Cyclopentene Carbocyclic Nucleosides Related to the Antitumor Nucleoside Clitocine and Their Conversion to 8-Aza-neplanocin Analogues. Synthesis and Antiviral Activity

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Dedicated to the memory of Dr. Roland K. Robins

Synthesis of the cyclopentene carbocyclic analogue of the naturally occurring nucleoside clitocine (**1**) is reported. Starting with racemic cyclopentenylamine (**10**), the heterocyclic moieties of the clitocine analogue **4** and related 1,6-dihydro-6-oxo, **5**, and 2-amino-1,6-dihydro-6-oxo, **6**, analogues were constructed. These compounds were respectively converted to 8-aza-neplanocin A (**7**), 8-aza-neplanocin D (**8**, the inosine analogue), and the corresponding 8-aza-guanosine analogue **9** after reduction of the nitro group followed by nitrous acid cyclization. Extensive antiviral evaluation revealed that only 8-aza-neplanocin A (**7**) had enough antiviral activity to warrant further studies. This compound showed weak antiviral activity against HSV-1, HSV-2, and the thymidine kinase deficient (TK-) HSV-1. However, it displayed good antiviral activity against human cytomegalovirus (HCMV) at a concentration of 0.40-2.50 μg/ml, well below the cytotoxicity threshold. This activity profile is consistent with a mechanism of action involving the inhibition of the enzyme adenosylhomocysteine hydrolase.

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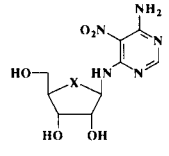
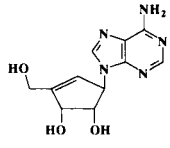
Clitocine (**1**) is a naturally occurring exocyclic amino nucleoside first isolated from the mushroom *Clitocybe inversa* [1]. It was later synthesized independently in two laboratories [2,3] and shown by the Robins group to be a potent inhibitor of L1210 cell growth in culture (ID_{50} = 301 n*M*), as well as a substrate and inhibitor of the enzyme adenosine kinase (K_i = 3 μ*M*) [3]. Synthesis of the corresponding carbocyclic analogue of clitocine **2** has also been achieved but the biological properties of this molecule are unknown [4].

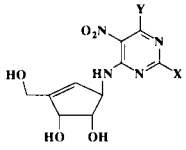
Figure 1



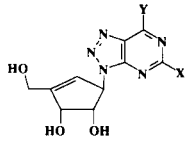
1, X = O (Clitocine)
2, X = CH_2 (C-Clitocine)



3, Neplanocin A



4, X = H, Y = NH_2
5, X = H, Y = OH
6, X = NH_2, Y = OH

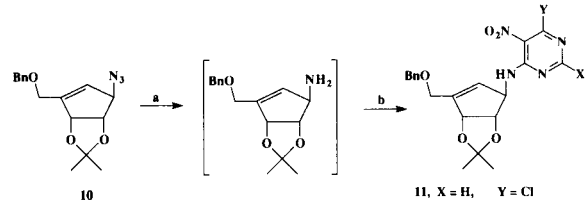


7, X = H, Y = NH_2
8, X = H, Y = OH
9, X = NH_2, Y = OH

In continuation of our work in the area of neplanocin-like nucleosides, we wished to investigate the synthesis and biological properties of compounds containing the clitocine aglycon and related heterocyclic bases. Among the neplanocins, neplanocin A (**3**) is the prototypical cyclopentene carbocyclic nucleoside and this compound possesses excellent antitumor and antiviral properties [5-7]. Based on these considerations, the cyclopentene analogue of clitocine (**4**) was conceived as a potential antitumor and antiviral target. Variants of this molecule, which included the corresponding 1,6-dihydro-6-oxo, **5**, and 2-amino-1,6-dihydro-6-oxo, **6**, were also synthesized.

The X-ray crystallographic structure and nmr spectrum of clitocine revealed the existence of two strong intramolecular hydrogen bonds connecting the nitro group and the hydrogen atoms on the two adjacent amino functions

Scheme 1



10

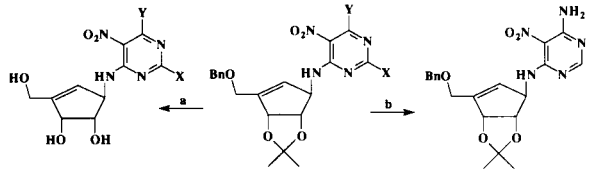
11, X = H, Y = Cl
12, X = NH_2, Y = OH

Reagents: (a) 1. Ph_3P/ THF 2. H_2O/Δ (b) 4,6-dichloro-5-nitropyrimidine/Et_3N/THF/rt or 6-chloro-3,4-dihydro-5-nitro-4-oxopyrimidine/Et_3N/THF/Δ

[3]. These hydrogen bonds contribute to force the aglycone to adopt a fairly planar configuration which approximates that of a bicyclic purine ring system. Therefore, the corresponding bicyclic aglycone compounds **7-8**, having a formal five-membered ring built through a nitrogen bridge, were also considered as attractive targets and these 8-aza-neplanocin analogues **7-8** also were synthesized and evaluated for their biological activity.

