Syntheses of antivirals from the family of acyclic nucleoside phosphonates and their prodrugs

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Acyclic nucleoside phosphonates (ANPs) are compounds which contain a nucleobase attached to an aliphatic chain having a phosphonomethyl residue. They were developed originally in our Institute in the eighties of last century and they are noted for their broad spectrum of biological activities, especially antiviral, cytostatic, immunomodulatory and antiparasitic ones. Some of them are commercially available pharmaceuticals effective against serious viral infections – cidofovir, adefovir and tenofovir (1,2).

The ANPs represent three different types: HPMP, i.e. (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] derivatives (e.g. HPMPC, cidofovir), PME, i.e. 2-(phosphonomethoxy)ethyl derivatives (e.g. PMEA, adefovir) and PMP, i.e. (*R*)-2-(phosphono-methoxy)propyl derivatives, e.g. PMPA, tenofovir.

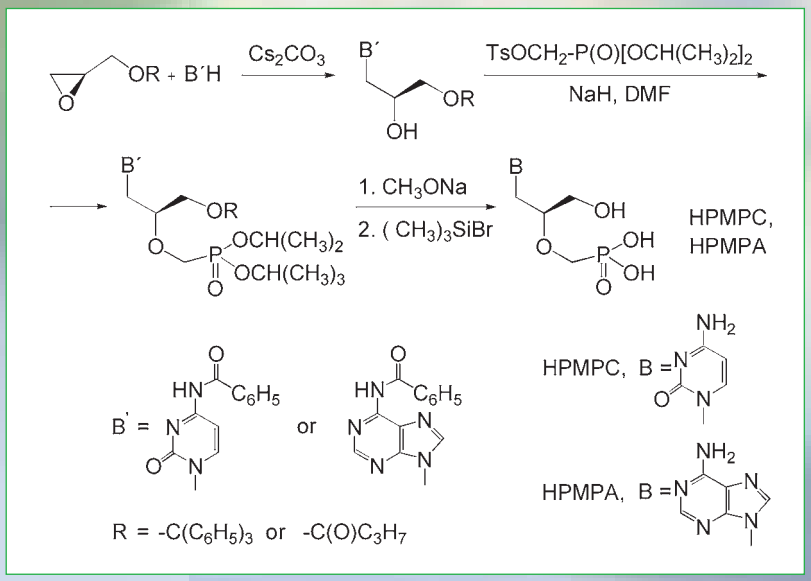

Figure 1 – Synthesis of HPMPC (cidofovir) and HPMPA

CIDOFOVIR AND OTHER HPMP DERIVATIVES

Currently, the synthesis of (*S*)-HPMP derivatives mostly utilizes base-catalyzed nucleophilic opening of the oxirane ring in (2*S*)-2-[(trityloxy)methyl] oxirane or (*R*)-glycidol butyrate (4) with appropriate nucleobase, e.g. N^4-benzoylcytosine, N^6-benzoyladenine or 2,6-bis(benzoyl-amino)purine (3,4). This reaction proceeds regioselectively to N^9 position of the purine or N^1 position of the pyrimidine base. The intermediary formed 3´-O-protected 2,3-dihydroxypropyl derivatives with free 2´-hydroxyl group are subsequently etherified with dialkyl (diethyl or

