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O-ALKYL-5',5'-DINUCLEOSIDE-PHOSPHATES AS COMBINED PRODRUGS OF ANTIVIRAL AND ANTIBIOTIC COMPOUNDS

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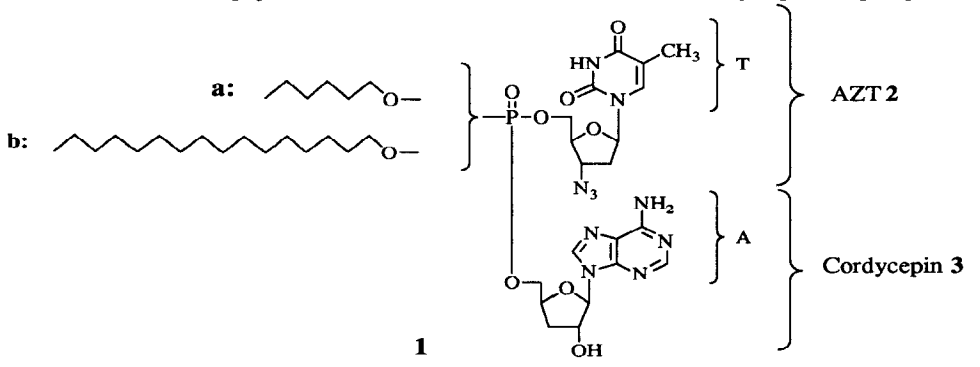
Summary: The chemical syntheses of two phosphotriester derivatives **1a,b** containing two different 5',5'-linked antiviral and antibiotic nucleosides as well as an aliphatic chain of different length are described. These compounds may act as prodrugs of biological active nucleoside analogues.

Recently it has been shown that combination treatment of anti HIV agents shows advantages with regard to single drug treatment in AIDS and the AIDS-related complex (ARC) because this permits a reduction in individual doses and would decrease toxicity side effects[1]. One approach to combination therapy involves the use of dimers of antiviral nucleosides which are linked via a phosphate bridge[2]. Unfortunately, charged phosphodiesters cannot penetrate the cell membranes or the blood brain barrier because the ability of a drug to penetrate a membrane is correlated to its lipophilic properties.

In this work, we want to report the syntheses of two phosphotriesters which combine the two requirements mentioned above. The described phosphotriesters **1a,b** contain: a) 3'-azido-3'-deoxythymidine (AZT) **2** as antiviral nucleoside (anti HIV), b) 3'-deoxyadenosine ("Cordycepin") **3** as antiviral and antibiotic nucleoside[3] and c) an alkyl-residue (**1a**: R=C_6H_{13} and **1b**: R=$C_{16}H_{33}$; these two alkyl residues were chosen to investigate the effects of different lipophilicities of **1a** and **1b**) esterified at the third acid group of the phosphate.



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We hypothesized that our phosphotriesters could act as lipophilic prodrugs and provide superior pharmacological properties for the following reasons:

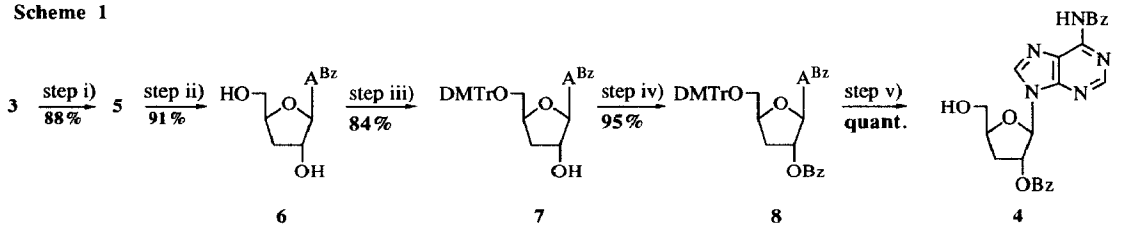
a) the aliphatic chains facilitate the passive membrane transport of the nucleoside analogues,

b) among the different ways of hydrolysis of **1a,b**, one could liberate the two nucleoside analogues as a phosphate diester and

c) further hydrolysis would yield at the same time two different nucleosides as their 5'-mononucleotides and their non-phosphorylated nucleosides, respectively. This is of special interest with respect to antiviral nucleosides which require intracellular kinase to convert them into 5'-nucleotides[4].