For the preparation of the carbocyclic clitocine series **4-6**, the requisite cyclopentenyl amine was generated from the corresponding azide **10** either by catalytic hydrogenation over Lindlar's catalyst [8], or more conveniently, by refluxing the azide in aqueous THF in the presence of triphenyl phosphine (Scheme 1). The *in situ* generated amine was immediately reacted with either 4,6-dichloro-5-nitropyrimidine, or 6-chloro-3,4-dihydro-5-nitro-4-oxo-2-aminopyrimidine [9,10], to give key intermediates **11** and **12**. The 6-chlorine in compound **11** was then easily displaced with ammonia to give the protected cyclopentenyl clitocine analogue **13** which, after treatment with boron trichloride, gave the cyclopentenyl clitocine analogue **4** (Scheme 2). In contrast to clitocine, the free base of **4** was somewhat unstable and, during several recrystallization attempts from water, the material turned dark and crystals were not obtained. When the neutralization step after the boron trichloride deprotection was omitted, the more stable hydrochloride salt, **4**·HCl, was obtained. Quite the opposite was found for compounds **11** and **12** which formed very stable free bases, **5** and **6**, after deblocking with boron trichloride and neutralization (Scheme 2). It is important to note that during this deblocking reaction the carbon-chlorine bond of compound **11** was simultaneously hydrolyzed to provide, in one step, the desired inosine analogue **5**.

Scheme 2

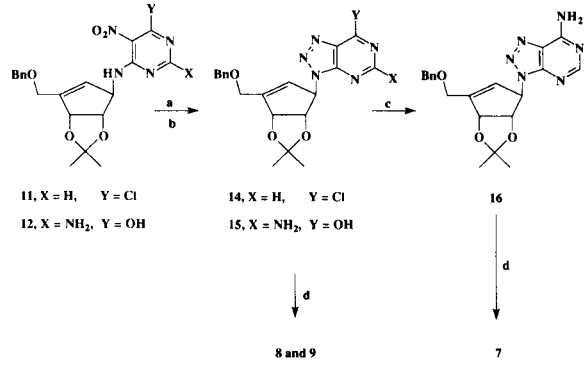


Reagents: (a) 1. BCl_3/CH_2Cl_2 (-78 °C) 2. MeOH, rt (b) NH_3/MeOH, rt

As in the case of clitocine, the nmr spectra of these carbocyclic analogues indicated the existence of two strong intramolecular hydrogen bonds to the nitro group. These hydrogen bonds were more evident in the protected nucleosides **11-13**, in agreement with the data reported for protected clitocine [3] where a similar set of signals was observed. The deuteriochloroform nmr spectrum of compound **13** shows a doublet at δ 8.87 for the 4-NH proton, and two singlets, at δ 8.45 and δ 6.25 that correspond to the two individual 6-NH_2 signals that experience different environments due to hydrogen bonding to the nitro group. The same pattern was also observed for the protected guanosine analogue, **12**, with similar signals in deuteriochloroform at δ 9.14 (doublet), δ 8.26 (singlet) and δ 6.80 (singlet). For the protected chloro compound **11**, however, the 4-NH doublet in deuteriochloroform was hidden under the phenyl aromatic multiplet at δ 7.30 and a solvent change to DMSO-d_6 was required to uncover the 4-NH signal (δ 8.45, doublet). It is interesting to observe, that with the exception of the latter compound, the 4-NH signals resisted exchange on the addition of deuterium oxide. A similar tendency was observed even in the case of the deprotected compounds **4-6**, except that in the cases complete exchanged was eventually achieved. The positive ion FAB mass spectra of these compounds revealed a strong peak for the cationized molecular ion and an unusual peak indicative of loss of oxygen. Only in the case of compound **4** was the peak corresponding to the cationized molecular ion rather weak (m/z 284, 6% relative abundance). Instead, a strong MH^+-18 peak (m/z 266, 100% relative abundance) became the dominant signal of the spectrum. That the loss of water appears to occur from the aglycon moiety is surmised by the presence of a peak at m/z 111 (b-H_2O).

Ring closure to the 8-azaneplanocins required first the reduction of the nitro groups of compounds **11** and **12** (Scheme 3). Stannous chloride-sodium borohydride reduction [11] worked very well for compound **11**, but failed in the case of **12**. In this latter instance, however, reduction

Scheme 3



Reagents: (a) 1. $SnCl_2.2H_2O$/EtOH 2. $NaBH_4$ 3. $NaNO_2$/AcOH/H_2O (X = H, Y = Cl)
(b) 1. Zn/AcOH/Δ 2. $NaNO_2$/AcOH/H_2O (X = NH_2, Y = OH)
(c) NH_3/MeOH, rt, 3 days
(d) BCl_3/CH_2Cl_2 (-78 °C) 2. MeOH, rt

