Synthesis of a Novel Ring-Expanded ("Fat") Nucleotide Analogue of Phosphonomethoxyethylguanine (PMEG) Containing the Imidazo[4,5-*e*][1,3]diazepine Ring System

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The synthesis of 6-amino-4,5-dihydro-8*H*-1-(2-phosphonylmethoxyethyl)imidazo[4,5-*e*][1,3]diazepine-4,8-dione (**7**), a novel ring-expanded ("fat") acyclic nucleotide analogue of phosphonomethoxyethylguanine (PMEG), has been reported. It was prepared in 4 steps in 51% overall yield starting from dimethyl imidazole-4,5-dicarboxylate.

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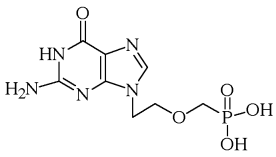
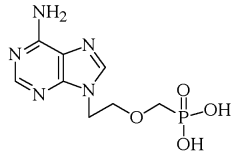
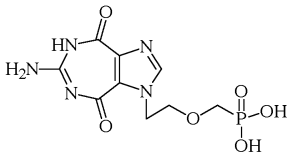
PMEG (**1**) [9-((2-phosphonomethoxy)ethyl)guanine] [1,2] and its adenine counterpart PMEA (**2**) [3,4] are two acyclic nucleoside phosphonate (nucleotide) analogues of purine that have been reported to possess broad spectrum antiviral and/or antitumor properties [4-6]. We report here the synthesis of a novel ring-expanded ("fat") analogue of PMEG (*f*PMEG), 6-amino-4,5-dihydro-8*H*-1-(2-phosphonylmethoxyethyl)imidazo[4,5-*e*]-[1,3]diazepine-4,8-dione (**7**).



1 (PMEG)



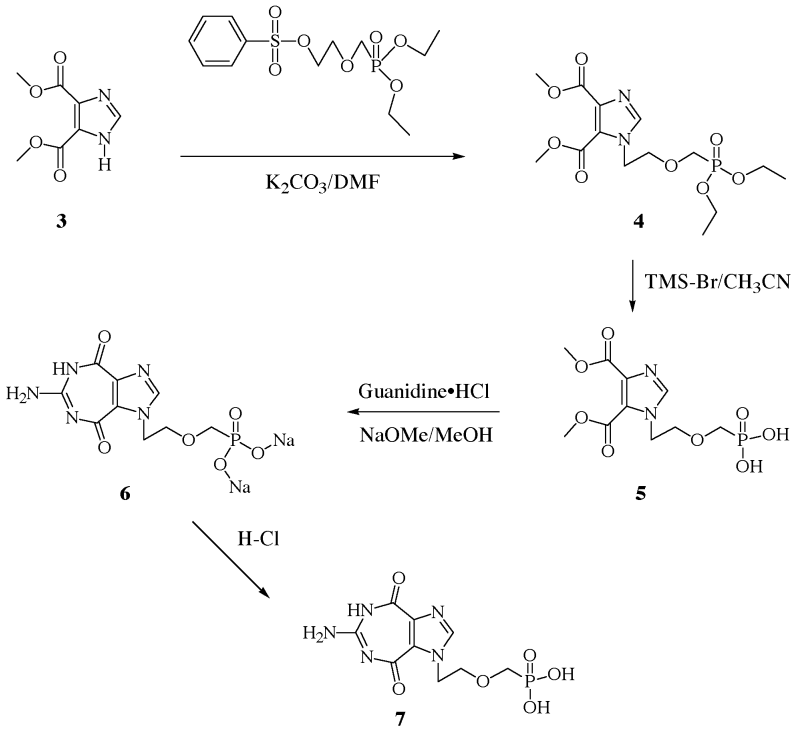
2 (PMEA)



7 (*f* PMEG)

The synthesis of target **7** (Scheme 1) commenced with the condensation of dimethyl 4,5-imidazoledicarboxylate (**3**) [7,8] with diethyl 2-*p*-toluenesulfonyloxyethoxy-methanephosphonate [9] in the presence of potassium

Scheme 1



3 **4** **5** **6** **7**

carbonate in *N*,*N*-dimethylformamide (DMF). The purity of the phosphonate reagent was crucial for the success of this reaction as the impure reagent gave intractable material containing several byproducts, from which the isolation and purification of the desired product was difficult. The product, dimethyl 1-(2-diethoxyphosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (**4**) was obtained in 65% yield. The phosphonyldiethoxy groups of **4** were selectively deprotected over its ester methoxy groups at 4,5-positions by reaction with bromotrimethylsilane in anhydrous acetonitrile at room temperature to obtain the corresponding phosphonic acid **5** in 84% yield. Compound **5** was condensed with guanidine hydrochloride at room temperature in a solution of sodium methoxide in anhydrous methanol to obtain the disodium salt **6** of the target compound in 93% yield. Because of its insolubility in methanol, it could be easily isolated by simple filtration. The salt was quantitatively converted to the target free acid **7** by neutralization with 2 *N* hydrochloric acid. The ^{1}H and ^{13}C NMR as well as elemental microanalytical and mass spectral data of **7** are consistent with the assigned structure.

