + \sin 72°)$$

where θ_0, θ_1, θ_2, θ_3 and θ_4 are the five torsion angles about C12–C13*, C13*–C14*, C14*–C10, C10–C11 and C11–C12 for the two independent molecules **16aD** and **16aU**, respectively (see values in Table S1 in the Supporting Information).

In the conformer **16aD** the purine ring occupies the more favourable space, from the steric point of view, in a pseudo-equatorial position around the cyclopentene ring, and the imidazolyl hydrogen atom points out of the plane of the cyclopentene ring. In the other case, **16aU**, the purine occupies a pseudoaxial position that shifts the hydrogen atom of the imidazole ring just over the plane of the cyclopentene ring. Presumably this conformer, although more stable, is less populated when the C=C double bond is oriented to receive the addition by the nitrile oxide or by the nitrosocarbonyl intermediate in the 1,3-dipolar cycloaddition or ene reaction, respectively.

An analogous conformational analysis was conducted on the ethylamino and cyclopropylamino cycloadducts to the bromonitrile oxide **18a** and **18b**, these being the compounds found to be active against the flu virus. Figure 4 shows the calculated structures for both series of conformers. The two ethylamino conformers differ more significantly in energy then the cyclopropylamino ones, with **18aD** being more stable by 2.15 kcal mol^{-1}. The reduced flexibility of the cyclopentane moiety is caused by the fusion with the bromoisoxazoline ring, and presumably this increases the difficulties in interconversion between the two forms; in addition, the ethylamino-purine ring can be easily located in the wider space area created by the cyclopentane ring in the envelope conformation as in **18aD**.

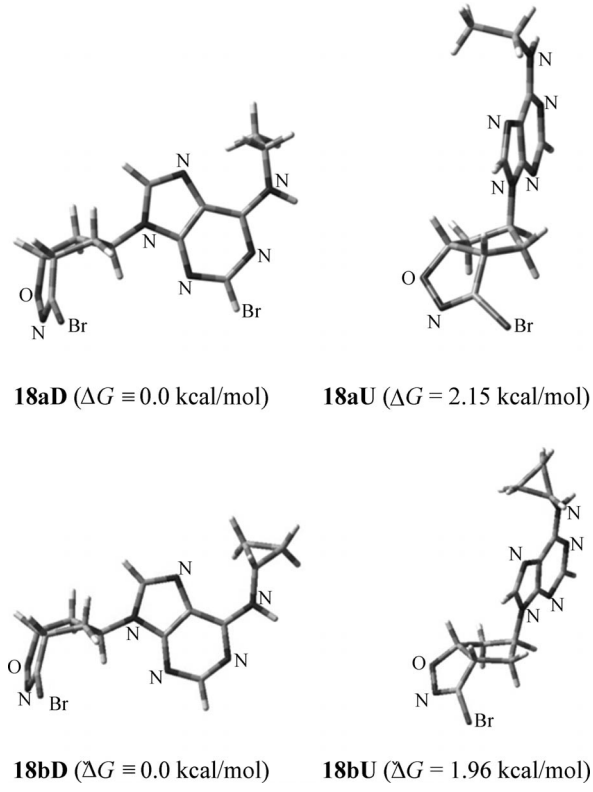

Figure 4. Calculated structures of the conformers **18aD**, **18bD**, **18aU** and **18bU** and relative energies. Deviations from planarity are reported in degrees.

Analogous considerations apply to comparison of the two cyclopropylamino derivatives **18bD** and **18bU**.

These conformational studies should serve for the planned programmed docking studies on HA and NA receptors, allowing for the evaluation of the levels of binding between the active compounds and the proteins responsible for the viral activity. This pivotal step should suggest structural modifications for introduction to improve the activities of the selected molecules.

