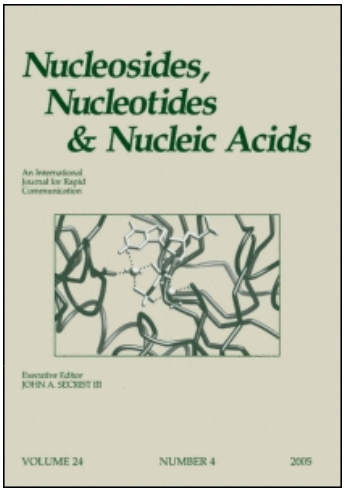

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BENZYL-FUNCTIONALIZED *cyclo*Sal-d4T MONOPHOSPHATES

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ABSTRACT

Synthetic routes to benzyl-functionalized *cyclo*Sal-d4T monophosphates ($7CH_2X$-*cyclo*Sal-d4TMP) have been developed. Their hydrolytic behavior in basic aqueous solution (pH = 7.3) was studied and their hydrolysis half-lives were determined. It turned out that two different degradation pathways are leading to different products: beside the formation of the expected d4TMP and a styrene type derivative, a phenyl-d4T-phosphodiester was obtained as well. The product distribution was specified.

INTRODUCTION

*Cyclo*Saligenyl-(*cyclo*Sal-) pro-nucleotides have been developed to deliver anti-HIV active nucleotides in cells via a pH-driven selective chemical hydrolysis [1]. During hydrolysis most probably a quinone methide intermediate analogous to **6** is formed that is quenched by water to yield a salicylic alcohol as the by-product. In this paper we report the synthesis of benzyl-functionalized *cyclo*Sal-d4TMPs **1**–**5**. This new type of *cyclo*Sal pro-nucleotide has been developed to influence the hydrolysis pathway towards forming a styrene-type derivative **7** as the by-product instead. Particularly, residues bearing electron-withdrawing groups used

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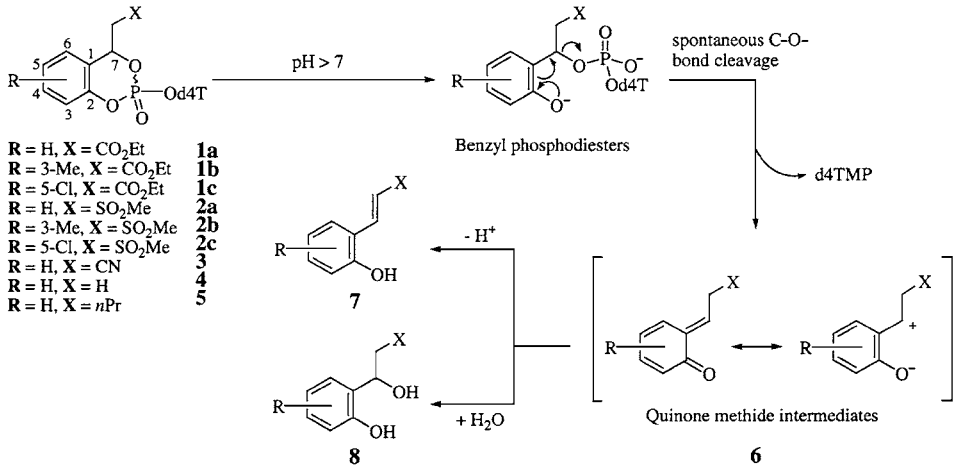

Figure 1. Possible hydrolysis pathway of benzyl-functionalized *cyclo*Sal-d4TMPs.

as benzyl-substituents should facilitate an α-proton removal in the quinone methide intermediate **6** to yield the styrene-type derivative **7** (Fig. 1).

RESULTS

The benzyl-functionalized prodrugs 7-CH_2X-*cyclo*Sal-d4TMP **1**–**5** (Fig. 1) have been synthesized in a three-step synthesis each, starting from salicylic aldehyde or its 3-Me-/5-Cl-derivative, respectively. First the salicylic alcohols were obtained after aldol-type additions of appropriate nucleophiles to the salicylic aldehydes. These alcohols then were used to prepare cyclic chlorophosphites. After their preparation under previously published conditions [1] they were not further purified but the raw products were used as phosphitylating agents for d4T in the third step. The synthesis was completed by oxidation of the intermediately obtained phosphite triesters to the corresponding phosphotriesters with *tert*-butyl hydroperoxide.

Their hydrolysis half-lives were determined in 12.5 mM PBS solution (phosphate buffer Soerensen, pH = 7.3) and compared to that of *cyclo*Sal-d4TMP (Table 1), which bears no further benzyl substituent (**7H**). In contrast to the original *cyclo*Sal-phosphotriesters, further hydrolysis products beside the expected d4TMP were identified for the benzyl-functionalized prodrug systems **1**–**5**. The product ratio was determined by comparing suitable integrated HPLC-chromatograms at the end of hydrolysis (Table 1). The relevant products were identified by their HPLC retention times, UV-spectra and ESI^+-MS as well as ESI^+-MS/MS spectrometry. The formation of two different types of hydrolysis products in the case of the benzyl-substituted prodrug systems **1**–**5** can be rationalized on the basis of two competing degradation pathways (Fig. 2). First, nucleophilic attack of the hydroxide anion to the phosphorus atom (path ***a*** in Fig. 2) would lead to a benzyl phosphodiester.

