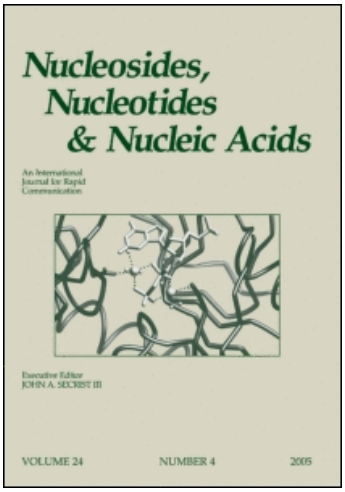


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# ACYCLIC NUCLEOTIDES RELATED TO CLITOCINE: SYNTHESIS AND ANTI-HIV ACTIVITY

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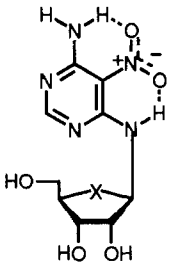
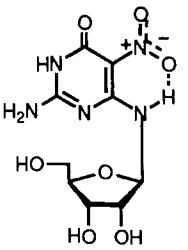
**Abstract.** The syntheses and antiviral activity of analogues of the anti-HIV agents PMEA, PMEDAP, (*R*)-PMPA, (*R*)-PMPDAP are described. In these analogues the adenine moiety is replaced by 4,6-diamino-5-nitro-pyrimidine (the aglycon of clitocine) or 2,4,6-triamino-5-nitro-pyrimidine. The synthesis of similar acyclic phosphonates related to PMEG and (*R*)-2'-methyl-PMEG is also reported. Some compounds proved to be active as anti-HIV agents.



Clitocine, [6-amino-5-nitro-4-(β-D-ribofuranosylamino)pyrimidine] (1) is a natural exocyclic nucleoside isolated from the mushroom *Clitocybe inversa*. It has been shown to possess strong insecticidal activity and potent cytostatic effects against several leukemia cell lines through inhibition of adenosine kinase.[1,2] The carbocyclic clitocine analogue **2** has been found to be readily phosphorylated by adenosine kinase and significantly active both *in vitro* and *in vivo* against influenza A virus.[3]



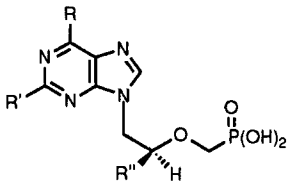
Clitocine X = O (**1**)
X = $CH_2$ (**2**)



(**3**)

From a structural view point, clitocine shows interesting biogenetic relationships with adenosine and possesses a planar aglycone moiety with each oxygen atom of the nitro group bound to the two adjacent amino hydrogens (4-NH and 6-NH), as revealed by X-ray crystal data and NMR spectroscopy.[2] A similar relationship has been found between guanosine and the guanosine-type analogue of clitocine **3**.[4]

The structural analogy of **1** with adenosine prompted us to prepare new analogues of the anti-HIV agents PMEA,[5] (*R*)-PMPA,[6] PMEDAP,[5] (*R*)-PMPDAP[6] in which the adenine moiety was replaced by 4,6-diamino-5-nitro-pyrimidine (compounds **4** and **5**) or 2,4,6-triamino-5-nitro-pyrimidine (compounds **6**