Conclusions

We report the synthesis of heterobase-functionalized cyclopentene derivatives as valuable substrates for the introduction of suitable substituents through pericyclic reactions. The use of 6-chloropurine in this specific case was considered more convenient because of the further functionalization made possible by the presence of the chlorine atom in the 6-position in the heterobase and the robust methods of derivatization already applied in previous syntheses. The problem of the high symmetry that characterizes the chloropurine cyclopentene makes the structural attribution of the further derivatized compounds somewhat dif-

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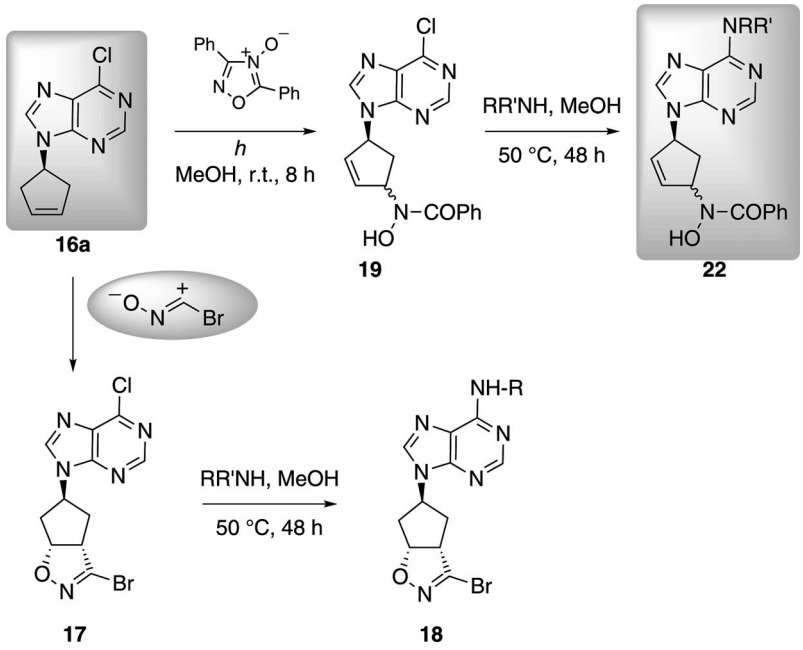

Scheme 8.

ficult, although both the ene and 1,3-dipolar cycloaddition reactions seem to offer new contributions to the search for biologically active compounds (Scheme 8).

Future developments – in particular, the application of the mild and high-yielding Al(Hg) N–O bond cleavage of the isoxazoline ring for the convenient introduction of new functionalities – are currently under study.

This scaled-up strategy for the synthesis of new nucleoside analogues will be applied to prepare new products to be tested in secondary screening against the virus found to be most promising in the previous observations.

Experimental Section

General: All melting points are uncorrected. Elemental analyses were performed with a C. Erba 1106 elemental analyzer. IR spectra (Nujol mulls) were recorded with a Perkin–Elmer RX-1 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE 300 instrument in the specified deuterated solvents. Chemical shifts (δ) are expressed in ppm from internal tetramethylsilane. Column chromatography and tlc: silica gel 60 (0.063–0.200 mm, Merck), eluent cyclohexane/ethyl acetate from 9:1 to 5:5. MPLC chromatographic separations were performed with a Biotage Flash Master Personal apparatus and eluent cyclohexane/ethyl acetate from 9:1 to 5:5. The identification of samples from different experiments was achieved through mixed m.p.s and superimposable IR spectra.

Materials: Cyclopentadiene was freshly distilled from the dimer purchased from Sigma–Aldrich. 6-Chloropurine (**14**) was purchased from Sigma–Aldrich. All other reagents and solvents were purchased from Sigma–Aldrich or Alfa–Aesar and used without any further purification.

Synthesis of Adducts of 6-Chloropurine (14) and Cyclopentene Mesylate 13: 6-Chloropurine (**14**, 3 g, 19 mmol) was suspended in anhydrous DMF, and a slight excess (1.2 equiv.) of NaH (95%) was added portionwise with vigorous stirring at 0 °C over a couple of hours to generate the corresponding sodium salt **15**. Cyclopentene mesylate **13** (1.5 g, 9 mmol) was added dropwise to the solution, and the reaction mixture was stirred at room temperature for 48 h. After this, the reaction was quenched by pouring the mixture into ice, and the products were extracted from the water phase with dichloromethane. The residue obtained upon evaporation of the solvent was subjected to chromatographic separation to isolate the products. Regioisomers **16a** and **16b** were obtained in 36% and 23% yields, respectively, and fully characterized.