Before introducing the purine nucleoside **3** (3'-deoxyadenosine)[5] into the triesters **1a,b**, we needed 2',N^6-dibenzoyl-3'-deoxyadenosine **4** as a protected derivative of **3**. Scheme 1 shows the five step synthetic pathway starting from **3** to give **4** as a crystalline, colourless product in an overall yield of 65%. The critical step after perbenzoylation to **5** and selective debenzoylation to **6** is the selective introduction of the 5'-O-dimethoxytrityl (DMTr) protecting group (step iii). Reaction of **6** with DMTrCl (1.1eq.) in the presence of the *Huenig* base diisopropylethylamine (DIPEA, 1.1eq.) and a catalytic amount of dimethylaminopyridine (DMAP, 0.1eq.) in pyridine at room temperature yielded **7** in 84%[6]. It has to be outlined, that the substitution of the DIPEA by DMAP or omission of these bases (only the solvent pyridine acting as base) resulted in a strong decrease in the yield of **7** because of formation of a mixture of **7**, the O^5',N^6-ditritylated derivative and the N^6-monotritylated product. The selective 2'-O-benzoylation (step iv) to give **8** was carried out analogously to a described literature procedure with an excellent yield of 95%[7].

Scheme 1

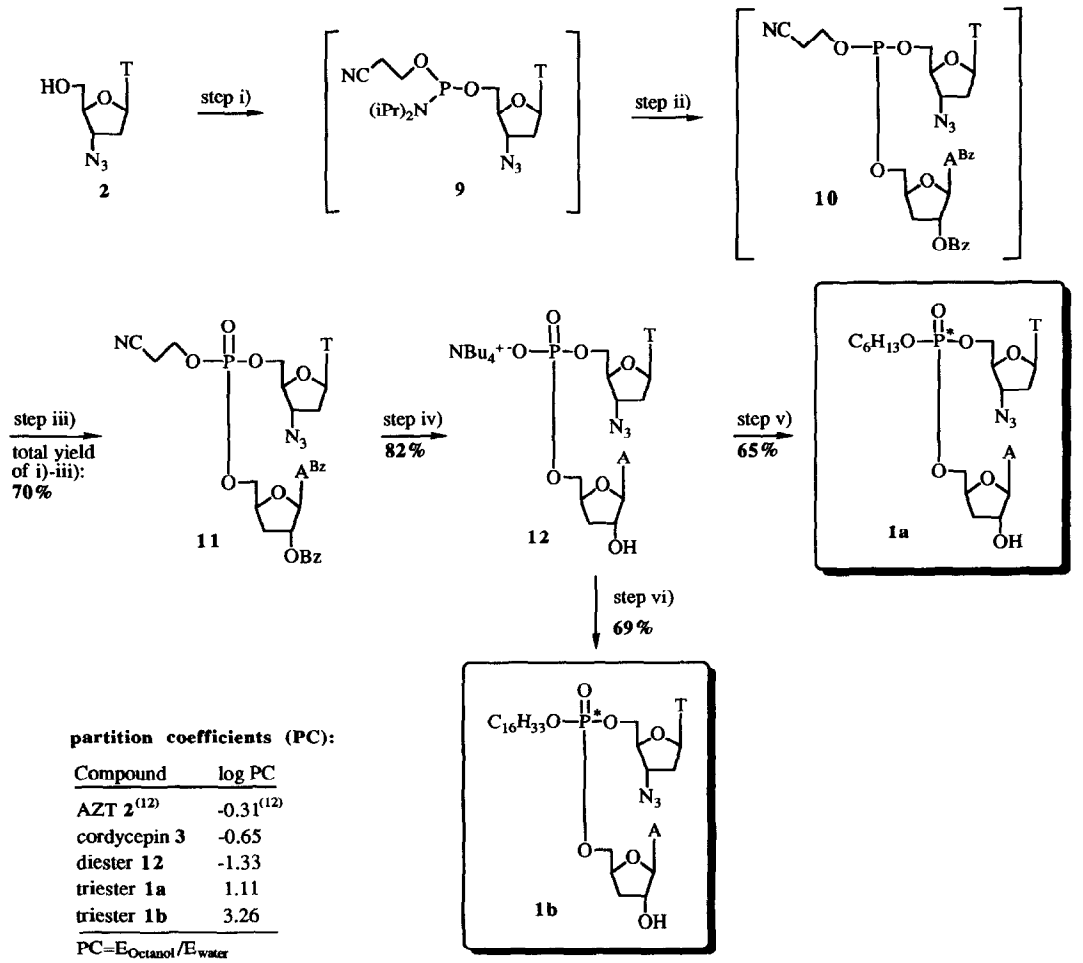


steps: i) 6eq BzCl, pyridine, 2.5h, 0°C[8]; ii) 1.5M NaOH/H_2O, MeOH/THF (1.1), 30 min, 0°C[8]; iii) 1.1eq. DMTrCl, pyridine, 1.1eq. DIPEA, 0.1eq. DMAP,10h, rt; iv) 1.1eq PhC(O)OC(O)Ph, CH_3CN, DMAP(cat.), NEt_3, 1 5h, rt; v) 2%BSA in CH_2Cl_2/MeOH, 10min, rt.

The following syntheses of the triesters **1a,b** were achieved as summarized in scheme 2. For the coupling of the two nucleosides we used the phosphoramidite chemistry (step i and ii). Because of great instability of the intermediates **9** and **10**, the first three steps were carried out as a one-pot reaction. Concerning the first step, no starting material and complete conversion could be detected by TLC after 10 min (solvent: ethylacetate/ petroleum ether 4:1; R_f=0.65). Interestingly, when we replaced