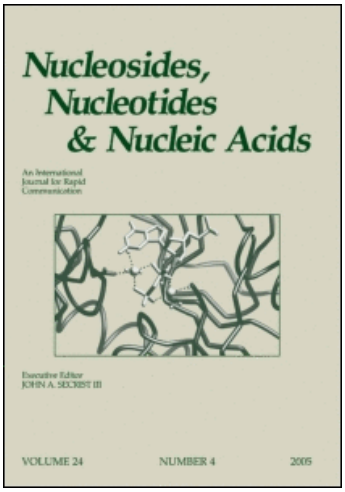

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New CycloAMB-Nucleoside Phosphonate Prodrugs

U. Görbig[a]; J. Balzarini[b]; C. Meier[a]
[a] Department of Chemistry, Organic Chemistry, Faculty of Science, University of Hamburg, Hamburg, Germany [b] Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium

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NEW *CYCLO*AMB-NUCLEOSIDE PHOSPHONATE PRODRUGS

U. Görbig □ *Department of Chemistry, Organic Chemistry, Faculty of Science, University of Hamburg, Hamburg, Germany*

J. Balzarini □ *Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium*

C. Meier □ *Department of Chemistry, Organic Chemistry, Faculty of Science, University of Hamburg, Hamburg, Germany*

□ cyclo*Sal- and* cyclo*Amb-nucleoside phosphonate prodrugs of PMEA were synthesized and characterized. Each of these compounds showed different disadvantages in hydrolysis. Thus, a new series of* cyclo*Aminobenzyl(*cyclo*Amb)-PMEA prodrugs was synthezised and studied with regard to their hydrolysis properties and biological activity.*

Keywords Nucleoside phosphonates; prodrugs; antiviral activity; hydrolysis; benzyl cation; phenylphosphate diester

INTRODUCTION

The nucleoside phosphonates PMEA **1**, PMPA **2**, and HPMPC **3** show very broad antiviral activity against DNA- and retro-viruses.[1]

The therapeutic use of these phosphonates is limited by their poor oral bioavailability due to the negatively charged phosphonate moiety that is present at physiological pH.[2] Therefore, neutral and membrane-permeable prodrugs were synthesized. Bis-(POM)-PMEA **4** and bis-(SATE)-PMEA **5,** for example, can penetrate cellular membranes and deliver the parent PMEA by enzymatic activation.[3]

The *cyclo*Sal pronucleotide system has been developed for the intracellular delivery of therapeutically active nucleoside monophosphates (NMPs) and has already been applied to different nucleoside analogs successfully, e.g., the anti-HIV active 3′-deoxy-2′,3′-didehydothymidine **6** (d4T).[4] We already reported on the synthesis and characterization of *cyclo*Sal- and *cyclo*Amb- PMEA prodrugs.[5] The *cyclo*Sal derivatives show very low hydrolysis half-lives limiting their antiviral potency. The *cyclo*Amb compounds show

Address correspondence to Ulf Görbig, University of Hamburg, Martin-Luther-King Platz 6, Hamburg D-20146, Germany. E-mail:ulfgoerbig@gmx.de

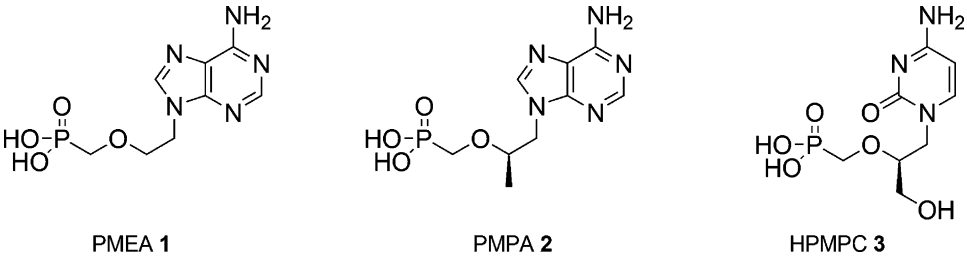

FIGURE 1 Stuctures of nucleoside phosphonates **1**, **2**, **3**.

higher hydrolysis half-lives, partially in optimal ranges. But their hydrolysis led to a quite stable hydrolysis intermediate **8** (Figure 1) that delivered PMEA very slowly, so their antiviral potency was also limited.[5] Here, we present novel *cyclo*Amb-PMEA prodrugs with new substitution patterns in order to destabilize the hydrolysis intermediate and accelerate the delivery of PMEA **1** to achieve higher antiviral activity.

