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5′-Esters of 2′-deoxyadenosine and 2-chloro-2′-deoxyadenosine with cell differentiation-provoking agents★✪

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Phenylacetic and retinoic acids are carboxyacidic cell differentiating agents displaying anticancer activities. We report on a new class of compounds including the 5′-esters of 2′-deoxyadenosine (dA) or 2-chloro-2′-deoxyadenosine (cladribine, 2CdA) and the aforementioned acids. The rationale behind the synthesis of these esters was that if they are hydrolyzed inside the lymphoid cells, either dA will be removed from the intracellular environment by deamination, or 2CdA will be phosphorylated and accumulated. In either case targetted delivery of the differentiating agent to the lymphoid cells may be envisaged. The said compounds were synthesized by the Mitsunobu procedure employing triphenylphosphine and azadicarboxylic acid esters, and their stability was tested against various esterases. Esters of dA and 2CdA with phenylacetic acids were found to be resistant to enzymatic hydrolysis, whereas those with retinoic acids were efficiently hydrolyzed by commercially available hepatic esterase as well as by esterases present in the blood plasma and in diluted human lymphocyte lysate. Susceptibility to enzymatic hydrolysis was found to be a prerequisite of cytotoxic and/or differentiating activity of these esters in leukemic cell lines.

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Abbreviations: PA, phenylacetate; PB, phenylbutyrate; o-Br-PB, *ortho*-bromo-phenylbutyrate; dA, 2′-deoxyadenosine; 2CdA, 2-chloro-2′-deoxyadenosine (cladribine); dCF, deoxycoformycin; AZT, 3′-azido-2′,3′-dideoxythymidine (zidovudine); ATRA, all-*trans*-retinoic acid; dCK, deoxycytidine kinase; ADA, adenosine deaminase; Me_2SO, dimethylsulfoxide.

Phenylacetate (PA) and its analogs [1, 2], as well as several retinoic acids [3] are inducers of cell differentiation. In various assay systems these carboxyacidic differentiating agents have been found to display anticancer (cytotoxic and/or proapoptotic) properties [4, 5], and some of them are being tested in treatments of chosen neoplastic diseases including leukemias [5, 6]. Combinations of a differentiating agent with various cytotoxic drugs are also being considered [7]. For example, phenylbutyric acid (PB) enhances growth inhibition of fluorodeoxyuridine-treated human colon cell lines *in vitro* [8]. Combination of all-*trans*-retinoic acid (ATRA) with cytarabine has been recently evaluated in newly diagnosed acute promyelocytic leukemia cases [9] and in high-risk myelodysplastic syndrome [10]. Treatment with combination of deoxyadenosine (dA) and the potent adenosine deaminase (ADA) inhibitor deoxycoformycin (dCF) greatly enhanced granulocytic differentiation of acute myeloid leukemia cells *in vitro* by ATRA [11].

Lymphocytes display exceptionally high activity of deoxycytidine kinase (dCK), the enzyme which controls phosphorylation of dA and some of its analogs, such as cladribine (2-chloro-2′-deoxyadenosine, 2CdA) [12]. While dA alone is not toxic to these cells because it is efficiently eliminated by ADA, dA combined with the ADA inhibitor dCF results in intracellular accumulation of dA phosphates leading to the depletion of lymphocytic ATP and selective lymphocyte death [13]. In a similar mechanism 2CdA, which is resistant to ADA but is efficiently phosphorylated by dCK, accumulates several hundred-fold inside the lymphocytes and also exerts selective lymphocytotoxicity [14] (Fig. 1).

Considering the high deamination rate of dA and the significant intracellular accumulation of 2CdA phosphates in lymphoid cells we designed a series of 5′-esters of dA and 2CdA with carboxyacidic derivatives known of their cell differentiating properties: PA, *ortho*-bromo-phenylacetate (o-Br-PA), PB, ATRA and 13-*cis*-retinoate. We hypothesized that