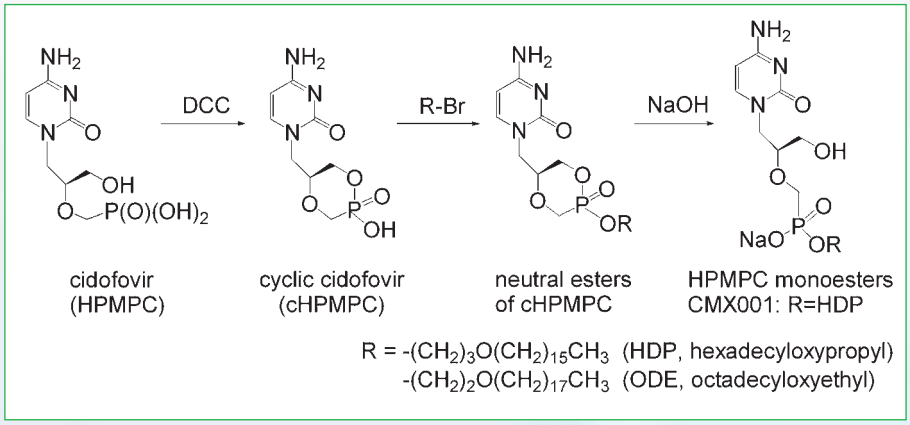

Figure 2 – Transformation of cidofovir to alkoxyalkyl esters

diisopropyl) ester of tosyloxymethanephosphonate in the presence of sodium hydride. After removal of benzoyl groups, trityl and phosphonic ester groups are deprotected by treatment with bromotrimethylsilane followed by hydrolysis (Figure 1). Preparation of diisopropyl tosyloxymethanephosphonate consists in the treatment of diisopropyl phosphite with paraformaldehyde and triethylamine followed by tosylation (5). Most recently, introduction of phosphonomethyl ether group using diisopropyl bromomethylphosphonate (6) was also recommended (7,8).

PRODRUGS OF HPMP DERIVATIVES

All acyclic nucleoside phosphonates have two negative charges in the molecule which cause their high polarity and make their transport through cell membranes more difficult. It is a reason of their low oral bioavailability. To overcome this problem, there is a general tendency to their transformation to appropriate prodrugs (esters or amidates). In contrast to PME and PMP series, there is no commercially available prodrug so far available for HPMP derivatives: cidofovir, approved for the treatment of cytomegalovirus retinitis in AIDS patients, is applied as an intravenous infusion of the free acid. Syntheses of neutral lipophilic diesters are problematic due to a presence of hydroxyl group in an aliphatic chain which takes part easily in intramolecular esterification forming a stable six-membered ring of the cyclic phosphonate. At present, there are three types of prodrugs of HPMPC (and/or other HPMP derivatives eventually): cyclic phosphonates, neutral esters derived from a cyclic form (alkyl salicylates, alkoxyalkyl esters, peptidomimetics) and alkoxyalkyl monoesters (9-11). The representative of last mentioned, hexadecyloxypropyl ester of cidofovir (CMX001) is currently developed by Chimerix (CA, USA.) as an antipox virus agent. At present, the company has initiated the first Phase II multi-dose clinical trial in patients (12).

Alkoxyalkyl esters of cidofovir are prepared by alkylation of *N,N,*-dicyclohexyl-4-morpholinocarboxamidinium salt of cyclic cidofovir (cHPMPC) with appropriate alkoxyalkyl bromides. Thus formed esters of cHPMPC are subsequently cleaved to corresponding monoesters (HDP-CDV, ODE-CDV) by heating with 2M solution of sodium hydroxide according to Figure 2 (13,14).

An alternative route was developed for alkoxyalkyl esters of HPMPA. It is based on etherification of appropriate hydroxy derivate with alkoxyalkyl tosyloxymethanephosphonate, a phosphonomethyl residue-containing agent having the lipophilic group preattached (15). This compound can be prepared in three steps from diethyl tosyloxymethanephosphonate: deprotection of ethyl ester groups with bromotrimethylsilane, transformation to chloridate by the action of oxalyl chloride and reaction with alkoxyalkanols under