EXPERIMENTAL

The ^{1}H and ^{13}C nmr spectra were recorded on a General Electric QE-300 nmr spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. The data are reported in the following format: Chemical shift (all relative to Me_4Si), multiplicity (s= singlet, d=doublet, dt=double triplet, dd=double doublet, t=triplet, q=quartet, m=multiplet, br=broad, coupling constants, integration and assignment). Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. Evaporations were done under reduced pressure on a rotary evaporator. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} (0.2 mm thickness). Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Dry solvents were prepared as follows: methanol was distilled from calcium hydride and was stored over molecular sieves (type 3Å); methylene chloride was distilled from calcium hydride and was stored over molecular sieves (type 3Å); *N*,*N*-dimethylformamide (DMF) was dried over calcium oxide and then distilled under reduced pressure from calcium hydride, and was subsequently stored over molecular sieves (type 3Å). All starting materials were purchased from Aldrich Chemical Co. All solvents were reagent grade and were purchased from VWR Scientific. All yields reported are for dry compounds that require no further purification for use in other reactions.

Dimethyl 1-(2-Diethoxyphosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (**4**).

A mixture of dimethyl imidazole-4,5-dicarboxylate (**3**) [7,8] (1.0 g, 5.4 mmoles) and potassium carbonate (0.75 g, 5.4 mmoles) in 40 ml of anhydrous DMF was stirred at 100 °C for 3 hours. A solution of diethyl *2-p*-toluenesulfonyloxyethoxymethanephosphonate [9] (2.75 g, 7.5 mmoles) in 5 ml of dry dimethylformamide was added, and the mixture was continued to stir for 48 hours at 100 °C. The reaction mixture was evaporated *in vacuo*. The residue was dissolved in chloroform and successively washed with 0.1 *M* hydrochloric acid and water. After drying over anhydrous magnesium sulfate, the chloroform solution was filtered and evaporated to dryness *in vacuo*. The resulting residue was purified by flash chromatography over silica gel, eluting with chloroform to afford **4** as a colorless oil (1.33 g, 65%), R_f 0.74 [chloroform:methanol (10:1)]; ^{1}H nmr (deuteriochloroform): δ 7.67 (s, 1H, imidazole), 4.44 (t, 2H, J=4.8 Hz, H-1'), 4.10 (dq, 4H, $J_{P\text{-}OCH2}$=8.1 Hz, J=6.9 Hz, CH_2CH_3), 3.92 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.88 (t, 2H, J=4.8 Hz, H-2'), 3.75 (d, 2H, $J_{P\text{-}CH}$=8.1 Hz, OCH_2P), 1.31 (t, 6H, J=6.9 Hz, CH_2CH_3); ^{13}C nmr (deuteriochloroform): δ 16.40 (s, CH_3), 16.48 (s, CH_3), 46.79 (s, C-1'), 52.17 (s, OCH_3), 52.40 (s, OCH_3), 62.33 (s, OCH_2CH_3), 62.41 (s, OCH_2CH_3), 65.17 (d, $J_{P,C}$=166.4 Hz, OCH_2P), 71.50 (d, $J_{P,C\text{-}2'}$=9.9 Hz, C-2'), 137.08 (C-4 or 5), 139.81 (C-5 or 4), 140.63 (C-2), 160.44 (C=O), 162.74 (C=O); ms (EI): m/z 379 (M+1), 378 (M^+), 363 (M-15), 347 (M-31), 333 (M-45), 319, 287, 257, 241, 225, 211 (100%), 193, 179, 165, 153, 139, 125, 95, 72; ms: (FAB) m/z 379 [MH^+]; hrms (FAB): Calcd for $C_{14}H_{24}N_2O_8P$: 379.1270; Found 379.1268.

Anal. Calcd. for $C_{14}H_{23}N_2O_8P\bullet H_2O$: C, 42.64; H, 6.39; N, 7.10. Found: C, 42.29; H, 6.26; N, 6.84.

Dimethyl 1-(2-Phosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (**5**).