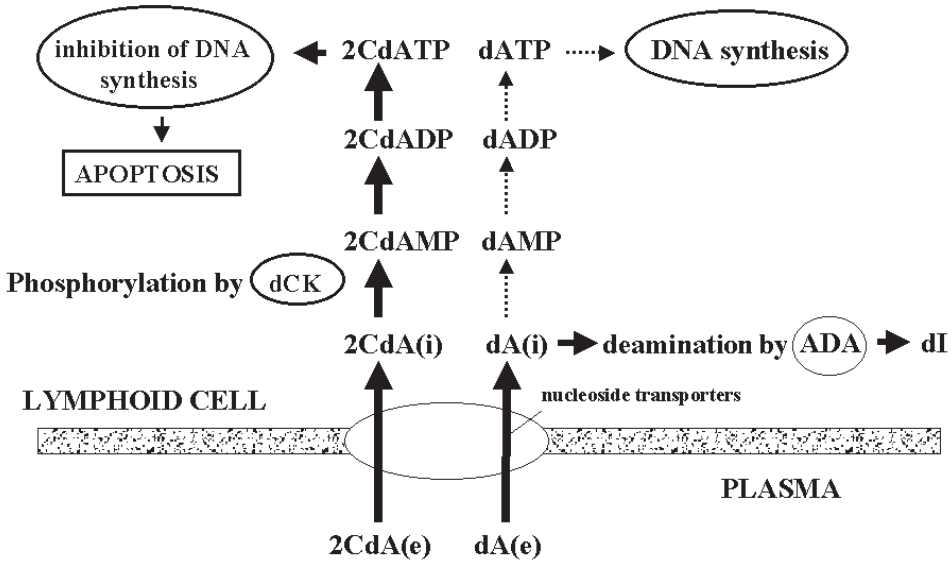

Figure 1. Metabolism of 2′-deoxyadenosine (dA, right side of the scheme) and 2-chloro-2′-deoxyadenosine (2CdA, left side of the scheme) in lymphoid cells.

These nucleosides are taken up from extracellular milieu (e) by nucleoside transporting proteins located at the cellular membranes. Lymphoid cells contain uniquely high activity of deoxycytidine kinase (dCK), the regulatory enzyme for the tri-step purine nucleoside phosphorylation pathway. Inside these cells (i) dA is phosphorylated to mono-, di- and triphosphates in quantities matched to the requirement for DNA synthesis, and the excess is efficiently deaminated by adenosine deaminase (ADA) to 2′-deoxyinosine (dI). 2CdA follows similar route, except that it is not deaminated by ADA. The resistance to deamination results in selective accumulation of 2CdA phosphates inside the lymphoid cells, leading to DNA synthesis inhibition and induction of apoptosis.

such esters could serve as vehicles for targeted delivery of the said differentiating agents to lymphocytes. The removal of the nucleoside product of hydrolysis of these esters (dA or 2CdA) by deamination and phosphorylation, respectively, should lead to selective concentration of the differentiating agent inside lymphoid cells (Fig. 2).

Here we describe the synthesis and some physico-chemical properties of these esters, and report on their susceptibility to enzymatic hydrolysis *in vitro*.

MATERIALS AND METHODS

General methods. Chemicals, solvents and enzymes were purchased from Sigma-Aldrich. Melting points (uncorr.) were measured in open capillary tubes on a Gallenkamp-5 melting point apparatus. Ultraviolet absorption spectra were recorded on a Kontron Uvikon 940 spectrophotometer.

^{1}H-NMR spectra (in ppm) were measured with Varian Gemini (200 MHz) and Varian UNITYplus (500 MHz) spectrometers at 298

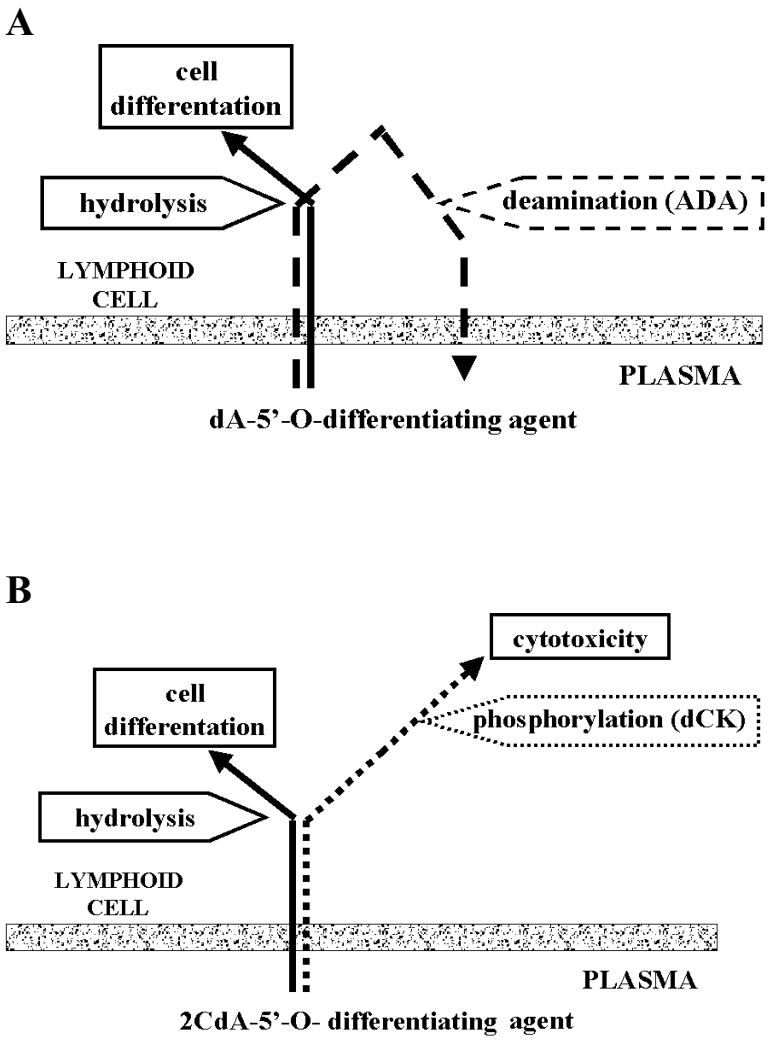

Figure 2. Scheme explaining the concept of targetted delivery of carboxyacidic differentiating agents to lymphoid cells using their 5′-esters with dA or 2CdA as drug delivery system.