Results

All new *cyclo*Amb prodrugs were synthesized as published before (6–25% yield).[5] 2-Aminobenzyl alcohols were synthezised by reduction of the corresponding anthranilic acids with lithium aluminiumhydride (4-Me: 61%, 6-Me: 71% yield). 7-Me-2-aminobenzyl alcohol was prepared by reduction of 2-amino-acetophenone with sodium borhydride (99% yield). 7,7-Di-me-anthranil alcohol was prepared by treating anthranilic acid methyl ester with a methyl Grignard reagent (54% yield).

Donor substituents *ortho* or *para* to the benzyl substituent (4- and 6-position) of 2-aminobenzyl alcohols stabilize a formed benzyl cation so that the C-O-bond cleavage of the hydrolysis intermediate (Figure 2) should be accelerated. Donor substituents in the benzyl position (7-postion) probably will show a stronger effect. However, tracking the hydrolysis of the new *cyclo*Amb-PMEA prodrugs by ^{31}P-NMR-spectroscopy, we found that none of the new compounds show an observable faster degradation of the hydrolysis

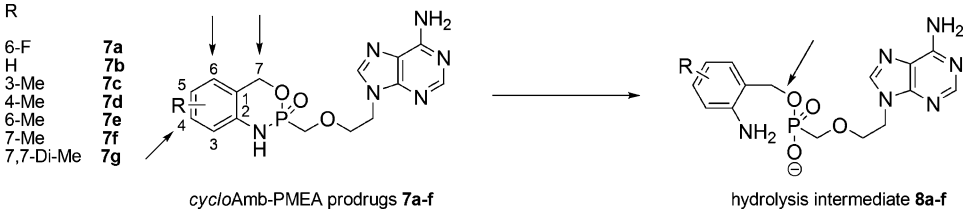

FIGURE 2 New substitution patterns may accelerate the C-O-bond cleavage of the hydrolysis intermediate.

TABLE 1 Properties of *cyclo*Amb-PMEA prodrugs

Compound	Hydrolysis half-life[a] [h]	Anti-HIV activity[b] (IC_{50} [μM]) HIV-1	Anti-HIV activity[b] (IC_{50} [μM]) HIV-2	CC_{50}^{c} [μM]
7a	1.3	28.0 ± 0.0	26.0 ± 2.1	244 ± 8.5
7b	4.0	20.0 ± 7.1	21.0 ± 9.2	183 ± 24.0
7c	21.3	29.1 ± 29.1	56.0 ± 43.4	>250
7d	4.9	17.5 ± 3.5	30.0 ± 0.0	>250
7e	11.5	15.0 ± 0.0	15.0 ± 0.0	175 ± 11.3
7f	28.8	53.3 ± 5.8	43.3 ± 20.8	>250
7g	900	> 250	> 250	> 250
PMEA	—	10.0 ± 6.4	10.0 ± 0.0	50 ± 13

[a] Hydrolytic stability in phosphate buffer pH 7.3, 37°C.

[b] 50% effective concentration for the prevention of HIV-1 or HIV-2 replication *in vitro* (wild type CEM/0 cells).

[c] Cytotoxic concentration.

intermediate (Figure 2). This is an unexpected and surprising result. Even more surprising is the fact that the 7-methylation led to a vastly increase of the hydrolysis half-lives (Table 1).

The methyl groups obviously shield the phosphorus atom, so the nucleophile H_2O/OH^- cannot attack as effectively as in the unsubstituted case. This interesting result is in contrast to the observation of the behavior of 7-Me-*cyclo*Sal-d4T-monophosphate. Here, we found that the C-O-bond cleavage takes place within minutes before a nucleophile can attack the phosphorus atom, leading to a phenylphosphate diester, that proved stable to further hydrolysis.[6]

In conclusion, we missed the aim to accelerate the decay of the hydrolysis intermediate by methylating the 4-, 6-, or 7-position of the masking unit. Moreover, by substitution of the 7-position of the masking unit the first hydrolysis step of forming the intermediate is significantly decreased. According to these results we found antiviral activities of the new prodrugs that do not reach the activity of parent PMEA (Table 1). Compared to the published *cyclo*Amb-PMEA prodrugs[5] 4-Me- and 6-*cyclo*Amb-PMEA show a slight improvement of antiviral activity. Surprisingly, the cytotoxicity of the new prodrugs is much lower compared to parent PMEA **1**, though their values of antiviral activity are in a similar range (Table 1).

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