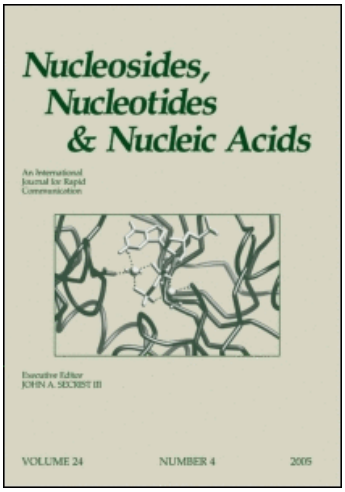

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Aryl-Containing Esters Of Triphosphoric Acid As Substrates Of Terminal Deoxynucleotidyl Transferase

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ARYL-CONTAINING ESTERS OF TRIPHOSPHORIC ACID AS SUBSTRATES OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE

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□ *A new group of terminal deoxynucleotidyltransferase (TDT) substrates, namely, non-nucleoside triphosphates (NNTP) bearing 5-substituted 2,4-dinitrophenyl fragments instead of nucleoside residues was synthesized.*

Keywords Non-nucleoside triphosphates; terminal deoxynucleotidyltransferase

INTRODUCTION

Terminal deoxynucleotidyltransferase (TDT, EC 2.7.7.31) is a unique template-independent DNA polymerase. TDT belongs to the X polymerase family, a subclass of an ancient nucleotidyltransferase superfamily, which includes nucleic acid polymerases such as DNA polymerases β, λ, μ, and some others. Unlike any other DNA polymerases, TDT incorporates both ribo- and deoxyribonucleotides *in vitro* with the equal efficacy as well as a large array of unnatural nucleoside triphosphates. It was shown in our laboratory that thriphosphate analogues bearing different bulky alkyl and aryl groups instead of a nucleoside residue can serve as substrates for calf thymus TDT, thus, demonstrating that the presence of a nucleic base in the substrate molecule is not a determining factor for the binding to the enzyme active site and incorporation into the growing DNA chain.[1–3] The efficacy of these compounds depends on substituent and linker structures and length.[4,5] It was shown that the affinities of some of the compounds of this series towards TDT were similar to those of natural substrates. Moreover, non-nucleoside triphosphates (NNTP) were demonstrated to be inhibitors and/or substrate terminators of other polymerases of the X family, particularly, of human β and λ polymerases.[6]

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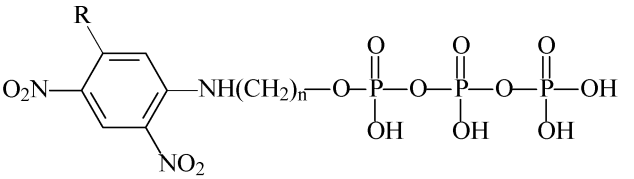
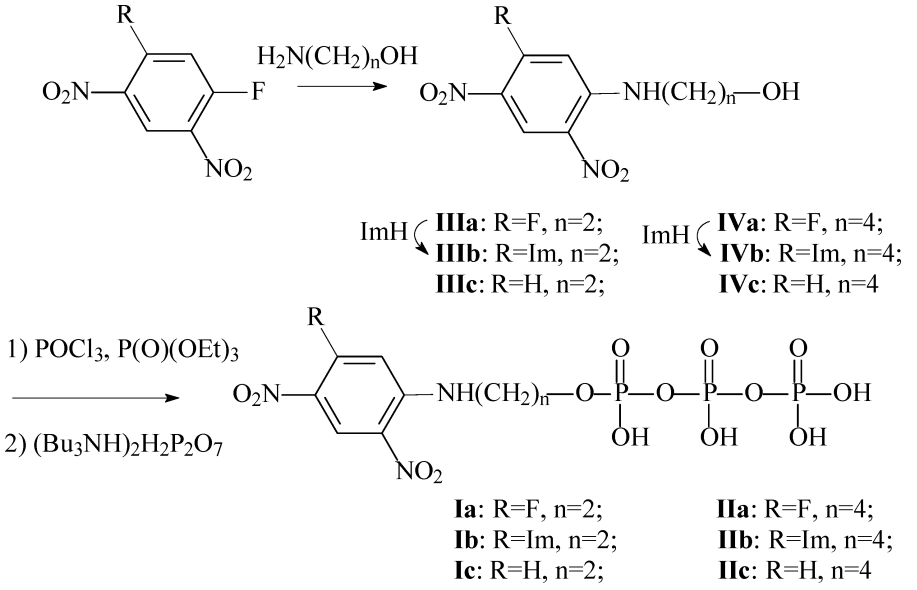
RESULTS AND DISCUSSION

We present in this work the synthesis of new NNTPs of the following structures:



Ia: n=2, R=F; **Ib**: n=2, R=Im; **Ic**: n=2, R=H; **IIa**: n=4, R=F; **IIb**: n=4, R=Im; **IIc**: n=4, R=H

Compounds (**IIIa**, **IIIc**, **IVa**, and **IVc**) were obtained by the reaction of the corresponding aminoalcohol with 1,3-difluoro-4,6-dinitrobenzene (for **IIIa** and **IVa**) or 1-fluoro-2,4-dinitrobenzene (for **IIIc** and **IVc**). For the preparation of the imidazole derivative (**IIIb** and **IVb**), the corresponding fluoro-containing precursors were treated with a solution of imidazole in DMF. The Ludwig triphosphorylation[7] carried out without the isolation of intermediate monophosphates gave the target triphosphates (Scheme).