A. Intracellular hydrolysis of a dA ester releases dA which in lymphoid cells is efficiently removed by deamination. **B.** Intracellular hydrolysis of a 2CdA ester releases free 2CdA which is not deaminated, but is removed through phosphorylation and concentrated specifically in lymphoid tissues. In both cases removal of the free nucleoside should result in selective intralymphocytic accumulation of the differentiating agent.

K in $D_6(Me_2SO)$ using tetramethylsilane as internal standard. Flash chromatography was performed with the use of Merck silica gel 60 (200–400 mesh). Analytical TLC was carried out on pre-coated silica gel F_{254} (Merck) plates (0.25 mm thickness) with CH_2Cl_2-MeOH (95:5, v/v) as developing solvent.

Synthesis of 5′-*O*-acylnucleosides

General procedure. A sample of 2′-deoxyadenosine or 2-chloro-2′-deoxyadenosine and of corresponding carboxylic acid (3 mmol each) was twice evaporated with pyridine (× 20 ml) and suspended in dry pyridine (25 ml for 2′-deoxyadenosine or 20 ml for 2-chloro-2′-deoxyadenosine) at room temperature. To the stirred reaction mixture triphenylphosphine (1.05 g, 4 mmol) and diisopropyl azodicarboxylate (810 mg, 4 mmol) were added portionwise. This was stirred additionally for 30 min and evaporated to an oil, and three times coevaporated with toluene to remove the rest of pyridine. The residue was applied on a silica gel 60 column (3 × 25 cm) and chromatographed with CH_2Cl_2 (300 ml), then with CH_2Cl_2-MeOH (95:5). The product-containing fractions were evaporated to a solid foam.

5′-O-*phenylacetyl-2′-deoxyadenosine* (**2a**): From 2′-deoxyadenosine (**1a**) and phenylacetic acid. Yield: (24%). UV (50% MeOH): 259 (12300). ^{1}H NMR (D_6-Me_2SO): 2.32 and 2.80 (2m, H-2′ and H-2′′), 3.62 (m, H-CH_3), 4.02 (m, H-4′), 4.18 and 4.30 (2m, H-5′ and H-5′′), 4.45 (m, H-3′), 5.48 (bs, HO-3′), 6.38 (t, H-1′), 7.10–7.20 (m, H-phenyl and NH_2), 8.15 and 8.26 (2s, H-2 and H-8). TLC: Rf 0.16. Elemental analysis: calculated for $C_{18}H_{19}N_5O_4$ (369.38): C, 58.53; H, 5.18; N, 18.96. Found: C, 58.69; H, 5.27; N, 18.83.

5′-O-*(*p-*bromophenylacetyl)-2′-deoxyadenosine* (**2b**): From **1a** and *p*-bromophenylacetic acid. Yield:19%. UV (50% MeOH): 230 (sh), (12000), 265 (11200). ^{1}H NMR (D_6-Me_2SO): 2.34 and 2.72 (2m, H-2′ and H-2′′), 3.65 (m, H-methylene), 4.00 (m, H-4′), 4.19 and 4.30 (2m, H-5′ and H-5′′), 4.48 (m, H-3′), 5.50 (bs, HO-3′), 6.34 (t, H-1′), 7.17 and 7.46 (2d, H-phenyl), 7.27 (bs, NH_2), 8.14 and 8.28 (2s, H-2 and H-8). TLC: Rf 0.16. Elemental analysis: calculated for $C_{18}H_{18}N_5BrO_4$ (448.28): C, 48.23; H, 4.05; N, 15.62. Found: C, 48.34; H, 4.16; N, 15.48.

5′-O-*(3-phenylbutyryl)-2′-deoxyadenosine* (**2c**): From **1a** and 3-phenylbutyric acid. Yield: 56%. UV (50% MeOH): 259 (13600). ^{1}H NMR (D_6-Me_2SO): 1.76, 2.28 and 2.54 (3m, H-methylene), 2.32 and 2.86 (2m, H-2′ and H-2′′), 4.00 (m, H-4′), 4.16 and 4.28 (2m, H-5′ and H-5′′), 4.48 (m, H-3′), 5.48 (bs, HO-3′), 6.34 (t, H-1′), 7.10–7.30 (m, H-phenyl and NH_2) 8.13 and 8.28 (H-2 and H-8); TLC: Rf 0.18. Elemental analysis: calculated for $C_{20}H_{23}N_5O_4$ (397.44): C, 60.44; H, 5.83; N, 17.62. Found: C, 60.57; H, 5.96; N, 17.45.