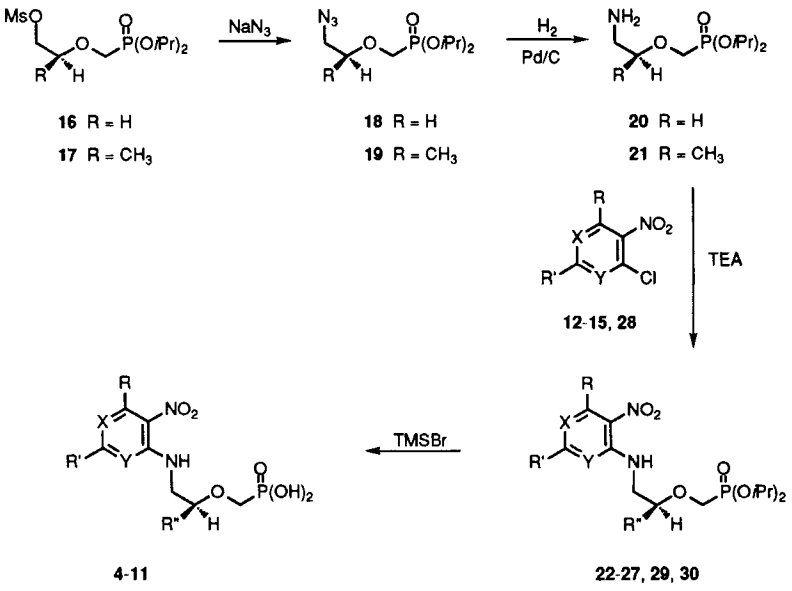
| | |
|---|---|
| PMEA | R = $NH_2$, R', R" = H |
| (*R*)-PMPA | R = $NH_2$, R' = H, R" = $CH_3$ |
| PMEDAP | R, R' = $NH_2$, R" = H |
| (*R*)-PMPDAP | R, R' = $NH_2$, R" = $CH_3$ |
| PMEG | R = OH, R' = $NH_2$, R" =H |
| (*R*)-PMPG | R = OH, R' = $NH_2$, R" = $CH_3$ |

and **7**). 1-Deaza- and 3-deaza-analogues of **4** (compounds **8** and **9**) were also prepared.

Furthermore, we also synthesized the 2-amino-5-nitro-6-[2-(phosphonomethoxy)ethylamino]-4(3H)-pyrimidinone (**10**) and (*R*)-2-amino-5-nitro-6-[2-(phosphonomethoxy)propylamino]-4(3H)-pyrimidinone (**11**), analogues of the antiviral agents PMEG[5] and (*R*)-PMPG,[7] in which the guanine moiety was replaced by 2,6-diamino-5-nitro-4(3H)-pyrimidinone.

Compounds **4-9** were prepared by condensation of a suitable nitro-chloro-derivative (**12-15**) with the diisopropyl ester of 2-phosphonomethoxy-ethylamine (**20**) or (*R*)-2-phosphonomethoxy-propylamine (**21**), which in turn were prepared by reaction of 2-*O*-[diisopropylphosphono)-methyl]-1-*O*-(methylsulfonyl)-1,2-ethanediol (**16**)[8] or (*R*)-2-*O*-[diisopropylphosphono)methyl]-1-*O*-(methylsulfonyl)-1,2-propanediol[7] (**17**) with sodium azide, followed by reduction of the azido-derivatives **18** and **19** (Scheme I). Deprotection of the phosphonic acid moiety of dialkyl phosphonates **22-27** with bromotrimethylsilane in acetonitrile afforded phosphonates **4-9**. In a similar way, starting from 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (**28**), the phosphonates **10** and **11** were obtained. As observed in clitocine, the presence of a strong hydrogen bonding in solution between the $NO_2$ group and the 4-NH group in compounds **4-11** was confirmed by $^1$H-NMR spectroscopy in DMSO-$d_6$. In fact the 4-NH appeared as a broad triplet at unusually low field in the range of 9.32-9.61 ppm.

**Biological evaluation.** Activity of compounds **4-11** against HIV-1 ($III_B$ strain) and HIV-2 (CBL-20 strain) multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathogenicity in MT-4 and C8166 cells, respectively. Briefly, 50 µL of culture medium (RPMI 10% FCS) containing $1x10^4$ cells were added to each well of flat bottomed microtiter trays containing 50 µL of RPMI with or without various concentrations of the test compounds. 20 µL of an HIV-1 or HIV-2 suspension containing 100 and 1000 $CCID_{50}$, respectively, were then added. After a 4 days incubation (5 days for HIV-2) at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[9] The cytotoxicity of compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method. PMEA, PMEDAP, PMEG and (*R*)-PMPG were used as reference compounds.