Table 1

Antiviral Activity of 8-Azaneplanocin A

Compound	Minimum Inhibitory Concentration [a] (μg/ml)							
	Minimum Cytotoxic Conc/ [b] (μg/ml)	Herpes simplex Virus–1 (KOS) [c]	Herpes simplex Virus-2 (G) [c]	Vaccinia Virus [c]	Vesicular Stomat. Virus (VSV) [c]	Herpes simplex Virus-1 TK-Cheng C158/77 [c]	Resp. Syncytial Virus str. Long [d]	Influenza Virus B str. Ishikawa [e]
8-Aza-neplanocin A(**7**)	>400 [c] >100 [d] >20 [e]	20	7	300	70	7	20	20
BVDU	>400 [c]	0.01	20	0.7	>400	0.1	–	–
Ribavirin	>400 [c] >200 [d,e]	300	>400	70	40	100	4	4
3-Deaza-C-Ado [f]	>400	>400	>150	2	0.2	20	–	–
Neplanocin A	40 [c]	20	20	2	0.2	2	–	–

[a] Required to reduce virus-induced cytopathogenicity by 50%. [b] Required to cause a microscopically detectable ateration of normal cell morphology. [c] E_6SM cells. [d] HeLa cells. [e] MDCK cells. [f] 3-Deazaaristeromycin (3-deaza-carbocyclic adenosine).

Table 2

Activity of 8-Azaneplanocin A Against CMV in Human Embryonic Lung (HEL) Cells

Compound	Antiviral Activity [IC_{50} (μg/ml) [a]				Cytotoxicity [IC_{50} (μg/ml) [b]
	AD-169 Strain		Davis Strain		
	Assay 1	Assay 2	Assay 1	Assay 2	
8-Aza-neplanocin A (**7**)	2.50	7.33	0.40	1.00	>50
Neplanocin A	0.40	0.66	0.25	0.04	21.48
DHPG	0.08	0.40	0.40	0.33	153.83

[a] Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units. [b] Inhibitory concentration required to reduce cell growth by 50%.

with zinc powder gave the desired reduced product. The reduced compounds were not purified further and were immediately cyclized to the 8-aza analogues in the presence of nitrous acid to give intermediates **14** and **15**. After converting the 6-chloro analogue **14** to the corresponding 8-aza-adenosine **16**, all protecting groups in compounds **14-16** were removed with boron trichloride, as for the clitocine analogues, to give the desired targets **7-9**. In the same manner as for compound **11**, the 6-chloro analogue **14** was converted directly to the inosine derivative **8** under the deblocking reaction conditions. Both proton and carbon nmr spectra for compounds **7-9** were in excellent agreement with the proposed structures. The positive ion FAB mass spectra of the 8-aza-neplanocins were typical showing characteristic strong molecular ion peaks and the corresponding b + 2H peaks of the cleaved aglycons.

Biological Activity.

None of the compounds synthesized displayed significant cytotoxicity in the NCI *in vitro* cancer screen [12]. Extensive antiviral evaluation revealed 8-aza-neplanocin A (**7**) as the most interesting compound from either series. The antiviral activity of this compound against some representative viruses is shown in Table 1. Compound **7** displayed some activity against herpes simplex viruses HSV-1 and HSV-2, and also against the thymidine kinase deficient strain (TK- HSV-1). More importantly, however, 8-aza-neplanocin A was active against two variants of human cytomegalovirus (HCMV) at concentrations well below the cytotoxicity threshold (Table 2). This type of activity is consistent with a mechanism involving inhibition of *S*-adenosylhomocysteine hydrolase [13]. The effects of 8-aza-neplanocin on this enzyme are under investigation.

EXPERIMENTAL

Melting points are uncorrected and were determined in a Mel-Temp apparatus, Laboratory Devices, USA. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN. Silica gel column chromatography was performed on silica gel 60 (E. Merck, 230-400 mesh) and analytical thin layer chromatography (tlc) was performed on Analtech Uniplates silica gel GF with the solvents indicated. Detection of compounds by tlc was done either by uv light or by methanol spray (10% sulfuric acid) followed by heating on a hot plate. Proton and ^{13}C nmr spectra were recorded in the solvents indicated at 250 MHz and 62.9 MHz, respectively, on a Bruker AC-250 instrument. Chemical shifts are expressed as δ values with reference to TMS. Positive-ion fast atom bombardment (FAB) mass spectra were obtained by using samples dissolved in a glycerol matrix, and ionization was effected by a beam of xenon atoms derived by neutralizing xenon ions accelerated through 8.6 kV.

(±)-4-[[rel-(1*R*,4*R*,5*S*)-3-[(Benzyloxy)methyl]-4,5-(isopropylidenedioxy)-2-cyclopenten-1-yl]amino]-5-nitro-6-chloropyrimidine (**11**).