Compound 16a: 0.73 g (36%), m.p. 53–54 °C from acetone. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 2.78 (dd, J = 15, 8 Hz, 2 H, CH_2), 2.97 (dd, J = 15, 5 Hz, 2 H, CH_2), 5.37 (m, 1 H, N-CH), 5.90 (s, 2 H, CH=CH), 8.66 (s, 1 H, CH=N), 8.79 (s, 1 H, CH=N) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 39.2, 54.3, 129.2, 131.5, 146.2, 149.3, 151.6, 152.0 ppm. IR: $\tilde{\nu}$ = 1592 (C=C), 1557 (C=N) cm^{-1}. $C_{10}H_9ClN_4$ (220.66): calcd. C 54.43, H 4.11, N 25.39; found C 54.6, H 4.2, N 25.2.

Compound 16b: 0.47 g (23%). Yellowish oil. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 2.02 (dd, J = 16, 2 Hz, 2 H, CH_2), 2.29 (m, 2 H, CH_2), 4.99 (m, 1 H, N-CH), 5.14 (s, 2 H, CH=CH), 7.87 (s, 1 H, CH=N), 7.99 (s, 1 H, CH=N) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 38.7, 55.8, 80.4, 125.4, 126.8, 127.0, 138.5, 144.4, 146.3, 150.3 ppm. IR: $\tilde{\nu}$ = 1592 (C=C), 1538 (C=N) cm^{-1}. $C_{10}H_{20}ClN_4$ (220.66): calcd. C 54.43, H 4.11, N 25.39; found C 54.2, H 4.1, N 25.5.

Cycloaddition between Bromonitrile Oxide and Adduct 16a: Sodium hydrogen carbonate (0.8 g, 9.5 mmol) was suspended in a solution of adduct **16a** (1.0 g, 4.5 mmol) in AcOEt (150 mL). An AcOEt solution (80 mL) of the bromoxime shown in Scheme 4 (1.84 g, 9.1 mmol) was added dropwise with vigorous stirring at room temp. The reaction mixture was stirred at room temperature for 72 h. After this, the inorganic salts were filtered, and the solvent was removed under vacuum. The residue obtained was subjected to chromatographic purification to isolate the cycloadduct **17** in 98% yield.

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Compound 17: 1.52 g (98%), m.p. 223–225 °C from methanol. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 2.66 (m, 4 H, CH_2), 4.22 (t, J = 9 Hz, 1 H, 4-H isox.), 4.89 (m, 1 H, N-CH), 5.35 (dd, J = 9, 5 Hz, 1 H, 5-H isox.), 8.79 (s, 1 H, CH=N), 8.81 (s, 1 H, CH=N) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 33.9, 53.4, 55.1, 84.3, 131.4, 141.2, 146.5, 149.1, 151.3, 151.9 ppm. IR: $\tilde{\nu}$ = 1589 (C=N), 1558 (C=N) cm^{-1}. $C_{11}H_9BrClN_5$ (342.58): calcd. C 38.57, H 2.65, N 20.44; found C 38.6, H 2.4, N 20.3.

Syntheses of the Amino Derivatives 18a and 18b. General Method: Solutions of chloronucleoside **17** (30 mg, 0.09 mmol) in MeOH (2 mL) were either saturated with gaseous ethylamine and kept in a sealed tube at 50 °C for 48 h or, in the case of the liquid cyclopropylamine, an excess (50 equiv.) was added to the solution and the reaction was allowed to proceed in a sealed tube under the same conditions. The solvent was evaporated, and the residues were taken up with methanol to allow crystallization of the nucleoside derivatives **18a** and **18b**, which were fully characterized.

Compound 18a: 27.7 mg (90%), m.p. 204–205 °C from methanol. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 1.19 (t, J = 9 Hz, 3 H, CH_3), 2.60 (m, 4 H, CH_2), 3.59 (br, 2 H, N-CH_2), 4.17 (t, J = 9 Hz, 1 H, 4-H isox.), 4.78 (m, 1 H, N-CH), 5.33 (dd, J = 9, 5.5 Hz, 1 H, 5-H isox.), 7.54 (br, 1 H, NH), 8.19 (s, 1 H, CH=N), 8.20 (s, 1 H, CH=N) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 15.0, 30.6, 34.1, 34.9, 52.6, 55.3, 84.5, 119.5, 139.3, 141.1, 152.2, 154.6 ppm. IR: $\tilde{\nu}$ = 3270 (NH), 1621 (C=N) cm^{-1}. $C_{13}H_{15}BrN_6O$ (351.20): calcd. C 44.46, H 4.30, N 23.93; found C 44.5, H 4.4, N 23.9.