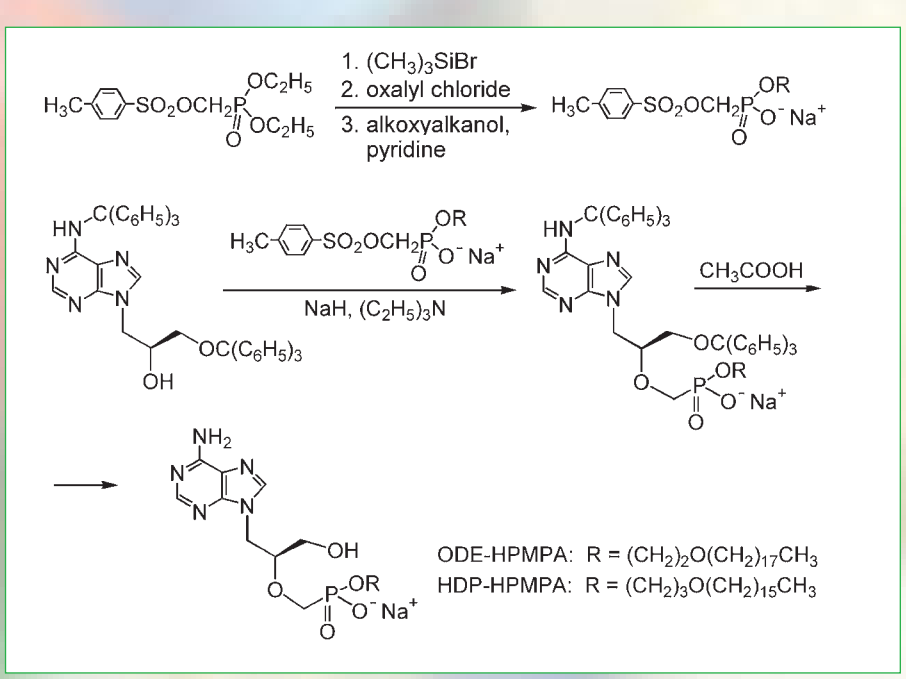

Figure 3 – Alkoxyalkyl esters of HPMPA. An alternative approach to alkoxyalkyl monoesters

