Pergamon

Tetrahedron Letters 40 (1999) 607–610

TETRAHEDRON LETTERS

Bis(*N,N*-Dimethylcarbamoyloxymethyl) 2', 3'-dideoxyuridine 5'-monophosphate (DM_2-ddUMP): A Potential ddUMP Prodrug.

Saeed R. Khan and David Farquhar*

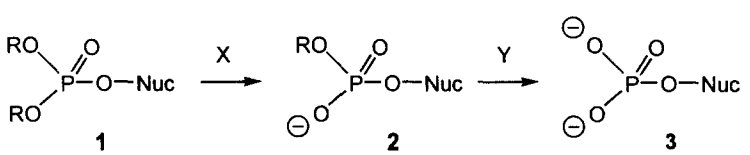
Department of Clinical Investigation, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Received 10 September 1998; accepted 11 October 1998

Abstract: Bis(*N,N*-dimethylcarbamoyloxymethyl) 2', 3'-dideoxyuridine 5'-monophosphate (DM_2-ddUMP) (**1a**) was synthesized as a potential membrane-permeable prodrug of ddUMP (**3**). Unlike the bis(pivaloyloxymethyl) analog, **1b**, **1a** was completely resistant to degradation by plasma enzymes - a property that should enhance its bioavailability.

5'-Mononucleotides (nucleoside 5'-monophosphates) play a key role in many biochemical pathways including the biosynthesis of nucleic acids. Unlike their nucleoside counterparts, 5'-mononucleotides possess limited potential as therapeutic agents. This is due to the fact that they are dianionic at physiologic pH and penetrate poorly into cells.[1,2] To overcome this limitation, we reported a series of neutral bis(pivaloyloxymethyl) [PIV_2] phosphotriesters, **1b**, as potential membrane-permeable prodrugs of the parent 5'-mononucleotides.[3-6] We showed that these compounds penetrated facilely into cells and reverted to the parent 5'-mononucleotides, **3**, after successive cleavage of the PIV groups by carboxylate esterases (to give **2b**) and phosphodiesterases, respectively, (Scheme 1).

Scheme 1



a, R = $(CH_3)_2NCOOCH_2$
b, R = $(CH_3)_3CCOOCH_2$

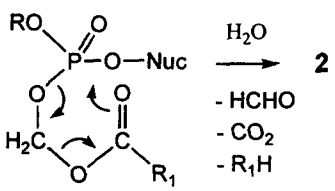
Nuc = Nucleosid-5'-yl
X = Carboxylate esterase
Y = Phosphodiesterase

Despite this success, a significant limitation of the PIV_2 prodrugs as potential clinical therapeutic agents is that they are rapidly degraded in human plasma and other tissues by carboxylate esterases[7]. To overcome this problem, we have investigated the potential of bis(*N,N*-dimethylcarbamoyloxymethyl) phosphotriesters, **1a**, as membrane permeable 5'-mononucleotide prodrugs. The rationale is that carbamates are likely to be far more resistant to hydrolytic cleavage by plasma enzymes than carboxylate esters. Neutral carbamoyloxymethyl phosphotriesters, on the other hand, should be more chemically labile than their acyloxymethyl counterparts. Thus,

0040-4039/99/$ - see front matter
PII: S0040-4039(98)02446-0

the rearrangement pathway depicted in Scheme 2 is expected to be more facile with **1a** than **1b** because of the greater nucleophilic character of the carbamoyl group compared to the acyl group. Once formed, the monoanionic intermediate, **2a**, should be fairly stable. In the presence of cellular phosphodiesterases, however, **2a** should be readily converted to the free 5'-mononucleotide, **3**.