5′-O-*(6-phenylhexanoyl)-2′-deoxyadenosine* (**2d**): From 1a and 6-phenylhexanoic acid. Yield: (45%). UV (50% MeOH): 259 (13600). ^{1}H NMR (D_6-Me_2SO): 1.23, 1.50, 2.26 and 2.50 (4m, H-methylene), 2.32 and 2.85 (2m, H-2′ and H-2′′), 3.98 (m, H-4′), 4.14 and 4.28 (2m, H-5′ and H-5′′), 4.48 (m, H-3′), 5.47 (bs, HO-3′), 6.35 (t, H-1′), 7.10–7.30 (m, H-phenyl and NH_2), 8.13 and 8.28 (2s, H-2 and H-8). TLC: Rf 0.20. Elemental analysis: calculated for $C_{22}H_{27}N_5O_4$ (425.49): C, 62.10; H, 6.40; N, 16.46. Found: C, 61.93; H, 6.53; N, 16.33.

5′-O-*(all-*trans-*retinoil)-2′-deoxyadenosine* (**2e**): From **1a** and all-*trans*-retinoic acid. Yield: (38%). UV (50% MeOH): 259 (16800), 365 (35400). ^{1}H NMR (D_6-Me_2SO): 0.8–2.90 (m, H-CH_3, H-cyclohexane and H-2′), 4.03 (m, H-4′), 4.18 and 4.31 (2m, H-5′ and H-5′′), 4.50 (H-3′), 5.48 (bs, HO-3′), 5.80–6.45 (m, H-olefinic and H-1′), 7.06 (m, H-olefinic), 7.80 (bs, NH_2), 8.13 and 8.28 (2s, H-2 and H-8). TLC: Rf 0.25. Elemental analysis: calculated for $C_{29}H_{36}N_5O_4$ (518.64): C, 67.16; H, 7.00; N, 13.50. Found: C, 67.29; H, 7.13; N, 13.35.

5′-O-*(13-*cis-*retinoil)-2′-deoxyadenosine* (**2f**): From **1a** and 13-*cis*-retinoic acid. Yield: (31%). UV (50% MeOH): 260 (19600), 360 (16500). ^{1}H NMR (D_6-Me_2SO): 1.0–2.90 (m, H-CH_3,

H-cyclohexane and H-2′), 4.05 (m, H-4′), 4.18 and 4.33 (2m, H-5′ and H-5′′), 4.53 (m, H-3′), 5.46 (bs, HO-3′), 6.20–6.40 (m, H-olefinic and H-1′), 7.06 (m, H-olefinic),7.63 (d, H-olefinic), 7.75 (bs, NH_2), 8.22 and 8.38 (2s, H-2 and H-8). TLC: Rf 0.28. Elemental analysis: calculated for $C_{29}H_{36}N_5O_4$ (518.64): C, 67.16; H, 7.00; N, 13.50. Found: C, 67.31; H, 7.15; N, 13.39.

5′-O-phenylacetyl-2-chloro-2′-deoxyadenosine (**2g**): From **1b** and phenylacetic acid. Yield: (22%). UV (50% MeOH): 264 (11800). ^{1}H NMR (D_6-Me_2SO): 2.33 and 2.70 (2m, H-2′ and H-2′′), 3.63 (m, H-methylene), 4.00 (m, H-4′), 4.18 and 4.30 (2m, H-5′ and H-5′′), 4.42 (m, H-3′), 5.50 (bs, HO-3′), 6.38 (t, H-1′), 7.10–7.20 (m, H-phenyl), 7.28 (bs, NH_2), 8.28 (s, H-8). TLC: Rf 0.22. Elemental analysis: calculated for $C_{18}H_{18}N_5ClO_4$ (403.83): C, 53.54; H, 4.49; N, 17.34. Found: C, 53.44; H, 4.44; N, 17.45.

*5′-O-(*p*-bromophenylacetyl)-2-chloro-2′-deoxyadenosine* (**2h**): From **1b** and *p*-bromophenylacetic acid. Yield: (21%). UV (50% MeOH): 229 (12000), 256 (12300). ^{1}H NMR (D_6-Me_2SO): 2.34 and 2.72 (2m, H-2′ and H-2′′), 3.64 (m, H-methylene), 4.00 (m, H-4′), 4.18 and 4.28 (2m, H-5′ and H-5′′), 4.46 (m, H-3′), 5.50 (bs, HO-3′), 6.27 (t, H-1′), 7.18 and 7.44 (2d, H-phenyl) 7.82 (bs, NH_2), 8.28 (s, H-8). TLC: Rf 0.24. Elemental analysis: calculated for $C_{18}H_{17}BrClN_5O_4$ (482.73): C, 44.84; H, 3.55; N, 14.51. Found: C, 44.90; H, 3.66; N, 14.38.