Compound 18b: 29.3 mg (92%), m.p. 207–210 °C from methanol. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 0.62 (m, 2 H, CH_2 cycloprop.), 0.71 (m, 2 H, CH_2 cycloprop.), 2.55 (m, 4 H, CH_2), 3.05 (br, 2 H, N-CH cycloprop.), 4.17 (t, J = 9 Hz, 1 H, 4-H isox.), 4.76 (m, 1 H, N-CH), 5.32 (dd, J = 9, 5.5 Hz, 1 H, 5-H isox.), 7.88 (d, J = 1 Hz, 1 H, NH), 8.23 (s, 1 H, CH=N), 8.24 (s, 1 H, CH=N) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 6.4, 23.8, 34.0, 39.8, 52.5, 55.2, 84.4, 119.6, 139.5, 141.2, 152.1, 155.6 ppm. IR: $\tilde{\nu}$ = 3271 (NH), 1614 (C=N) cm^{-1}. $C_{14}H_{15}BrN_6O$ (363.21): calcd. C 46.30, H 4.16, N 23.14; found C 46.4, H 4.2, N 23.2.

Photochemical Derivatization of Adduct 16a: 3,5-Diphenyl-1,2,4-oxadiazole 4-oxide (2.9 g, 1.5 equiv.) was added portionwise with stirring to a methanol solution of **16a** (1.8 g, 8.2 mmol), over a period of a couple of hours during irradiation with 2 × 15 W lamps centred at 310 nm. The reaction mixture was stirred for a further 3 h for completion. After evaporation of the solvent, the residue was subjected to chromatographic separation to purify the ene adducts **19syn** and **19anti**, obtained in 26% yields as an inseparable mixture of diastereoisomers. The mixture was fully characterized spectroscopically; in the NMR spectra we report the chemical shift of the major diastereoisomer, with the signals of the minor one in square brackets.

Compound *synlanti*-19: 0.75 g (26%), m.p. 176–179 °C from acetone. ^{1}H NMR (300 MHz, DMSO, 80 °C): δ = 2.44 [2.10] (m, 1 H, H-CH), 3.02 [2.63] (m, 1 H, HC-H), 6.28 [5.67] (m, 1 H, N-CH), 5.95 (m, 2 H, CH=CH), 7.42 (m, 3 H, arom.), 7.66 (m, 2 H, arom.), 8.69 [8.46] (s, 1 H, CH=N), 8.77 [8.82] (s, 1 H, CH=N), 9.82 [9.73] (s, 1 H, OH) ppm. ^{13}C NMR (75 MHz, DMSO, 80 °C): δ = 33.8 [34.2], 39.5 [39.8], 60.4 [58.3], 127.8, 128.2, 130.2, [131.3], [131.9], 132.9, [134.8], 134.9, 135.8, [136.1], [145.4], 146.2, [149.0], 151.3, 151.5, [151.6], 168.9 [169.0] ppm. IR: $\tilde{\nu}$ = 3200 (OH), 1634 (C=O), 1592 (C=C), 1557 (C=N) cm^{-1}. $C_{17}H_{14}ClN_5O_2$ (355.78): calcd. C 57.39, H 3.97, N 19.68; found C 57.5, H 4.0, N 19.6.

Synthesis of the Ethylamino Derivative 20: A solution of chloronucleosides **19** (30 mg, 0.08 mmol) in MeOH (2 mL) was saturated with gaseous ethylamine and kept in a sealed tube at 50 °C for 48 h. After this, the solvent was evaporated and the residues were taken up with methanol to allow crystallization of compound **20**, which was fully characterized.