Table 1. Anti-HIV and Hydrolysis Data of Compounds **1–5**, **7H** and d4T

	EC_{50}[a] (μM)					
	CEM/O		CEM/TK$^-$	CC_{50}[b] (μM)		Phenyl phosphodiester
	HIV-1	HIV-2	HIV-2	CEM/O	$t_{1/2}$ (h)	(%)
1a	0.25	0.44	6	37.6	4.14	49.7
1b	0.19	0.95	2	61.4	6.82	57.6
1c	0.12	0.50	4	37.1	1.51	32.4
2a	0.28	0.4	20.0	224	0.30	62.4
2b	0.28	1	115	116	0.40	74.2
2c	0.07	0.03	21.5	38	0.11	38.7
3	—	—	—	—	1.06	58.8
4	0.33	0.43	20.0	≥250	0.66	100
5	—	—	—	—	0.31	100
7H	0.12	0.12	0.65	60.2	7.83	0
d4T	0.075	0.12	50	97.3	—	—

a) 50% Effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%; b) 50% Cytotoxic concentration or concentration required to reduce CEM cell viability by 50%.

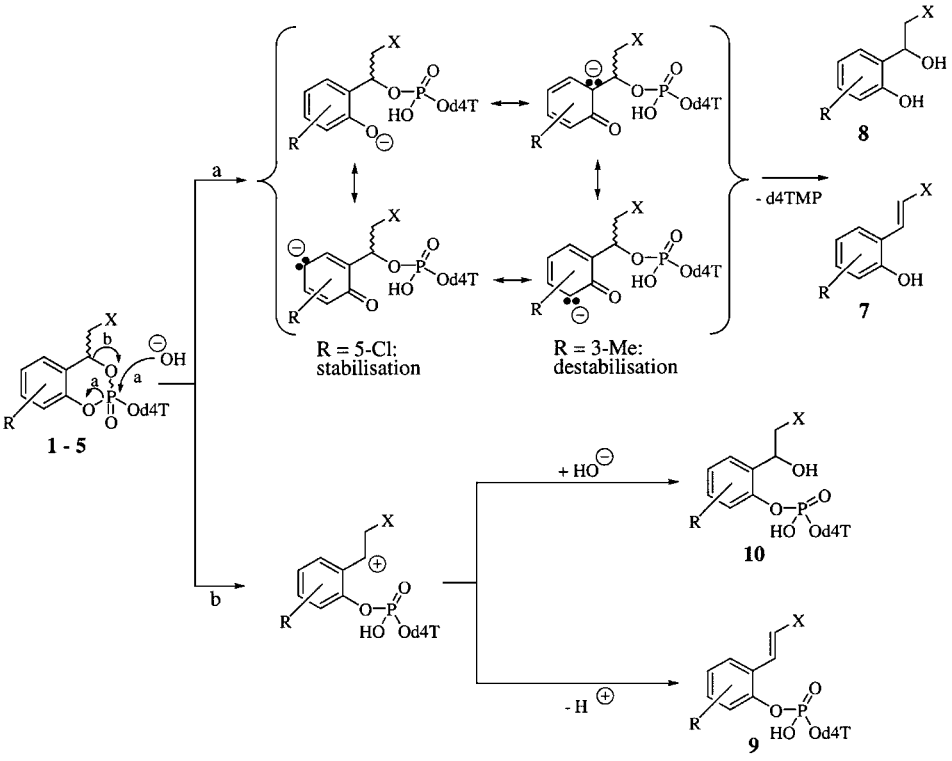

Figure 2. Competing degradation pathways of 7-CH_2X-*cyclo*Sal-d4TMP phosphotriesters.

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Subsequent cleavage of the benzyl-C–O bond leads to d4TMP and the styrene type by-product **7** in the case of the prodrugs bearing alkyl residues with electron-withdrawing substituents in the benzyl position (**1**–**3**). The benzyl-functionalized prodrugs **4** and **5** with pure alkyl substituents should give salicylic alcohol derivatives **8** and d4TMP after hydrolysis as already described for the *cyclo*Sal-prodrugs without further benzyl substituents. A spontaneous cleavage of the benzyl-C–O bond leading to a benzyl-cation is the competing hydrolysis pathway (path ***b*** in Fig. 2). This cation is stabilized by the phenyl system and is even further stabilized by the benzyl substituent. This latter effect is the major difference compared to **7H**. For the prodrug systems **1**–**3** subsequent α-proton removal results in a styrene-type phenyl phosphodiester **9** whereas in fact the only hydrolysis product obtained in the case of **4** and **5** was a phenyl phosphodiester **10** bearing a benzyl alcohol in the aromatic side chain.

The different capabilities of the benzyl substituents to stabilize the benzyl cation may be the reason for the different hydrolysis half-lives as well as the different product ratio during hydrolysis of **1**–**5** (Table 1).

The prodrugs **1**, **2** and **4** were further tested for their anti-HIV activity and the results were compared with the data available for d4T itself and *cyclo*Sal-d4TMP **7H** (Table 1). It was found that the EC_{50} data obtained in wild-type CEM cells for the benzyl- functionalized prodrugs **1**, **2** and **4** was up to 4 times higher than the data obtained for d4T and *cyclo*Sal-d4TMP (**7H**). But in TK-deficient cells prodrugs **1**, **2** and **4** except **2a** were up to 25 times more active against HIV-2 than d4T. This might be due to at least partial delivery of d4TMP by the prodrugs **1**, **2** and **4** which has been observed in hydrolysis studies. The fact that *cyclo*Sal-d4TMP **7H** is still better in activity may reflect the different rates of hydrolysis and degradation product ratio obtained for **1**, **2** and **4** since d4TMP is the only precursor of the anti-HIV active species but not the phenyl phosphodiesters **9** and **10**.

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