5′-O-(3-phenylbutyryl)-2-chloro-2′-deoxyadenosine (**2i**): From **1b** and 3-phenylbutyric acid. Yield: (47%). UV (50% MeOH): 264 (12500). ^{1}H NMR (D_6-Me_2SO): 1.78, 2.20 and 2.60 (3m, H-methylene), 2.35 and 2.78 (2m, H-2′ and H-2′′), 4.00 (m, H-4′), 4.16 and 4.28 (2m, H-5′ and H-5′′), 4.45 (m, H-3′), 5.50 (bs, HO-3′), 6.27 (t, H-1′), 7.10–7.30 (m, H-phenyl), 7.81 (bs, NH_2), 8.31 (s, H-8). TLC: Rf 0.24. Elemental analysis: calculated for $C_{20}H_{22}ClN_5O_4$ (431.88): C, 55.62; H, 5.13; N, 16.22. Found; C, 55.77; H, 5.24; N, 16,03.

5′-O-(6-phenylhexanoyl)-2-chloro-2′-deoxyadenosine (**2j**): From **1b** and 6-phenylhexanoic acid. Yield: (46%). UV (50% MeOH): 264 (13200). ^{1}H NMR (D_6-Me_2SO): 1.20, 1.50, 2.24 and 2.58 (4m, H-methylene), 2.36 and 2.75 (2m, H-2′ and H- 2′′), 3.99 (m, H-4′), 4.14 and 4.25 (2m, H-5′ and H-5′′), 4.45 (m, H-3′), 5.50 (bs, HO-3′), 6.27 (t, H-1′), 7.10–7.30 (m, H-phenyl), 7.80 (bs, NH_2), 8.30 (s, H-8). TLC: Rf 0.26. Elemental analysis: calculated for $C_{22}H_{26}ClN_5O_4$ (459.938): C, 57.45; H, 5.70; N, 15.23. Found: C, 57.60; H, 5.83; N, 15.04.

*5′-O-(all-*trans*-retinoil)-2-chloro-2′-deoxyadenosine* (**2k**): Yield: (32%). UV (50% MeOH): 263 (17600), 354 (17300). ^{1}H NMR (D_6-Me_2SO): 0.85–2.80 (m, H-CH_3, H-cyclohexane and H-2′), 4.03 (m, H-4′), 4.17 and 4.30 (2m, H-5′ and H-5′′), 4.48 (H-3′), 5.48 (bs, HO-3′), 5.78–6.45 (m, H-olefinic and H-1′), 7.05 (m, H-olefinic), 7.80 (bs, NH_2), 8.31 (s, H-8); TLC: Rf 0.32. Elemental analysis: calculated for $C_{29}H_{35}ClN_5O_4$ (553.09): C, 62.98; H, 6.38; N, 12.66. Found: C, 62.88; H, 6.31; N, 12.50.

*5′-O-(13-*cis*-retinoil)-2-chloro-2′-deoxyadenosine* (**2l**): From **1b** and 13-*cis*-retinoic acid. Yield: (27%). UV (50% MeOH): 265 (18800), 360 (23000). ^{1}H NMR (D_6-Me_2SO): 1.0–2.85 (m, H-CH_3, H-cyclohexane and H-2′), 4.02 (m, H 4′), 4.17 and 4.32 (2m, H-5′ and H-5′′), 4.48 (m, H-3′), 5.62 (bs, HO-3′), 6.18–6.34 (m, H-olefinic and H-1′), 7.06 (m, H-olefinic),7.65 (d, H-olefinic), 7.82 (bs, NH_2), 8.30 (s, H-8); TLC: Rf 0.33. Elemental analysis: calculated for $C_{29}H_{35}ClN_5O_4$ (553.09): C, 62.98; H, 6.38; N, 12.66. Found: C, 62.83; H, 6.30; N, 12.49.

Stability and enzymatic hydrolysis of dA- and 2CdA-5′-esters. Blood was collected from young healthy male human volunteers using heparin as an anticoagulant. Plasma was isolated by centrifugation (1200 × ***g*** for 10 min). Lymphocytes were isolated from 3 ml aliquots of blood using Lymphoprep (Nycomed Pharma AS) and the procedure recommended by the manufacturer. The isolated lymphocytes (volume about 10 μl) were frozen and thawed thrice, and cellular debris was removed from the lysate by centrifugation.