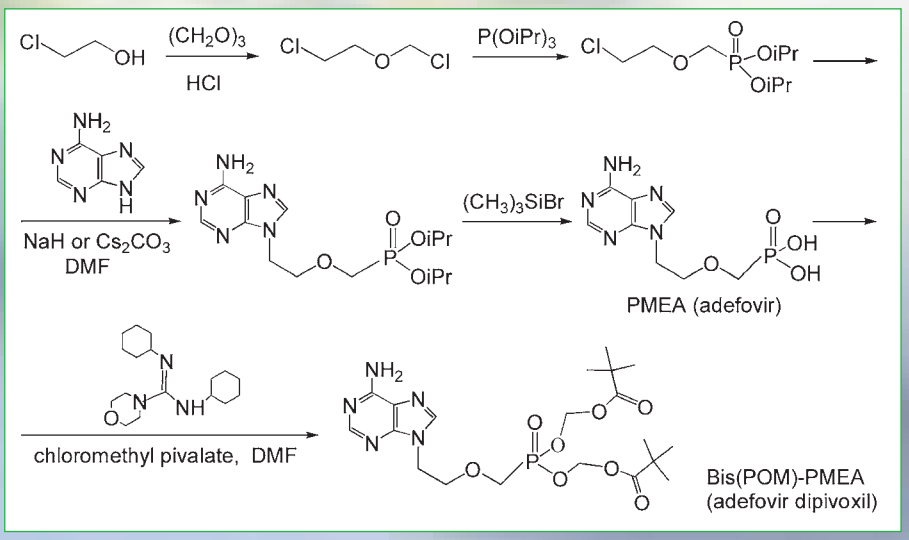

Figure 4 – Synthesis of adefovir and its prodrug, Bis(POM)-PMEA

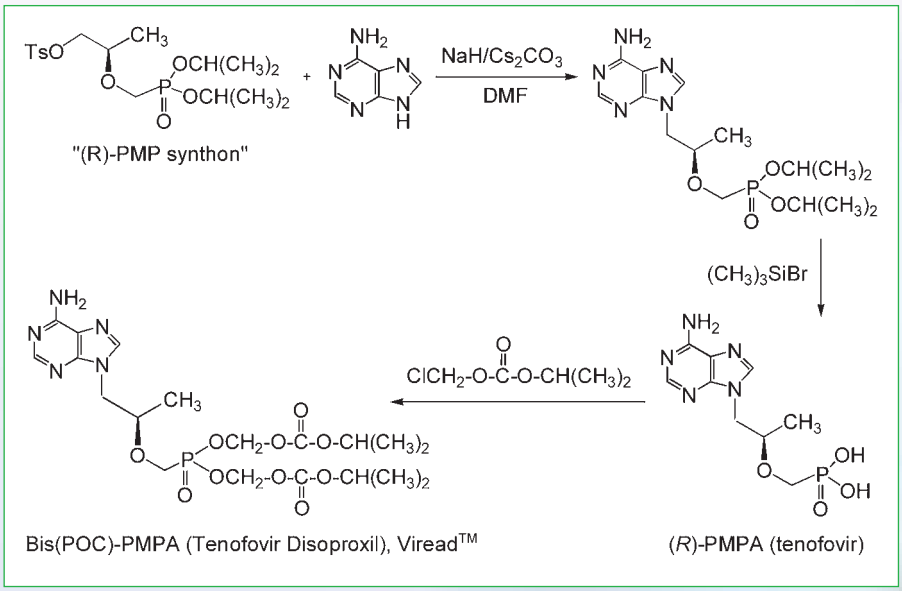

Figure 5 – Synthesis of tenofovir and its prodrug form

basic conditions. The starting material for syntheses of HPMPA esters was (*S*)-9-(3-trityloxy-2-hydroxypropyl)-*N* ^{6}-tritylladenine, an intermediate in preparation of HPMPA according to Webb's method (16). The whole process is outlined in Figure 3.

PHOSPHONATES OF PME TYPE. ADEFOVIR

Synthesis of acyclic nucleoside phosphonates of PME and PMP type consists in base-catalyzed condensation of a purine or pyrimidine base with appropriate synthon, i.e. the whole aliphatic part of the molecule containing a phosphonomethyl residue and an appropriate leaving group – mostly tosyl or halogen.

N-2-(Phosphonomethoxy)ethyl (PME) derivatives are thus synthesized from dialkyl (diethyl or diisopropyl) 2-chloroethoxy-methylphosphonates. These synthons are accessible by the treatment of trialkyl phosphites with 2-(chloroethoxy)methyl chloride (17,18). The synthesis of PMEA (adefovir) according to this procedure is outlined in Figure 4. The analogous procedure was applied also to the preparation of PMEG (guanine analogue) and a series of *N*^{6}-substituted PMEDAP [N-2-(phosphonomethoxy)ethyl-2,6-diaminopurines] including 9-(2-phosphonomethoxyethyl)-*N*^{6}-cyclopropyl-2,6-diaminopurine (cPrPMEDAP). In these cases condensation reaction of diisopropyl 2-chloroethoxy-methylphosphonate is performed with 2-amino-6-chloropurine; the intermediary formed 2-amino-6-chloropurine PME derivative is used for further transformations: acid hydrolysis (to PMEG) or reaction with amines, e.g. cyclopropyl amine to give cPrPMEDAP (19). An alternative way to PME derivatives is reaction of heterocyclic bases with dialkyl 2-hydroxyethylphosphonate under Mitsunobu conditions (20).

Adefovir is clinically used for the treatment of hepatitis B in the form of its orally applicable prodrug, bis(pivaloyloxymethyl) ester – Adefovir Dipivoxil, Bis(POM)-PMEA, Hepsera™. Its synthesis proceeds from PMEA by the action of chloromethyl pivalate in the presence of the hindered base *N,N'*-dicyclohexylmorpholinocarboxamidine (Figure 4) (21).