A solution of dimethyl 1-(2-diethoxyphosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (**4**) (0.48 g, 1.3 mmoles) in 10 ml of dry acetonitrile was added bromotrimethylsilane (0.40 g, 2.6 mmoles), and the mixture was stirred for 24 hours at room temperature, when a tlc showed the reaction was complete. The reaction mixture was evaporated to dryness, and the residue was codistilled with acetonitrile (3 x 20 ml), mixed with water (30 ml), and the pH was adjusted to 8 with triethylamine. The mixture was allowed to stand for 1 hour, evaporated to dryness *in vacuo*, and the residue was codistilled with methanol (2 x 20 ml). It was purified by flash chromatography over silica gel, eluteing successively with chloroform and chloroform - methanol (10:1). The appropriate UV absorbing fractions were pooled and evaporated to afford pure **5** as a colorless syrup (350 mg, 84%), R_f 0.13 [chloroform:methanol:30% ammonium hydroxide (2:1:0.3)]; ^{1}H nmr (deuteriodimethylsulfoxide): δ 7.98 (s, 1H, imidazole), 5.64 (br s, 2H, OH, exchangeable with D_2O), 4.34 (t, 2H, J=4.8 Hz, H-1'), 3.81 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.77 (t, 2H, J=4.8 Hz, H-2'), 3.55 (d, 2H, $J_{P\text{-}CH2}$=8.4 Hz, OCH_2P); ^{13}C nmr (deuteriodimethylsulfoxide): δ 46.41 (s, C-1'), 52.51 (s, OCH_3), 53.07 (s, OCH_3), 66.36 (d, $J_{P,C}$=160.2 Hz, OCH_2P), 71.09 (d, $J_{P,C\text{-}2'}$=10.2 Hz, C-2'), 135.32 (C-4 or 5), 141.43 (C-5 or 4), 141.43 (C-2), 160.45 (C=O), 162.95 (C=O); ms: (FAB) m/z 323 [MH^+]; hrms (FAB): Calcd for $C_{10}H_{16}N_2O_8P$: 323.0644; Found 323.0649.

6-Amino-4,5-dihydro-8*H*-1-(2-disodiophosphonylmethoxyethyl)imidazo[4,5-*e*][1,3]diazepine-4,8-dione (**6**).

A solution of dimethyl 1-(2-phosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (**5**) (0.32 g, 1 mmole) and guanidine hydrochloride (0.48 g, 5 mmoles) in 50 ml of absolute methanol was stirred for 30 minutes at room temperature. Then 2 ml of 25% (w/v) solution of sodium methoxide in methanol was added. The resulting mixture was stirred for 48 hours at room

temperature. The precipitate that formed was filtered and washed with anhydrous methanol to give **6** as a white solid (335 mg, 93%), mp >250 °C; ^{1}H nmr (deuteriodimethylsulfoxide + deuterium oxide): δ 8.17 (s, 1H, imidazole), 4.55 (t, 2H, J=4.2 Hz, H-1'), 3.76 (t, 2H, J=4.2 Hz, H-2'), 3.56 (d, 2H, $J_{P\text{-}CH2}$=9.0 Hz, OCH_2P); ^{13}C nmr (deuteriodimethylsulfoxide + deuterium oxide): δ 46.77 (s, C-1'), 66.34 (d, $J_{P,C}$=160.2 Hz, OCH_2P), 70.62 (d, $J_{P,C\text{-}2'}$=10.0 Hz, C-2'), 144.99 (C-2, 3a, 4, 8, 8a), 149.97 (C-6); ms: (FAB) m/z 362 [MH^+], 340, 318; hrms (FAB): Calcd for $C_9H_{11}N_5O_6Na_2P$: 362.0243; Found 362.0240.

6-Amino-4,5-dihydro-8*H*-1-(2-phosphonylmethoxyethyl)-imidazo[4,5-*e*][1,3]diazepine-4,8-dione (**7**).

The above disodium salt (**6**) was dissolved in water and neutralized with 2 *N* hydrochloric acid to give free acid of **7** as a white solid after filtration and washing with water, mp >250 °C; ^{1}H nmr (deuteriodimethylsulfoxide): δ 10.65 (br s, 1H, NH, exchangeable with D_2O), 8.04 (s, 1H, imidazole), 7.44 (br s, 1H, NH, exchangeable with D_2O), 6.61 (br s, 1H, NH, exchangeable with D_2O), 4.53 (t, 2H, J=4.2 Hz, H-1'), 3.74 (t, 2H, J=4.2 Hz, H-2'), 3.52 (d, 2H, $J_{P\text{-}CH2}$=9.0 Hz, OCH_2P); ^{13}C nmr (deuteriodimethylsulfoxide): δ 46.44 (s, C-1'), 66.81 (d, $J_{P,C}$=161.4 Hz, OCH_2P), 71.10 (d, $J_{P,C\text{-}2'}$=10.1 Hz, C-2'), 144.20 (C-2, 3a, 4, 8, 8a), 149.99 (C-6); ms (FAB): m/z 318 [MH^+]; hrms (FAB): Calcd for $C_9H_{13}N_5O_6P$: 318.0604; Found 318.0598.

Anal. Calcd. for $C_9H_{12}N_5O_6P$: C, 34.08; H, 3.81; N, 22.08. Found: C, 34.18; H, 3.91; N, 22.21.

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