A solution of cyclopentenylazide [8] (**10**, 1.0 g, 3.3 mmoles) in anhydrous THF (20 ml) was treated with triphenylphosphine (0.89 g, 3.3 mmoles) and the resulting solution was stirred at room temperature overnight under a blanket of nitrogen. Water (0.12 ml, 6.6 mmoles) was added and the solution was heated to reflux for 6 hours. After cooling to room temperature, the cyclopentenylamine formed was treated immediately with 4,6-dichloro-5-nitropyrimidine (0.64 g, 3.3 mmoles) and triethylamine (0.46 ml, 3.3 mmoles). The resulting mixture was then stirred at room temperature for 5 hours and filtered. The filtrate was evaporated to dryness and the residual thick oil was purified by silica gel column chromatography using petroleum ether/ethyl acetate (19:1), followed by petroleum ether/ethyl acetate (9:1) as eluant mixtures. The yellow solid obtained was recrystallized from hexanes to give **11** (0.95 g, 66%), mp 106-107°; ^1H nmr (deuteriochloroform): δ 1.35 and 1.43 (2s, 6 H, 2 CH_3), 4.20 (s, 2 H, H-6′$_{a,b}$), 4.52 (d, J = 6.1 Hz, 1 H, H-5′), 4.61 (s, 2 H, CH_2Ph), 5.20 (m, 2 H, H-4′, H-1′), 5.75 (br s, 1 H, H-2′), 7.36 (m, 6 H, NH, Ph), 8.51 (s, 1 H, H-2).

Anal. Calcd. for $C_{20}H_{21}ClN_4O_5$: C, 55.49; H, 4.89; N, 12.94. Found: C, 55.41; H, 4.84; N, 12.98.

(±)-2-Amino-4-[[rel-(1*R*,4*R*,5*S*)-3-[(benzyloxy)methyl]-4,5-(isopropylidenedioxy)-2-cyclopenten-1-yl]amino]-1,6-dihydro-5-nitro-6-oxopyrimidine (**12**).

In the same manner as above, 0.79 g (2.6 mmoles) of cyclopentenylazide was reduced to cyclopentenylamine. The solution of the amine in THF (20 ml) was diluted further with absolute ethanol (20 ml) and treated with 6-chloro-3,4-dihydro-5-nitro-4-oxo-2-aminopyrimidine [9,10] and heated to reflux for 6.5 hours. The precipitate that was formed upon cooling was filtered off and washed with a small amount of THF/ethanol (1:1) to give **12** (0.75 g, 67%) as a white solid which was recrystallized from acetonitrile, mp >240° dec; ^1H nmr (deuteriochloroform): δ 1.40 and 1.45 (2s, 6 H, 2 CH_3), 4.15 (s, 2 H, H-6′$_{a,b}$), 4.55 (s, 2 H, CH_2Ph), 4.85 (br s, 1 H, H-5′), 5.05 (br s, 1 H, H-1′), 5.25 (d, J = 5.5 Hz, 1 H, H-4′), 5.80 (s, 1 H, H-2′), 6.80 (br s, 1 H, N*H*H, deuterium oxide exchanged), 7.40 (m, 5 H, Ph), 8.30 (br s, 1 H, NH*H*, deuterium oxide exchanged), 9.50 (d, J = 6.5 Hz, 1 H, N*H*, not exchangeable), 10.60 (br s, 1 H, CON*H*, deuterium oxide exchanged).

Anal. Calcd. for $C_{20}H_{23}N_5O_6 \cdot 0.5H_2O$: C, 54.08; H, 5.52; N, 15.98. Found: C, 53.96; H, 5.49; N, 15.93.

(±)-4-[[rel-(1*R*,4*R*,5*S*)-3-[(Benzyloxy)methyl]-4,5-(isopropylidenedioxy)-2-cyclopenten-1-yl]amino]-5-nitro-6-aminopyrimidine (**13**).

Chloropyrimidine **11** (0.2 g, 0.45 mmole) was suspended and stirred with 7 ml of saturated methanolic ammonia contained in a threaded resealable glass tube at room temperature. Gradually, the compound went into solution and five minutes later, a white precipitate formed. Stirring continued for 2 hours. The reaction mixture was then reduced to dryness and the white solid that remained was suspended in water and collected by filtration. The solid was recrystallized from ethanol to give 0.18 g (94%) of **13**, mp >240° dec; ^1H nmr (deuteriochloroform): δ 1.31 and 1.39 (2s, 6 H, 2 CH_3), 4.16 (s, 2 H, H-6′$_{a,b}$), 4.51 (d, J = 5.6 Hz, 1 H, H-5′), 4.56 (s, 2 H, CH_2Ph), 5.17 (m, 2 H, H-4′, H-1′), 5.74 (s, 1 H, H-2′), 6.25 (br s, 1 H, N*H*H, deuterium oxide exchanged), 7.28 (m, 5 H, Ph), 8.10 (s, 1 H, H-2), 8.46 (br s, NH*H*, deuterium oxide exchanged), 8.85 (br d, J = 7.1 Hz, 1 H, NH, not exchangeable).

Anal. Calcd. for $C_{20}H_{23}N_5O_5$: C, 58.10; H, 5.61; N, 16.94. Found: C, 58.17; H, 5.61; N, 16.88.

(±)-4-[[rel-(1*R*,4*R*,5*S*)-3-[Hydroxymethyl]-4,5-dihydroxy-2-cyclopenten-1-yl]amino]-5-nitro-6-aminopyrimidine (**4**).