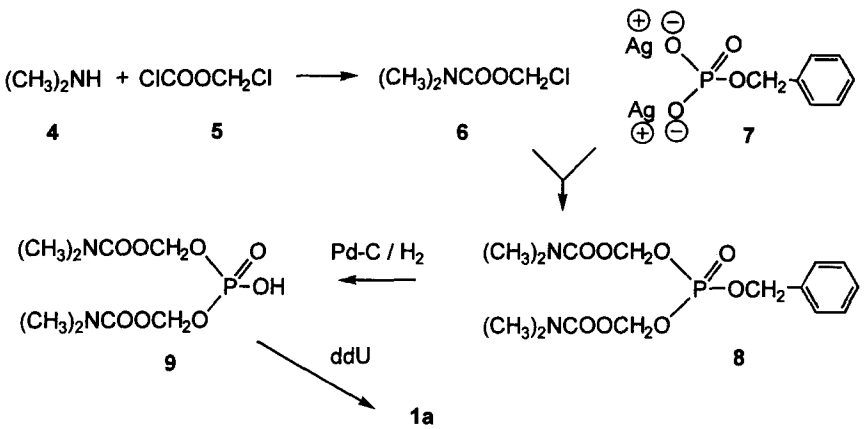
Scheme 2



R = $(CH_3)_2NCOOCH_2$
R' = $(CH_3)_2N$

To investigate the merits of this approach, we selected bis(*N,N*-dimethylcarbamoyloxymethyl) 2',3'-dideoxyuridine 5'-monophosphate (DM_2 ddUMP) (**1a**) as a model mononucleotide prodrug. In this communication, we report on the synthesis of **1a** (Scheme 3) and its stability in aqueous buffer and human plasma.

Scheme 3



A solution of dimethylamine (**4**) (20 g, 0.44 mole) in toluene (250 mL) was added dropwise over 30 min with vigorous stirring at 0 °C to chloromethylchloroformate (28.7 g, 20 mL, 0.22 mol). The mixture was stirred overnight at room temperature, then filtered to remove precipitated salts. The filtrate was concentrated under reduced pressure, and the residue was taken up in toluene (100 mL), and again filtered to remove additional precipitated salts. The filtrate was washed successively with 5% $NaHCO_3$ solution (50 mL) and H_2O (3 x 100 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure (30 mm Hg) at < 30 °C to give of *N*-dimethylcarbamoyloxymethyl chloride (**6**)[8] as a light yellow viscous liquid in 83 % yield. Since **6** was over 98% pure as evidenced by NMR, it was used in reaction without further purification. A solution of **6** (11.5 g, 0.083 mol) in dry toluene (100 mL), was added, dropwise, over 30 min to a suspension of finely divided disilver benzyl phosphate, **7**[3,6] (13.0 g, 0.033 mol) in toluene (50 mL). An exothermic reaction ensued. The

reaction mixture was refluxed with stirring for 24 h. The precipitated salt was filtered. The filtrate was washed successively with 5% $NaHCO_3$ solution (1 x 100 mL) and H_2O (3 x 50 mL), then dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded bis(*N,N*-dimethylcarbamoyloxymethyl) benzyl phosphate (**8**)[9] as a colorless, viscous oil (13.7 g, 89%). A solution of (**8**) (10.6 g, 0.03 mol) in cyclohexane (200 mL) was hydrogenated over 5% Pd/C (500 mg) at a pressure of 30 psi. After 1 h, an additional 500 mg of catalyst was added and hydrogenolysis was continued for a further 1 h. The catalyst was filtered and the solution was evaporated under reduced pressure to give bis(*N,N*-dimethylcarbamoyloxymethyl) hydrogen phosphate, **9**[10] as a colorless viscous oil. The yield was 7.3 g (68%). Reaction of **9** (297 mg, 0.99 mmol) with 2',3'-dideoxyuridine (140 mg, 0.66 mmol), triphenylphosphine (136 mg, 0.99 mmol) and diethylazodicarboxylate (0.16 mL, 0.99 mmol) in dimethylacetamide (0.5 mL) at 60 °C gave bis(*N,N*-dimethylaminocarbamoyloxymethyl) 2', 3'- dideoxyuridine 5'-monophosphate (**1a**)[11] as a viscous oil. Pure **1a** was obtained by silica column chromatography by using $CHCl_3$/MeOH (9:1) as eluent. Yield, 144 mg (45%).

Stability Studies of 1a and 1b.

When stirred at a concentration of 10^{-4} M in 0.05 M aqueous phosphate buffer solution,[12] pH 7.4, the half-life of **1a** was 4.5 h. The only product of reaction was DM_1-ddUMP (**2a**). Under similar conditions the half life of the PIV analog, **1b**, was 157 h. When incubated at the same concentration in human plasma,[13] the half life of **1a** was 4 h - nearly identical to that in buffer alone. By comparison, the half-life of the PIV analog **1b** in plasma was only 5 minutes. This finding supports the principle inherent in the design of **1a**, namely that the carbamate prodrugs are more unstable in solution than their acyl analogs but that the carbamoyloxymethyl groups are far more resistant to hydrolysis by plasma carboxylate esterases than their acyloxymethyl counterparts. In further studies we have shown that **2a** is a good substrate for phosphodiesterase I and is readily converted to the free 5'-mononucleotide, ddUMP. Further studies of bis(carbamoyloxymethyl) phosphotriesters as potential nucleotide prodrugs are underway and will be the subject of a future communication.