β-cyanoethyl-(diisopropylamino)-chlorophosphoramidite by bis(diisopropylamino)-chlorophosphoramidite no reaction took place and we isolated the starting material **2**. The second step was completed within 20 min (TLC; solvent: dichloromethane/methanol 9:1; R_f=0.55). Oxidation of **10** in step iii) under standard conditions yielded **11**, which was purified by silica gel column chromatography and isolated in an overall yield of 70%. The structure of **11** was confirmed by means of ^{1}H-, ^{13}C-, ^{31}P-NMR as well as by the ^{1}H-^{13}C-correlation NMR technique. The removal of the β-cyanoethyl-residue as well as the benzoyl-protecting groups in **11** by treatment with 1% sodium methylate solution gave after Dowex ion-exchange the 5',5'-linked phosphodiester **12**[9]. The final triesters **1a,b** were obtained as 1:1-mixtures of

two diastereomers (R_p, S_p) from the tetrabutylammonium salt **12** by direct nucleophilic displacement of 1-iodohexane (**1a**) and 1-iodohexadecane (**1b**) in boiling acetonitrile (60% yield)[10]. During the purification of **1a,b**, we were able to isolate both diastereomers of **1a,b** by silica gel chromatography, which were characterized by ^1H-, ^{13}C-, ^{31}P- and COSY-NMR as well as by FAB-mass. The purity was checked by elemental analysis and HPLC separation (99% purity)[11].

Scheme 2



partition coefficients (PC):

Compound	log PC
AZT **2**[12]	-0.31[12]
cordycepin **3**	-0.65
diester **12**	-1.33
triester **1a**	1.11
triester **1b**	3.26

$PC = E_{Octanol}/E_{water}$

steps: i) 1.1eq. $(NCCH_2CH_2O)(N(iPr)_2)PCl$, DIPEA, CH_3CN, 0°C, 10min; ii) **4**, tetrazole, CH_3CN, rt, 30min; iii) I_2, H_2O/THF/pyridine, rt, 5min; iv) a) 1% NaOMe, MeOH, rt, 16h; b) Dowex H^+; c) Dowex Bu_4N^+; v) $C_6H_{13}I$, CH_3CN, 80°C, 6h, vi) $C_{16}H_{33}I$, CH_3CN, 80°C, 20h.

Determination of the partition coefficents of **1a,b** (see scheme 2) shows that both compounds are much more lipophilic than the parent anti HIV-nucleoside analogue AZT **2** and the previously described phosphotriester derivatives[10].