Free Base.

The protected precursor **13** (0.29 g, 0.7 mmole) was dissolved in anhydrous dichloromethane (45 ml) and cooled with a dry-ice/acetone bath while being kept under nitrogen. Boron trichloride (1*M* solution in dichloromethane, 5.6 ml, 5.6 mmoles) was added and the reaction mixture was stirred for 2 hours. The cooling bath was removed and stirring was continued for 30 minutes more. Immediately, 30 ml of methanol was added and the solution was evaporated to dryness. This addition-evaporation cycle was repeated three times. The resulting material was dissolved in methanol (50 ml) and a solution of saturated methanolic ammonia was added dropwise until a precipitate was formed. The precipitate was collected and washed with water to give crude **4** (0.16 g, 84%) as a yellow solid. This solid proved to be quite unstable and quickly turned dark during heating in a mixture of methanol-water. Although we failed repeatedly to obtain a satisfactory elemental analysis with this compound (*vide infra*) the nmr spectrum was very well resolved and provided useful structural information; ^1H nmr (DMSO-d_6): δ 4.09 (AB d, 2 H, H-6′$_{a,b}$), 4.44 (d, J = 7.4 Hz, 1 H, H-5′), 4.53 (br d, J = 4.9 Hz, becomes a singlet after deuterium oxide exchanged, H-1′), 5.07 (m, 2 H, OH, deuterium oxide exchanged), 5.22 (d, J = 7.6 Hz, 1 H, H-4′), 5.33 (d, J = 5.7 Hz, 1 H, OH, deuterium oxide exchanged), 5.78 (s, 1 H, H-2′), 8.07 (s, 1 H, H-2), 8.91 (br s, 1 H, NH, deuterium oxide exchanged), 9.16 (br s, 2 H, NH, deuterium oxide exchanged).

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.40; H, 4.63; N, 24.73. Found: C, 45.18; H, 4.39; N, 25.57.

Hydrochloride Salt.

Starting with 0.47 g (1.1 mmoles) of **13**, the same procedure as described above was follwed except that treatment with methanolic ammonia was omitted. The white solid obtained after the repeated addition-evaporation cycle with methanol was suspended in ether and filtered to give 0.3 g of the hydrochloride salt of **4**. This compound could not be recrystallized and was partially

purified by inducing its precipitation from a methanolic solution with ether; ^{1}H nmr (DMSO-d_6): δ 3.35 (br s, 2 H, OH, deuterium oxide exchanged), 4.17 (s, 2 H, H-6′$_{a,b}$), 4.57 (d, J = 7.5 Hz, 1 H, H-5′), 4.75 (s, 1 H, H-1′), 5.30 (br s, 1 H, OH, deuterium oxide exchanged), 5.62 (br s, 1 H, OH, deuterium oxide exchanged), 5.91 (d, J = 7.5 Hz, H-4′), 5.96 (s, 1 H, H-2′), 8.61 (s, 1 H, H-2), 9.59 (s, 1 H, NH, deuterium oxide exchanged), 10.09 (s, 1 H, NH, deuterium oxide exchanged), 11.00 (s, 1 H, NH, deuterium oxide exchanged); ^{13}C nmr (DMSO-d_6): δ 57.08, 68.10, 70.28, 78.31, 110.20, 132.83, 141.85, 149.31, 150.39, 157.26; ms: FAB m/z (relative intensity) 284 (MH+, 6), 266 (MH-H_2O, 100), 250 (MH-O, 26).

(±)-4-[[rel-(1*R*,4*R*,5*S*)-3-[Hydroxymethyl]-4,5-dihydroxy-2-cyclopenten-1-yl]amino]-1,6-dihydro-5-nitro-6-oxopyrimidine (**5**).

Starting with compound **11** (0.3 g, 0.69 mmole) the deblocking procedure with boron trichloride (4.8 mmoles) was repeated exactly as described for compound **13**. The residual oil obtained after the repeated addition-evaporation cycle with methanol was purified by silica gel column chromatography using chloroform/-methanol (6:1) as eluant to give a light yellow oil. This material upon trituration with water generated **5** (0.15 g, 76%) as a white solid, which was recrystallized from water, mp 188-189°; ^{1}H nmr (DMSO-d_6): δ 4.08 (d, J = 5.3 Hz, 1 H, H-6′$_{a,b}$, becomes a singlet after deuterium oxide), 4.28 (d, J = 8.8 Hz, 1 H, H-5′), 4.62 (m, 1 H, H-1′), 5.00 (d, J = 8.7 Hz, 1 H, H-4′), 5.06 (t, J = 5.5 Hz, 1 H, OH, deuterium oxide exchanged), 5.20 (d, J = 6.2 Hz, 1 H, OH, deuterium oxide exchanged), 5.72 (s, 1 H, H-2′), 9.12 (d, J = 9.7 Hz, 1 H, H-2, becomes a singlet after deuterium oxide exchange), 9.52 (br s, 1 H, OH, deuterium oxide exchanged), 9.62 (br s, 1 H, NH, deuterium oxide exchanged), 11.30 (d, J = 9.7 Hz, 1 H, pyrimidine NH); ^{13}C nmr (DMSO-d_6): 58.05, 64.04, 67.73, 80.30, 106.87, 128.47, 145.63, 156.85, 162.72, 163.28; ms: FAB m/z (relative intensity) 285 (MH+, 100), 269 (MH-O, 50).