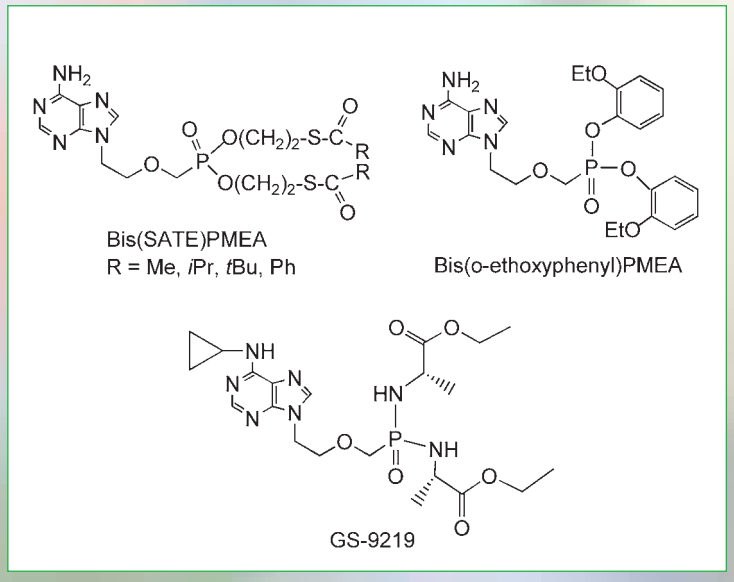

Figure 6 – Other structural types of PMEA and PMEG prodrugs

(PHOSPHONOMETHOXY)PROPYL (PMP) DERIVATIVES. TENOFOVIR

Syntheses of enantiomeric N-[2-(phosphonomethoxy)propyl derivatives are based on condensation of a nucleobase with appropriate chiral synthon. The starting compound for the synthesis of (*R*)-PMPA (tenofovir) is (*R*)-2-[bis(2-propyl)phosphonyl-methoxy]propyl] p-toluenesulfonate ["(*R*)-PMP-synthon"], the compound is prepared easily from (*R*)-1-benzyloxy-2-propanol by multi-step process involving chloromethylation, Arbuzov reaction with triisopropyl phosphite, catalytic hydrogenation and final tosylation (22). Condensation of adenine with this "PMP-synthon" followed by deprotection of ester groups gives (R)-9-[(2-phosphono-methoxy)propyl]adenine (PMPA, tenofovir). For clinical utilization this compound is further transformed to neutral prodrug, bis(isopropoxycarbonyloxymethyl) ester – Tenofovir disoproxil, Bis(POC)-PMPA. This prodrug is prepared from PMPA and chloromethyl isopropyl carbonate (Figure 5) (23,24). Tenofovir disoproxil is therapeutically used for the treatment of AIDS, namely as one-component drug Viread™ or in combination with other therapeutics (Truvada™, Atripla™).

OTHER TYPES OF PRODRUGS

Another type of diester prodrugs with a good oral bioavailability are bis(S-acyl-2-thioethyl esters (SATE-esters). Compared to Bis(POM)PMEA, the bis(tBu-SATE)PMEA was found to be more stable in human gastric juice and human serum (25). Very good oral bioavailability together with a good stability in intestinal tract was also found at substituted aryl esters, e.g. bis(*o*-ethoxyphenyl) ester (26). Structures of these prodrugs are shown in Figure 6.

Currently a special attention is paid to cyclic 1-aryl-1,3-propanyl esters designed specifically to be activated within the liver to treat liver-based diseases (HepDirect prodrugs). One of the representatives of this group, PMEA prodrug pradefovir was advanced into the Phase II of human clinical trials (27). Its synthesis consists in condensation of adefovir with 1-(3-chlorophenyl)-1,3-propanediol. The diol is accessible from 3-chloro-benzaldehyde by reaction with lithium enolate or alkyl acetate followed by ester reduction. Cyclic 1-aryl-1,3-propanyl esters contain two chiral centers in the molecule resulting in