Acknowledgments. This work was supported by grant NIH CA 71527

References and Notes

1. Lieberman, K.C.; Heidelberger, C. *J. Biol. Chem.* **1955**, *316*, 823-830.
2. Roll, P.M; Weinfeld, H.; Carroll, E.; Brown, G.B. *J. Biol. Chem.* **1956**, *220*, 439-454.
3. Farquhar, D.; Srivastava, D. N.; Kuttesch, N. J.; Saunders, P. P. *J. Pharm. Sci.* **1983**, *72*, 324-325.
4. Khan, S.; Nowak, B.; Plunkett, W.; Farquhar, D. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 425.
5. Sastry, K. J.; Nehete, P. N.; Khan, S.; Plunkett, W.; Arlinhaus, R. B.; Farquhar, D. *Mol. Pharmacol.* **1992**, *41*, 441-445.
6. Farquhar, D.; Khan, S.; Srivastva D. N.; Saunders, P. P. *J. Med. Chem.* **1994**, *37*, 3902-3909
7. Khan, S.; Farquhar, D. Manuscript is in preparation.
8. ^{1}H NMR (IBM-Bruker Model NR/200; $CDCl_3$): δ 5.78 (s, 2 H, CH_2), 2.97 (s, 6 H, $N(CH_3)_2$. Anal. ($C_4H_8NO_2Cl$) C, H, N.

9. ^{1}H NMR ($CDCl_3$): δ 7.23 (s, 5 H, C_6H_5), 5.63 (d, 4 H, OCH_2O, J = 14 Hz), 5.10 (d, 2 H, $C_6H_5CH_2$, J = 8 Hz), 2.93 (s, 12 H, $(CH_3)_2$). MS: m/z 391 (MH^+). Anal. ($C_{15}H_{23}N_2O_8P$) C, H, N.
10. ^{1}H NMR ($CDCl_3$): δ 5.64 (d, 4 H, OCH_2O, J = 14 Hz), 2.93 (s, 12 H, $N(CH_3)_2$). MS: m/z 301 (MH^+). Anal. ($C_8H_{17}N_2O_8P$) C, H, N.
11. ^{1}H NMR ($CDCl_3$): δ 7.65 (d, 1 H, H-6, J = 8 Hz), 6.0 (dd, 1 H, H-1'), 5.77 (d, 1 H, H-5, J = 8 Hz), 5.70 (d, 4 H, OCH_2O, J_{PH} = 12 Hz), 4.28 (m, 2 H, H-5'), 2.97 (s, 12 H, $N(CH_3)_2$), 2.22 (m, 2 H, H-3'), 2.15 (m, 1 H, H-4'), 1.71 (m, 2 H, H-2'). MS: m/z 495 (MH^+). Anal. ($C_{17}H_{27}N_4O_{11}P$): C, H, N.
12. Aliquots of a stock solution of DM_2-ddUMP in H_2O (10^{-3} M) were diluted with various buffers to a final concentration of 10^{-4} M. The solutions were stirred at room temperature. At selected time intervals (typically, 2, 4, 8, 12, 24, 30, 50, and 100 h) aliquots (50 μL) were removed and analyzed immediately for parent drug by HPLC on a μBondapak C-18 reversed phase column (150 x 3.90 mm, i.d.; Phenomenex) protected with a guard pak precolumn filter. The mobile phase was a linear gradient of MeOH:H_2O (1:1) and 0.05 M ammonium acetate, pH 4.4. The flow rate was 1 mL/min. Eluted compounds were monitored with a variable-wavelenth UV detector set at 268 and 0.005 AUFS sensitivity. The retention times for DM_2-ddUMP, DM_1-ddUMP, ddU, and ddUMP were respectively 28.81, 25.31, 13.88, and 8.46 min.
13. Human plasma was obtained from the Blood Bank of M. D. Anderson Cancer Center, Houston, TX. The reaction was started by adding 100 μL of stock solutions (1 x 10^{-2} M) of DM_2-ddUMP in EtOH to plasma (1900 μL) contained in 3 mL vials, such that the final concentrations of DM_2-ddUMP was 1 x 10^{-4} M. The samples were agitated for 20 seconds on a Vortex shaker to ensure thorough mixing and then incubated at 37 °C. Samples (100 μL) were withdrawn at 0, 1, 5, 15, 30, 60, 120, 180, 360, 1440 min and diluted with 4 volumes of ice cold MeOH. The mixture was agitated on a Vortex shaker for 1 min and then centrifuged at 10,000 rpm for 10 min to sediment precipitated protein. The mobile phase was a linear gradient of MeOH:H_2O (1:1) and 0.05 M ammonium acetate, pH 4.4. The supernatants were analyzed by HPLC for DM_2-ddUMP, DM_1-ddUMP, ddUMP, and ddU as described above.