Anal. Calcd. for $C_{10}H_{12}N_4O_6$: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.38; H, 4.31; N, 19.76.

(±)-2-Amino-4-[[rel-(1*R*,4*R*,5*S*)-3-[hydroxymethyl]-4,5-dihydroxy-2-cyclopenten-1-yl]amino]-1,6-dihydro-5-nitro-6-oxopyrimidine (**6**).

Compound **12** (0.30 g, 0.7 mmole) was dissolved in anhydrous dichloromethane (40 ml) and cooled with a dry-ice/acetone bath under a nitrogen atmosphere. Boron trichloride (1 *M* in dichloromethane, 7 ml, 7.0 mmoles) was added in one portion and the resulting mixture was stirred for 3 hours. The cooling bath was removed and stirring was continued for 10 minutes after which time methanol (30 ml) was added and the solution immediately reduced to dryness. This addition-evaporation cycle with methanol was repeated three times. The solid that remained was heated to boil in water and filtered to give a light yellow solution. Upon cooling, compound **6** (0.11 g, 53%) crystallized as a light yellow solid, mp >250° dec; ^{1}H nmr (DMSO-d_6): δ 3.30 (br s, 2 H, OH, deuterium oxide exchanged), 3.85 (t, J = 5.5 Hz, 1 H, H-5′), 4.00 (br s, 1 H, H-6′$_{a,b}$), 4.30 (d, J = 5.5 Hz, 1 H, H-4′), 4.80 (br s, 1 H, OH, deuterium oxide exchanged), 5.00 (br s, 1 H, H-1′), 5.60 (d, J = 1.6 Hz, 1 H, H-2′), 6.60 (br s, 1 H, N*H*H, deuterium oxide exchanged), 7.90 (br s, 1 H, NH*H*), 9.50 (d, J = 7.6 Hz, 1 H, NH, not exchangeable), 10.70 (s, 1 H, amide NH, deuterium oxide exchanged); ^{13}C nmr (DMSO-d_6): δ 58.40, 61.20, 72.00, 77.06, 110.56, 125.18, 148.72, 153.90, 156.03, 159.28; ms: FAB m/z (relative intensity) 300 (MH+, 100), 284 (MH-O, 70).

Anal. Calcd. for $C_{10}H_{13}N_5O_6$: C, 40.14; H, 4.38; N, 23.41. Found: C, 40.28; H, 4.40; N, 23.31.

(±)-3-[[rel-(1*R*,4*R*,5*S*)-3-[(Benzyloxy)methyl]-4,5-(isopropylidenedioxy)-2-cyclopenten-1-yl]-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**14**).

A mixture of compound **11** (0.2 g, 0.46 mmole) and stannous chloride dihydrate (0.52 g, 2.3 mmoles) in absolute ethanol (20 ml) was warmed to 60° in a water bath. To this suspension, sodium borohydride (8.7 mg, 0.23 mmole) in 10 ml of ethanol was added dropwise during the course of 10 minutes. After 40 minutes of stirring, the reaction mixture was removed from the water bath and cooled over an ice-water bath. The reaction mixture was then poured in cold water (80 ml) and a white precipitate formed. The *p*H was then adjusted to 7 with cold aqueous sodium bicarbonate and the mixture was extracted with ethyl acetate (4 x 75 ml). The combined organic layer was washed with brine (100 ml) and dried (magnesium sulfate). The filtrate was evaporated to give a dark yellow syrup (0.160 g, 85%) which was used immediately in the following reaction. The oil was dissolved in a mixture of acetic acid (28 ml) and water (15 ml) and cooled over an ice-water bath. To this solution, sodium nitrite (42 mg, 0.6 mmole) dissolved in 5 ml of water was added during 1 minute. The resulting mixture was stirred for 2 hours, removed from the cold bath and evaporated to dryness. The residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (9:1) as eluant to give **14** as an oil which solidified into a white solid upon trituration with hexanes (0.075 g, 46%). Recrystallization from hexanes afforded a pure sample of **14**, mp 91-92°; ^{1}H nmr (deuteriochloroform): δ 1.38 and 1.50 (2 s, 6 H, 2 C*H*$_3$), 4.30 (s, 2 H, H-6′$_{a,b}$), 4.59 (s, 2 H, C*H*$_2$Ph), 4.98 (d, J = 5.7 Hz, 1 H, H-5′), 5.52 (d, J = 5.7 Hz, 1 H, H-4′), 5.88 (s, 1 H, H-1′), 6.10 (s, 1 H, H-2′), 7.30 (m, 5 H, Ph), 8.90 (s, 1 H, H-5).