**4, 22** X, Y = N, R = $NH_2$, R', R" = H
**5, 23** X, Y = N, R = $NH_2$, R' = H, R" = $CH_3$
**6, 24** X, Y = N, R = R' = $NH_2$, R" = H
**7, 25** X, Y = N, R = R' = $NH_2$, R" = $CH_3$
**8, 26** X = CH, Y = N, R = $NH_2$, R', R" = H
**9, 27** X = N, Y = CH, R = $NH_2$, R', R" = H
**10, 29** X, Y = N, R = OH, R' = $NH_2$, R" = H
**11, 30** X, Y = N, R = OH, R' = $NH_2$, R" = $CH_3$

**12** X, Y = N, R = $NH_2$, R' = H
**13** X, Y = N, R, R' = $NH_2$
**14** X = CH, Y = N, R = $NH_2$, R' = H
**15** X = N, Y = CH, R = $NH_2$, R' = H
**28** X, Y = N, R = OH, R' = $NH_2$

**Scheme I**

In general, clitocine analogues appeared to be less cytotoxic than the corresponding purine phosphonates. Compounds **4-7** were active in protecting cell cultures against the HIV-1- and HIV-2-induced cytopathogenicity (Table 1). However, their potency and selectivity were lower than those of PMEA and PMEDAP. Against HIV-1, the analogues of PMEDAP and (*R*)-PMPDAP (compounds **6** and **7**) proved to be 4-fold more active than the analogues of PMEA and (*R*)-PMPA (compounds **4** and **5**); a similar increase of anti-HIV activity was observed when the adenine moiety of PMEA was substituted with 2-amino-adenine (PMEDAP).[5]

The substitution of N with a CH group at position 1 or 3 of the pyrimidine ring system (compounds **8** and **9**) resulted in inactive compounds. Also inactive were the phosphonate analogues of PMEG (**10**) and (*R*)-PMPG (**11**) (results not shown). The activity of compounds **4** and **5** confirmed that the 4,6-diamino-5-nitro-pyrimidine moiety could be considered a bioisostere of adenine, whereas the activity of **6** and **7**

**Table 1.** Comparative cytotoxicity and anti-HIV activity of phosphonates **4-7**.

| | | HIV-1 | | HIV-2 | |
|---|---|---|---|---|---|
| Compd | [a]$CC_{50}$ | [b]$EC_{50}$ | [c]SI | [b]$EC_{50}$ | [c]SI |
| **4** | > 342 | 118 | > 2.9 | 120 | > 2.8 |
| **5** | > 326 | 111 | > 2.9 | 115 | > 2.8 |
| **6** | > 325 | 28 | > 11.6 | 63 | > 5.2 |
| **7** | > 310 | 26 | > 12.5 | 60 | > 5.2 |
| PMEA | 229 | 5.3 | 43 | 26 | 8.8 |
| PMEDAP | 48 | 2.6 | 18.4 | ND | - |
| PMEG | 2.4 | 0.19 | 12.6 | 1.1 | 2.2 |
| (*R*)-PMPG | > 333 | 4.5 | > 74.0 | 5.0 | > 67 |

[a]Compound dose (μM) required to reduce the viability of mock-infected MT-4 cells by 50%. [b]Compound dose (μM) required to achieve 50% protection of MT-4 and C8166 cells against the cytopathic effect of HIV-1 and HIV-2, respectively. [c]Selective index: $CC_{50}/EC_{50}$ ratio.



showed that the 2,4,6-triamino-5-nitro-pyrimidine moiety was a bioisostere of 2-amino-adenine. The inactivity of PMEG and (*R*)-PMPG analogues **10** and **11** may be attributed to either poor transport of these compounds through the cell membrane or low affinity for the cellular enzyme(s) that convert phosphonates into their active metabolites (presumably the diphosphorylated derivatives).

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