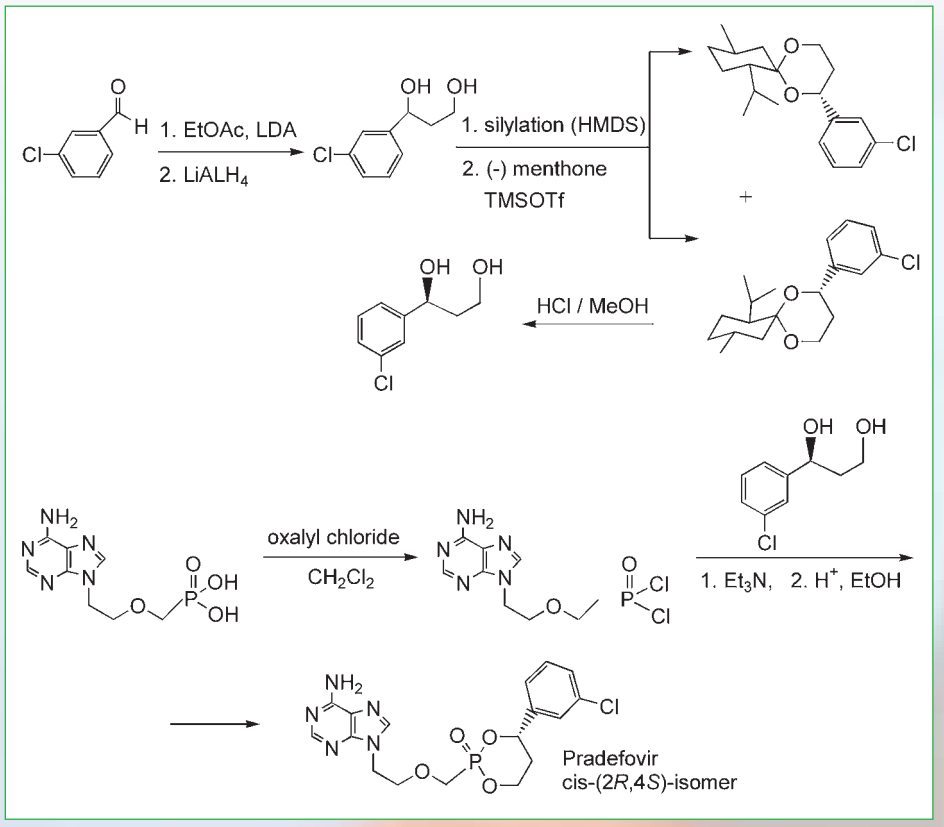

Figure 7 – Synthesis of pradefovir

existence of these compounds as a mixture of four diastereoisomers. Their separation and preclinical studies of each of them selected *cis*-(2*R*,4*S*)-isomer as the best candidate for further evaluation. Its stereoselective synthesis can be performed via resolution of racemic 1-(3-chlorophenyl)-1,3-dihydroxypropane through diastereomeric menthone adducts as intermediates (Figure 7) (28).

Besides cyclic phosphonate esters and diesters, a considerable attention is also paid to amidate prodrugs of PMEA, PMEG and PMPA, compounds bearing a phenyl ester group and an amino acid ester derivative linked by a phosphonamidate linkage (29). Unfortunately, a simultaneous presence of two different substituents at phosphorus atom gives rise to phosphorus atom asymmetry and necessity of – in some cases – difficult separation of diastereoisomers. To avoid this problem, the development of bis(phosphonamidate) type of prodrugs can be the solution. This type of prodrugs was successfully applied e.g. for cPrPMEDAP: its bis(phosphonamidate) with ethyl L-alanate (GS-9219, Figure 6) is currently investigated in clinical Phase I as the antineoplastic drug candidate against spontaneous non-Hodgkin's lymphoma (30). This "double prodrug" after reaching the target tissue finally reveals PMEG, 9-[2-(phosphono-methoxy)ethyl]guanine, a compound with strongly cytostatic effects. GS-9219 can be prepared from cPrPMEDAP by the action of L-alanine ethyl ester hydrochloride after activation of phosphonic acid residue with Aldrithiol and triphenylphosphine (31).

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