The esters **2a–2l** were dissolved in Me_2SO and added (1:9) to: a) deionized water; b) water suspension of porcine liver esterase (2 units/ml); c) human blood plasma; and d) human lymphocyte lysate diluted 2000 times. Reaction mixtures were then incubated at 37°C for several hours, and samples for the assay of released nucleosides were taken every hour. Free nucleosides (dA or 2CdA) were assayed by HPLC using a Merck Hitachi LaChrom chromatographic system, a Supelcosil C18 column, and 35 mM phosphate buffer (pH = 3) containing 9% acetonitrile as a mobile phase (injection volume 20 μl, flow rate 1 ml/min, detection at 264 nm).

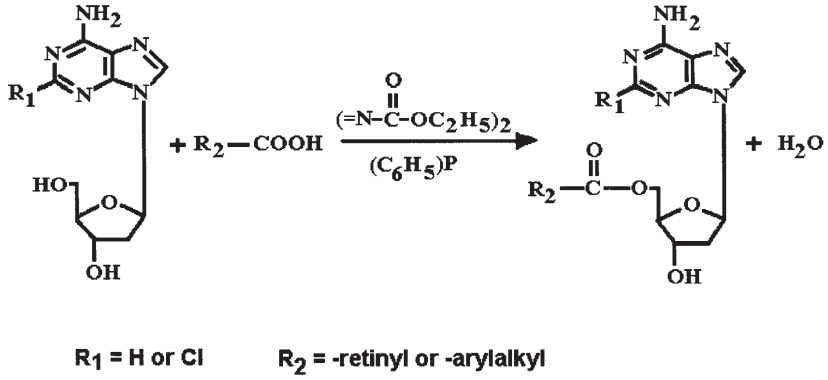

Figure 3. Scheme of the Mitsunobu reaction used to synthesize the esters of dA and 2CdA.

RESULTS

Chemical synthesis

Mitsunobu reaction is frequently employed for various organic reactions. Gentle reaction conditions (triphenylphosphine and dialkyldiazacarboxylate at room temperature) are very useful for chemical modification of biologically active compounds ([15], and references therein). It has been found that carboxylic acids specifically substitute 5′-hydroxyl group of ribo- and deoxyribonucleosides under the above mentioned conditions [15]. In this report we used Mitsunobu reaction for the synthesis of various 5′-O-acyl derivatives of 2′-deoxyadenosine and its structural analogue 2-chloro-2′-deoxyadenosine (Fig. 3). Due to the poor solubility of purine nucleosides 2′-deoxyadenosine (**1a**) and 2-chloro-2′-deoxyadenosine (**1b**) in diethyl ether or tetrahydrofurane which are usually used as solvents for Mitsunobu reaction, we applied pyridine as the reaction medium. According to the results of analytical TLC, single reaction products were formed. The yields of 5′-acylated nucleosides **2a–l** were lower than reported for acylation of alcohols in etheral solvents. The new nucleoside derivatives isolated by column flash chromatography were characterized by ^{1}H-NMR and elemental analysis. The presence of only 3′-OH signal in ^{1}H-NMR spectra of the reaction products isolated indicated the substitution of the primary 5′-hydroxyl group.

Susceptibility to enzymatic hydrolysis

In the absence of esterase activity no release of free nucleosides was observed from any of the esters. In the presence of esterases there was also no release of the nucleosides from the esters of dA and 2CdA with PA, o-Br-PA and PB (data not shown). However, continuous release of free dA or 2CdA in the presence of porcine esterase, blood plasma, or diluted human lymphocyte lysate were noted from the esters containing ATRA and 13-*cis*-retinoate. As an example, data concerning the release of 2CdA from the ATRA-5′-O-2CdA ester are shown (Fig. 4).

DISCUSSION

5′-Esters of dA and 2CdA with carboxyacidic differentiation inducers were designed for a targetted delivery of these cell differentiating compounds to lymphoid cells. The concept of targetted delivery is summarized in Fig. 2. Being much more lipophilic than the parent nucleosides, the esters should easily penetrate cell membranes and in the intracellular environment they may undergo esterase-catalyzed hydrolysis to the parent compounds. In lymphoid cells dA should be efficiently deaminated and removed, while 2CdA should be phosphorylated and concentrated several hundred-fold. In either case free carboxyacidic differentiating agent should concurrently be concentrated selectively in lymphoid cells.

A concept of lipophilic nucleoside 5′-esters with various carboxylic acids (amino acids, aliphatic acids, polyunsaturated fatty acids, and others) has been exploited by several groups for construction of prodrugs of 3′-azido-2′,3′-dideoxythymidine (AZT) (reviewed in [16]). Among them the 5′-ester of AZT with ATRA has been synthesized [17]. Lipophilic 5′-esters were expected to be preferentially deposited in cellular membranes providing for sustained intracellular release of AZT. Furthermore, some carboxylic acids used to esterify AZT display their own antiretroviral activity and their hydrolysis would release two active species with different target sites. This may result in improved antiviral efficacy and better tolerance, and may also retard the development of resistance. Interestingly, AZT-5′-ATRA improved cellular uptake of AZT *in vitro* four-fold. Furthermore, it was more cytotoxic than free AZT, which was attributed to the effect of ATRA released intracellularly [17].