Compound 20: 19.8 mg (91%), m.p. 175–178 °C from methanol. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 1.17 (t, J = 7 Hz, 3 H, CH_3), 2.73 (dd, J = 18.5, 3 Hz, 1 H, H-CH), 3.26 (dd, J = 18.5, 8 Hz, 1 H, HC-H), 3.52 (br, 2 H, N-CH_2), 5.77 (m, 1 H, N-CH), 6.63 (ddd, J = 12.5, 5.5, 2 Hz, 2 H, CH=CH), 7.71 (br, 1 H, NH), 8.04 (s, 1 H, CH=N), 8.19 (s, 1 H, CH=N), 10.90 (s, 1 H, OH) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 16.2, 34.0, 35.8, 40.8, 57.7, 133.9, 140.0, 142.5, 153.6, 155.7, 163.6 ppm. IR: $\tilde{\nu}$ = 3306 (NH), 3000 (OH), 1622 (C=C), 1585 (C=N) cm^{-1}. $C_{12}H_{14}N_6O$ (258.28): calcd. C 55.80, H 5.46, N 32.54; found C 55.6, H 5.4, N 32.5.

Syntheses of the Ethylamino Derivative 21: A solution of the chloronucleosides **16a** (30 mg, 0.14 mmol) in MeOH (2 mL) was saturated with gaseous ethylamine and kept in a sealed tube at 50 °C for 48 h. After this, the solvent was evaporated, and the residues were taken up with methanol to allow crystallization of compound **21**, which was fully characterized.

Compound 21: 28.1 mg (90%), m.p. 105–107 °C from methanol. ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 1.30 (t, J = 7 Hz, 3 H, CH_3), 2.68 (dd, J = 16, 3 Hz, 2 H, CH_2), 3.06 (dd, J = 16, 8 Hz, 2 H, CH_2), 3.72 (br, 2 H, N-CH_2), 5.36 (m, 1 H, N-CH), 5.75 (br, 1 H, NH), 5.94 (s, 2 H, CH=CH), 7.78 (s, 1 H, CH=N), 8.42 (s, 1 H, CH=N) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 15.0, 30.8, 35.7, 40.4, 52.4, 77.1, 119.5, 129.0, 137.4, 152.9, 154.7 ppm. IR: $\tilde{\nu}$ = 3276 (NH), 1618 (C=N) cm^{-1}. $C_{12}H_{15}N_5$ (229.28): calcd. C 62.86, H 6.59, N 30.54; found C 62.8, H 6.4, N 30.3.

Photochemical Derivatization of Compound 21: 3,5-Diphenyl-1,2,4-oxadiazole 4-oxide (0.8 g, 1.5 equiv.) was added portionwise with stirring over a period of a couple of hours to a methanol solution of **21** (0.5 g, 2.2 mmol), during irradiation with 2 × 15 W lamps centred at 310 nm. The reaction mixture was stirred for a further 3 h for completion. Upon evaporation of the solvent, the residue was subjected to chromatographic separation to purify the ene adducts *syn*-**22** and *anti*-**22**, obtained in 36% yield as an inseparable mixture of diastereoisomers. The mixture was fully characterized spectroscopically; in the NMR spectra we report the chemical shift of the major diastereoisomer, with the signals of the minor one in square brackets.

Compound *synlanti*-22: Orange oil, 0.29 g (36%). ^{1}H NMR (300 MHz, DMSO, 25 °C): δ = 1.16 (t, J = 7 Hz, 3 H, CH_3), 2.00 [2.32] (m, 1 H, H-CH), 3.00 [2.51] (m, 1 H, HC-H), 3.51 (br, 2 H, N-CH_2), 5.58–5.90 (m, 1 H, 1 H, N-CH), 6.18 (m, 2 H, CH=CH), 7.43 (m, 3 H, arom.), 7.63 (m, 2 H, arom.), 7.74 (br, 1 H, 1 H, NH), 7.95 [8.18] (s, 1 H, CH=N), 8.09 [8.22] (s, 1 H, CH=N), 9.77 [9.82] (s, 1 H, 1 H, OH) ppm. ^{13}C NMR (75 MHz, DMSO, 25 °C): δ = 14.9, 22.8, 30.7, 34.3, 34.5, 57.1, 59.1, 62.2, 63.4, 127.8, 128.2, 130.1, 132.8, 133.8, 134.9, 135.0, 135.1, 138.2, 138.8, 152.3, 152.4, 154.4, 168.8, 168.9 ppm. IR: $\tilde{\nu}$ = 3304 (OH), 1667 (C=O), 1621 (C=N), 1611 (C=N) cm^{-1}. $C_{19}H_{20}N_6O_2$ (364.40): calcd. C 62.62, H 5.53, N 23.06; found C 62.5, H 5.5, N 23.1.