Anal. Calcd. for $C_{20}H_{20}ClN_5O_3$: C, 58.04; H, 4.87; N, 16.92. Found: C, 58.15; H, 4.87; N, 16.92.

(±)-3-[[rel-(1*R*,4*R*,5*S*)-3-[(Benzyloxy)methyl]-4,5-(isopropylidenedioxy)-2-cyclopenten-1-yl]-5-amino-3,6-dihydro-7*H*-1,2,3-triazolo-[4,5-*d*]pyrimidin-7-one (**15**).

A suspension of nitro compound **12** (0.43 g, 1 mmole) and zinc powder (0.066 g, 10 mg-atoms) in 50% aqueous ethanol (10 ml) was treated with acetic acid (1 ml) and heated to reflux under nitrogen for 1 hour. The suspension was filtered and the zinc metal was washed with ethanol. The combined filtrate was evaporated to a light yellow foam and used immediately in the next step. The yellow foam was dissolved in a mixture of acetic acid (20 ml) and water (5 ml) and cooled over an ice-water bath. Sodium nitrite (0.090 g, 1.3 mmoles) dissolved in water (5 ml) was added over a period of 10 minutes with stirring and the resulting solution was stirred further for 2 hours. Some time after the removal of the cooling bath, a light tan solid precipitated out. The solid was removed by filtration and washed with ethanol. Recrystallization from ethyl acetate afforded **15** (0.35 g, 85%), mp 199-202°; ^{1}H nmr (DMSO-d_6): δ 1.27 and 1.36 (2 s, 6 H, 2 C*H*$_3$), 3.31 (br s, 2 H, N*H*$_2$), 4.18 (AB m, 2 H, H-6′$_{a,b}$), 4.55 (s, 2 H, C*H*$_2$Ph), 4.74 (d, J = 5.7 Hz, 1 H, H-5′), 5.35 (d, J = 5.7 Hz, 1 H, H-4′), 5.50 (s, 1 H, H-1′), 5.82 (s, 1 H, H-2′), 6.97 (br s, 1 H, NH), 7.30 (m, 5 H, Ph).

Anal. Calcd. for $C_{20}H_{22}N_6O_4$: C, 58.52; H, 5.40; N, 20.48. Found: C, 58.41; H, 5.43; N, 20.44.

(±)-3-[rel-(1*R*,4*R*,5*S*)-3-[(Benzyloxy)methyl]-4,5-(isopropylidenedioxy)-2-cyclopenten-1-yl]-7-amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**16**).

The chloro compound **14** (0.16 g, 0.39 mmole) was dissolved in saturated methanolic ammonia (8 ml) and sealed in a threaded

glass tube and kept under stirring at room temperature for 3 days. The reaction mixture was then evaporated to dryness and washed with water to give **16** as a white solid (0.14 g, 92%), which was recrystallized from ethanol, mp 169-170°; ^{1}H nmr (deuteriochloroform): δ 1.35 and 1.46 (2 s, 6 H, 2 CH_3), 4.27 (s, 2 H, H-6′$_{a,b}$), 4.58 (s, 2 H, CH_2Ph), 4.97 (br s, 1 H, H-5′), 5.49 (br s, 1 H, H-4′), 5.85 (s, 1 H, H-1′), 5.95 (s, 1 H, H-2′), 6.69 (br s, 2 H, NH_2), 7.30 (m, 5 H, Ph), 8.40 (s, 1 H, H-5).

Anal. Calcd. for $C_{20}H_{22}N_6O_3$: C, 60.90; H, 5.62; N, 21.31. Found: C, 61.03; H, 5.67; N, 21.34.

(±)-3-[rel-(1*R*,4*R*,5*S*)-3-[Hydroxymethyl]-4,5-dihydroxy-2-cyclopenten-1-yl]-7-amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**7**).

Compound **16** (0.20 g, 0.5 mmole) was dissolved in anhydrous dichloromethane (30 ml), cooled over a dry-ice/acetone bath and kept under nitrogen with stirring. To this solution, boron trichloride (1 *M* in dichloromethane, 3.5 ml) was added and the reaction mixture was stirred for 2.5 hours. The reaction mixture was allowed to reach room temperature and stirred at that temperature for 30 minutes more. Methanol (20 ml) was added and the solution was evaporated to dryness. This addition-evaporation cycle with methanol was repeated three times. The resulting thick oil was dissolved in methanol (20 ml), neutralized with saturated methanolic ammonia and reduced to dryness to give a tan solid. The compound was recrystallized from 50% aqueous ethanol to give **7** (0.12 g, 89%) as a white solid, mp >260°; ^{1}H nmr (DMSO-d_6): δ 4.10 (br d, 2 H, H-6′$_{a,b}$, becomes a singlet after deuterium oxide), 4.45 (m, 2 H, H-4′, H-5′), 4.95 (t, J = 5.5 Hz, 1 H, OH, deuterium oxide exchanged), 5.00 (d, J = 5.7 Hz, 1 H, OH, deuterium oxide exchanged), 5.21 (d, J = 6.5 Hz, 1 H, OH, deuterium oxide exchanged), 5.70 (m, 2 H, H-1′, H-2′), 7.80 (br s, 1 H, NH*H*, deuterium oxide exchanged), 8.30 (s, 1 H, H-5), 8.60 (br s, 1 H, N*H*H, deuterium oxide exchanged); ^{13}C nmr: δ 58.50, 67.20, 72.10, 76.55, 123.03, 124.06, 148.98, 150.25, 156.20, 156.58; ms: FAB m/z (relative intensity) 265 (MH+, 100), 137 (b+2H, 53).