Finally, the presented syntheses of the new phosphotriesters **1a,b** allow now further studies concerning the structure of the molecule in solution, confirmation of the determination of the stereochemistry on phosphorus (NMR techniques), the hydrolysis behaviour of **1a,b**, the passive membrane transport with unilamellar vesicles and at last testing for the biological activity against HSV1 and HIV. Data concerning these studies will be published in a forthcoming full paper.

Acknowledgement: Chris Meier is grateful to the *Fonds der Chemischen Industrie*, Frankfurt/Main; Germany for a "Liebig Stipendium". We are also grateful to the *ANRS 90-91 program* for financial support.

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(5) Cordycepin **3** was prepared by an improved method described recently: C.Meier; T.Huynh-Dinh, *Synlett* **2** (1991) 227.

(6) Recently, P.Herderwijn described the 5'-O-protection of **3** with tritylchloride with DMAP at 50°C with a yield of 85%. Under these conditions we isolated only 35% of the desired product **7**: P.Herderwijn; R.Pauwels; M.Baba; J.Balzarini; E.De Clercq, *J Med Chem* **30** (1987) 2131.

(7) A.Matsuda, *Synthesis* (**1986**) 385.

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(9) Spectroscopic data of **12**: ^{1}H-NMR(300MHz, D_2O): 1.68 (s, 3H); 2.09 (ddd,1H); 2.20 (mz, 3H); 4.01 (mz, 4H); 4.20 (mz, 1H); 4.40 (mz, 1H); 4.68 (mz, 1H); 4.82 (mz, 1H); 6.03 (d, 1H); 6.07 (t, 1H); 7.42 (s, 1H); 8.36 (s, 1H); 8.41 (s, 1H). ^{31}P-NMR(121MHz,D_2O): 0.836.

(10) a) F.Iglesias-Guerra, J.M.Neumann, T.Huynh-Dinh, *Tetrahedron Lett* **28** (1987) 3581, b) J.M.Neumann, M.Herve, J.-C.Debouzy, F.Iglesias-Guerra, C.Gouyette, B.Dupraz, T.Huynh-Dinh, *J.Am Chem.Soc.* **111** (1989) 4270.

(11) Spectroscopic data of **1a**: ^{1}H-NMR(300MHz, MeOD): 0.89 (t, 3H); 1.32 (mz, 6H); 1.62 (mz, 2H); 1.86 (s, 3H); 2.21 (mz,1H); 2.34 (mz, 3H); 3.97 (mz, 1H); 4.00 (q, 2H); 4.28 (mz, 4H); 4.38 (mz, 1H); 4.76 (mz, 1H); 4.83 (mz, 1H); 5.99 (2x d, 1H); 6.09 (2x t, 1H); 7.48 (s, 1H); 8.18 (s, 1H); 8.26 (2x s, 1H). ^{31}P-NMR(121MHz,D_2O): 0.857 (Rp), 0.914 (Sp). Spectroscopic data of **1b**: ^{1}H-NMR(300MHz, MeOD): 0.90 (t, 3H); 1.30 (mz, 26H); 1.60 (mz, 2H); 1.88 (s, 3H); 2.20 (mz,1H); 2.33 (mz, 3H); 3.98 (mz, 1H); 4.01 (q, 2H); 4.29 (mz, 4H); 4.37 (mz, 1H); 4.78 (mz, 1H); 4.83 (mz, 1H); 5.98 (2x d, 1H); 6.07 (2x t, 1H); 7.50 (s, 1H); 8.17 (s, 1H); 8.29 (2x s, 1H). ^{31}P-NMR(121MHz,D_2O): 0.885 (Rp), 0.976 (Sp). (The assignment of the Rp and Sp configuration of **1b** was achieved according to the ^{31}P-NMR chemical shifts and the mobility on silica gel according to B.V.L.Potter, F.Eckstein, *Nucl Acids Res* **11** (1983) 7087 (TLC, methylenchloride/ methanol 9:1): **1a**: Rp:0.70, Sp:0.64, **1b**: Rp:0.85, Sp:0.80).

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