X-ray Crystallographic Analysis of 16a: Unit cell dimensions for compound **16a** were obtained by least-squares fitting of 2θ values for 25 reflections, with an Enraf–Nonius CAD4 diffractometer and graphite-monochromated Mo–K_α radiation at the Centro Grandi Strumenti (CGS) of the University of Pavia, Italy. A summary of crystal data, data collection and structure refinement is presented in Table 4.

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Table 4. Crystal data, data collection and structure refinement.

Empirical formula	$C_{10}H_9N_4Cl$
Formula weight	220.661
Crystal size [mm]	$0.49 \times 0.42 \times 0.07$
Temperature [K]	293
Crystal system	monoclinic
Space group	*P* 21/m
a [Å]	12.104(4)
b [Å]	6.684(2)
c [Å]	12.880(3)
α	90.0
β	99.72(1)
γ	90.0
V [Å^3]	1027.1(5)
Z	4
$D_{calcd.}$ [g cm^{-3}]	1.427
Absorption coeff., *μ* [mm^{-1}]	0.339
Diffractometer/scan	Enraf–Nonius CAD-4, *ω*/2 *θ*
Radiation	MoK_α
λ [Å]	0.71073
F(000)	456
Range [°] for data coll.	$1.60 < \theta > 25.0$
Index ranges	$-14 < h > 14$, $0 < k > 7$, $0 < l > 15$
Reflections measured	1980
Unique reflections	1980
Corrections applied	Lorentz polarization
Refinement method	full-matrix, least-squares on F^2
Variables	211
Goodness-of-fit (1980)	1.057
R_1 (I) > 2 σ (I), (1355)	0.0466
R_1 (1980)	0.0754
(Δ*ρ*)max., min., e Å^{-3}	0.35, –0.32

The structure was solved by direct methods, and the E-map correctly revealed the non-hydrogen atoms in the molecules. The positions of the hydrogen atoms were located by difference Fourier synthesis, compared with those calculated from the geometry of the molecules and refined isotropically in the subsequent least-squares refinement. The programs SHELXL[34] was used to solve the structure. The ORTEP[35] program was used for molecular graphics.

Antiviral Assays: The National Institute of Allergy and Infectious Diseases (NIAID) established the AACF under a contract with Southern Research Institute. The NIAID, through the AACF, provides free and confidential services for suppliers interested in submitting compounds for evaluation for antiviral activity. Tested compounds were delivered in standard DMSO solutions. The methods applied for the different assays can be found at the URL via the internet at http://niaid-aacf.org.

The following control assays were applied: Herpes Simplex virus 1 (Crystal violet), Herpes Simplex virus 2 (Crystal violet), Varicella-Zoster virus (Crystal violet), Vaccinia virus (Crystal violet), Punta Toro virus (Visual), hepatitis B virus (DNA hybridization) and hepatitis C virus (Luciferase reporter/CytoTox-1). The antiviral activities of the above compounds were tested in vitro in cell line HFF (strain E-377 for HSV-1, strain G for HSV-2, strain Ellen for VZV and strain Copenhagen for VV), the Vero 76 cell line (strain Adames) for PTV, cell line 2.2.15 (strain ayw) for HBV and cell line Huh-Luc/Neo ET (strain CON-1) for HCV. The following control assays were applied: influenza A H1N1 cell line MDCK (strain California 7/2009; cellTiter-Glo) and neuramidase (NA) cell line HFF (strain NA; Neutral Red).

Supporting Information (see footnote on the first page of this article): X-ray crystallographic data for **16a** and copies of the ^{1}H NMR and ^{13}C NMR spectra of new compounds.

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