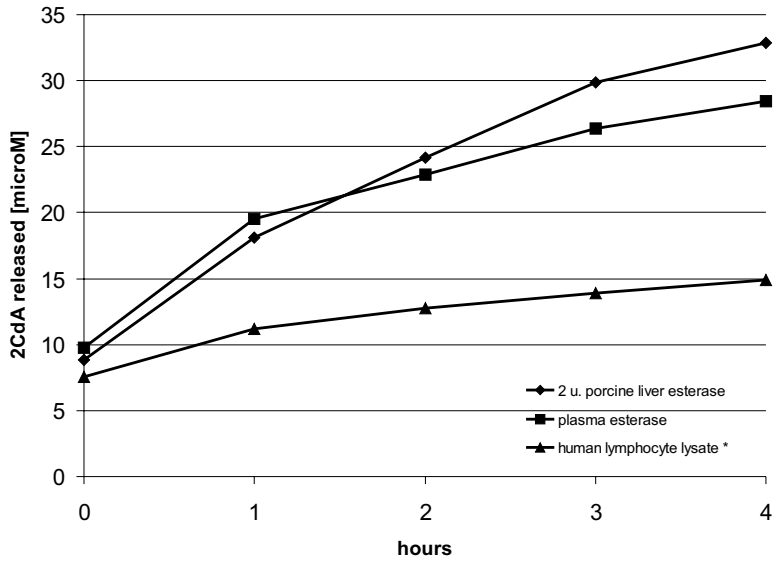

Figure 4. The kinetics of release of 2CdA from 2CdA-5′-ATRA in the presence of esterases from various sources.

Note that the human lymphocyte lysate was obtained from 3 ml of blood and diluted 2000 times. Therefore the esterase activity inside the lymphocytes is supposedly several thousand times higher than in the plasma.

In close analogy to the AZT-5′carboxylic acid esters, a prerequisite for the dA- or 2CdA-5′-esters-mediated targetted delivery of a differentiating agent to lymphoid cells is that the esters undergo intracellular hydrolysis to release the parent compounds. Since all these esters are stable in aqueous environment, esterase-mediated hydrolysis is required. The esters of dA and 2CdA with PA, o-Br-PA and PB were found totally resistant to esterase-mediated hydrolysis, therefore no pharmacological activity of these compounds should be expected. On the other hand, the esters with ATRA and 13-*cis*-retinoil were hydrolyzed by a commercially available porcine liver esterase, as well as by blood plasma and diluted lysates

of human lymphocytes. In agreement with these observations, experiments with leukemic cell cultures *in vitro* (which will be reported elsewhere) showed that the esters with PA, o-Br-PA and PB display neither cytotoxic nor cell-differentiating activity, whereas dA ester with ATRA was able to differentiate leukemic cells, and 2CdA ester with ATRA displayed both cytotoxic and cell-differentiating activity.

Targetted delivery of retinols to lymphatic tissues using their dA-5′-esters may help to reduce dangerous and sometimes fatal systemic retinoil toxicity known as "retinoic acid syndrome" [18, 19].

Although preliminary experiments suggest that under *in vitro* conditions the 2CdA ester with ATRA is on a molar basis less cytotoxic toward leukemia cell lines than the free 2CdA, this may not be predictive for its *in vivo* effects. Recently a critical role of angiogenesis in several hematological malignancies has been suggested, possibly mediated through the production by leukemic cells of vascular endothelial growth factor (VEGF). The production of VEGF may be abrogated by ATRA (see [20] and references therein). Concurrent sustained delivery of 2CdA and ATRA to leukemic cells by the 2CdA-5′-O-ATRA ester could produce a combination of cytotoxic and antiangiogenic effects.

Considering the above, assessment of possible therapeutic potential of dA- and 2CdA esters with ATRA and other retinoic acids is indicated.

REFERENCES

1. Newmark, H.L. & Young, C.W. (1995) Butyrate and phenylacetate as differentiating agents: Practical problems and opportunities. *J. Cell. Biochem.* **22** (Suppl.), 247–253.

2. Liu, L., Shack, S., Stetler-Stevenson, W.G., Hudgins, W.R. & Samid, D. (1994) Differentiation of cultured human melanoma cells induced by the aromatic fatty acids phenylacetate and phenylbutyrate. *J. Invest. Dermatol.* **103**, 335–340.

3. Rohwedel, J., Guan, K. & Wobus, A.M. (1999) Induction of cellular differentiation by retinoic acid *in vitro*. *Cells Tissues Organs* **165**, 190–202.