Anal. Calcd. for $C_{10}H_{12}N_6O_3$: C, 45.45; H, 4.58; N, 31.81. Found: C, 45.59; H, 4.63; N, 31.70.

(±)-3-[rel-(1*R*,4*R*,5*S*)-3-[Hydroxymethyl]-4,5-dihydroxy-2-cyclopenten-1-yl]-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (**8**).

The protected chlorotriazolopyrimidine **14** (0.37 g, 0.9 mmole) was dissolved in anhydrous dichloromethane (45 ml) and cooled over a dry-ice/acetone bath and kept under nitrogen with stirring. To this solution, boron trichloride (1 *M* in dichloromethane, 6.3 ml) was added and the reaction mixture was stirred for 2 hours. The reaction mixture was allowed to reach room temperature and stirred at that temperature for 30 minutes more. Methanol (30 ml) was added and the solution was evaporated to dryness. This addition-evaporation cycle with methanol was repeated three times. The residual solid was suspended in methanol (30 ml) and the solution was neutralized with saturated methanolic ammonia and reduced to dryness. Addition of water to the residue provided a sticky solid which was forced into solution by the gradual addition of ethanol. Removal of the solvent, once again, provided **8** as a white solid (0.17 g, 72%), mp 201-202°; ^{1}H nmr (DMSO-d_6): δ 4.10 (s, 2 H, H-6′$_{a,b}$), 4.40 (m, 2 H, H-4′, H-5′), 4.95 (t, J = 5.4 Hz, 1 H, OH, deuterium oxide exchanged), 5.00 (d, J = 5.4 Hz, 1 H, OH, deuterium oxide exchanged), 5.21 (d, J = 6.4 Hz, 1 H, OH, deuterium oxide exchanged), 5.68 (m, 2 H, H-1′, H-2′), 8.23 (s, 1 H, H-5), 12.7 (br s, 1 H, NH, deuterium oxide exchanged). Despite extensive drying the compound showed signals at δ 1.00 (t) and 3.40 (q) that corresponded to residual ethanol; ^{13}C nmr: δ 58.50, 67.58, 72.15, 76.83, 122.69, 129.80, 148.81, 149.52, 150.63, 155.40; ms: FAB m/z (relative intensity) 266 (MH+, 97), 138 (b+2H, 100).

Anal. Calcd. for $C_{10}H_{11}N_5O_4 \cdot 0.33$EtOH: C, 45.66; H, 4.63; N, 24.90. Found: C, 45.76; H, 4.66; N, 24.96.

(±)-3-[rel-(1*R*,4*R*,5*S*)-3-[Hydroxymethyl]-4,5-dihydroxy-2-cyclopenten-1-yl]-5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (**9**).

Protected **15** (0.30 g, 0.73 mmole) was dissolved in anhydrous dichloromethane (65 ml) and cooled over a dry-ice/acetone bath and kept under nitrogen with stirring. To this solution, boron trichloride (1 *M* in dichloromethane, 73 ml) was added and the reaction mixture was stirred for 2.5 hours. The reaction mixture was allowed to reach room temperature and was further stirred at that temperature for 30 minutes more. Methanol (30 ml) was added and the solution was evaporated to dryness. This addition-evaporation cycle with methanol was repeated three times. The residual solid was suspended in methanol (30 ml), neutralized with saturated methanolic ammonia and reduced to dryness again. The solid obtained was recrystallized from water to give compound **9** as a light tan solid (0.16 g, 78%), mp >260°; ^{1}H nmr (DMSO-d_6): δ 3.33 (br s, 1 H, OH, deuterium oxide exchanged), 4.10 (s, 2 H, H-6′$_{a,b}$), 4.40 (m, 2 H, H-4′, H-5′), 4.70-5.20 (br m, 2 H, OH, deuterium oxide exchanged), 5.40 (m, 1 H, H-1′), 5.62 (s, 1 H, H-2′), 6.87 (br s, 2 H, NH_2, deuterium oxide exchanged), 10.92 (s, 1 H, NH, deuterium oxide exchanged); ^{13}C nmr: δ 58.40, 66.23, 72.15, 76.12, 123.18, 124.37, 150.06, 151.39, 155.12, 155.65; ms: FAB m/z (relative intensity) 281 (MH+, 100), 153 (b+2H, 74).

Anal. Calcd. for $C_{10}H_{12}N_6O_4$: C, 42.85; H, 4.32; N, 30.00. Found: C, 42.86; H, 4.34; N, 29.94.

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