4. Hudgins, W.R., Shack., S., Myers, C.E. & Samid, D. (1995) Cytostatic activity of phenylacetate and derivatives against tumor cells. Correlation with lipophilicity and inhibition of protein prenylation. *Biochem. Pharmacol.* **50**, 1273–1279.

5. Miller, W.H., Jr. (1998) The emerging role of retinoids and retinoic acid metabolism blocking agents in the treatment of cancer. *Cancer* **83**, 1471–1482.

6. Gore, S.D., Samid, D. & Weng, L.J. (1997) Impact of the putative differentiating agents sodium phenylbutyrate and sodium phenylacetate on proliferation, differentiation, and apoptosis of primary neoplastic myeloid cells. *Clin. Cancer Res.* **3**, 1755–1762.

7. Witzig, T.E., Timm, M., Stenson, M., Svingen, P.A. & Kaufmann, S.H. (2000) Induction of apoptosis in malignant B cells by phenylbutyrate or phenylacetate in combination with chemotherapeutic agents. *Clin. Cancer Res.* **6**, 681–692.

8. Huang, Y. & Waxman, S. (1998) Enhanced growth inhibition and differentiation of fluorodeoxyuridine-treated human colon carcinoma cells by phenylbutyrate. *Clin. Cancer Res.* **4**, 2503–2509.

9. Lengfelder, E., Reichert, A., Schoch, C., Haase, D., Haferlach, T., Loffler, H., Staib, P., Heyll, A., Seifarth, W., Saussele, S., Fonatsch, C., Gassmann, W., Ludwig, W.D., Hochhaus, A., Beelen, D., Aul, C., Sauerland, M.C., Heinecke, A., Hehlmann, R., Wormann, B., Hiddemann, W. & Buchner, T. (2000) Double induction strategy including high dose cytarabine in combination with *all-trans* retinoic acid: Effects in patients with newly di-

agnosed acute promyelocytic leukemia. German AML Cooperative Group. *Leukemia* **14**, 1362–1370.

10. Venditti, A., Tamburini, A., Buccisano, F., Scimo, M.T., Del Poeta, G., Maurillo, L., Cox, M.C., Abruzzese, E., Tribalto, M., Masi, M. & Amadori, S. (2000) A phase-II trial of *all trans* retinoic acid and low-dose cytosine arabinoside for the treatment of high-risk myelodysplastic syndromes. *Ann. Hematol.* **79**, 138–142.

11. Niitsu, N. & Honma, Y. (1999) Adenosine analogs as possible differentiation-inducing agents against acute myeloid leukemia. *Leuk. Lymphoma* **34**, 261–271.

12. Eriksson, S., Arner, E., Spasokoukotskaja, T., Wang, L., Karlsson, A., Brosjo, O., Gunven, P., Juliusson, G. & Liljemark, J. (1994) Properties and levels of deoxynucleoside kinases in normal and tumor cells: Implications for chemotherapy. *Adv. Enzyme Regul.* **34**, 13–25.

13. Siaw, M.F., Mitchell, B.S., Koller, C.A., Coleman, M.S. & Hutton, J.J. (1980) ATP depletion as a consequence of adenosine deaminase inhibition in man. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6157–6161.

14. Liliemark, J. (1997) The clinical pharmacokinetics of cladribine. *Clin. Pharmacokinet.* **32**, 120–131.

15. Mitsunobu, O. (1981) The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* 1–28.

16. Parang, K., Wiebe, L.I. & Knaus, E.E. (2000) Novel approaches for designing 5′-O-ester prodrugs of 3′-azido-2′,3′-dideoxythymidine. *Curr. Med. Chem.* **7**, 995–1039.

17. Aggarwal, S.K., Gogu, S.R., Rangan, S.R. & Agrawal, K.C. (1990) Synthesis and biological evaluation of prodrugs of zidovudine. *J. Med. Chem.* **33**, 1505–1510.

18. Frankel, S.R., Eardley, A., Lauwers, G., Weiss, M. & Warrell, R.P., Jr. (1992) The "retinoic acid syndrome" in acute promyelocytic leukemia. *Ann. Intern. Med.* **117**, 292–296.

19. Vahdat, L., Maslak, P., Miller, W.H., Jr., Eardley, A., Heller, G., Scheinberg, D.A. & Warrell, R.P., Jr. (1994) Early mortality and the retinoic acid syndrome in acute promyelocytic leukemia: Impact of leukocytosis, low-dose chemotherapy, PMN/RAR-alpha isoform, and CD13 expression in patients treated with *all-trans* retinoic acid. *Blood* **84**, 3843–3849.

20. Kini, A.R., Peterson, L.C., Tallman, M.S. & Lingen, M.W. (2001) Angiogenesis in acute promyelocytic leukemia: Induction by vascular endothelial growth factor and inhibition by all-*trans*-retinoic acid. *Blood* **97**, 3919–3924.