The synthesized fluoro-containing triphosphates proved to be rather labile under the conditions of ion-exchange chromatography on DEAE (HCO_3^--form), and, therefore, were isolated in the following way. The reaction mixture was diluted with water and passed through a Dowex $50^{(H+)}$

column. The resulting solution was concentrated, and the target product was purified on a reversed-phase LiChroprep RP-18 column. Total yields of the synthesized triphosphates achieved 15–30% from the starting amino alcohol.

All the synthesized compounds demonstrated potent substrate properties in cell-free experiments with TDT.[8]

EXPERIMENTAL SECTION

N-(2,4-Dinitro-5-fluorophenyl)-2-aminoethyl triphosphate Ia. Yield 21%. UV (H_2O, pH 6): λ_{max} 265 nm (ε 9000), 349 nm (ε 15900). ^{1}H-NMR (D_2O): 9.10 (1H, d, *J* 8.1, H3), 7.04 (1H, d, *J* 14.6, H6), 4.22 (2H, dt, CH_2), 3.75 (2H, t, *J* 5.3, CH_2N). ^{31}P-NMR (D_2): -5.72 (1P, d, *J* 20.3, P_γ), -10.39 (1P, d, *J* 19.3, P_α), -21.50 (1P, dd, P_β). Mass (m/e): 484.1 [M^+-1].

N-(2,4-Dinitro-5-imidazolylphenyl)-2-aminoethyl triphosphate Ib. Yield 17%. UV (H_2O, pH 6): λ_{max} 272 nm (ε 5800), 365 nm (ε 7000) ^{1}H-NMR (D_2O): 9.24 (1H, s, H3), 8.96, 7.67 and 7.55 (3H, 3 br.s, Im), 7.50 (1H, s, H6), 4.40 (2H, m, CH_2), 3.83 (2H, t, *J* 5.3, CH_2N). ^{31}P-NMR (D_2): -10.24 (1P, d, *J* 19.3, P_γ), -10.83 (1P, d, *J* 20.3, P_α), -22.61 (1P, dd, P_β). Mass (m/e): 532.2 [M^+-1].

N-(2,4-Dinitrophenyl)-2-aminoethyl triphosphate Ic. Yield 24%. UV-VIS (H_2O, pH 6): λ_{max} 265 nm (ε 8300), 363 nm (ε 17500). ^{1}H-NMR (D_2O): 8.95 (1H, d, *J*2.5, H3), 8.23 (3H, dd, -5), 7.21 (1H, d,*J*9.65, H6), 4.01 (2H, m, CH_2), 3.72 (2H, t, *J* 5.9, CH_2N).^{31}P NMR (D_2O): δ -9.88 (d, 1P, *J* 21.4, P_γ), -10.32 (d, 1P, *J* 19.3, P_α), -21.77 (dd, 1P, P_β). Mass (m/e): 466.1 [M^+-1].

N-(2,4-Dinitro-5-fluorophenyl)-4-aminobutyl triphosphate IIa. Yield 15%. UV (H_2O, pH 6): λ_{max} 265 nm (ε 9100), 349 nm (ε 16000).^{1}H-NMR (D_2O): 9.10 (1H, d, *J* 8.1, H3), 6.94 (1H, d, *J* 15.6, H6), 4.01 (2H, m, CH_2), 3.50 (2H, t, *J* 6.8, CH_2N), 1.77 (4, m, $(CH_2)_2$). ^{31}P-NMR (D_2): -10.12 (1P, d, *J* 19.3, P_γ), -10.32 (1P, d, *J* 20.3, P_α), -22.65 (1P, dd, P_β). Mass (m/e): 512.1 [M^+-1].

N-(2,4-Dinitro-5-imidazolylphenyl)-4-aminobutyl triphosphate IIb. Yield 23%. UV (H_2O, pH 6): λ_{max} 272 nm (ε 5800), 365 nm (ε 7000).^{1}H-NMR (D_2O): 9.19 (1H, s, H3), 8.30, 7.45 and 7.33 (3H, 3 br.s, Im), 7.19 (1H, s, H6), 3.99 (2H, m, CH_2), 3.54 (2H, t, *J* 6.8, CH_2N), 1.82-1.72 (4, m, $(CH_2)_2$). ^{31}P-NMR (D_2): -10.10 (1P, d, *J* 19.3, P_γ), -10.31 (1P, d, *J* 20.3, P_α), -22.64 (1P, dd, P_β). Mass (m/e): 560.2 [M^+-1].

N-(2,4-Dinitrophenyl)-4-aminobutyl triphosphate IIc. Yield 30%. UV-VIS (H_2O, pH 6): λ_{max} 265 nm (ε 8300), 363 nm (ε 17500). ^{1}H-NMR (D_2O): 9.19 (1H, d, *J*2.8, H3), 8.30 (3H, dd, -5), 7.19 (1H, d, *J*9.65, H6), 3.99 (2H, m, CH_2), 3.54 (2H, t, *J* 6.8, CH_2N), 1.82-1.72 (4, m, $(CH_2)_2$). ^{31}P-NMR (D_2): -10.10 (1P, d, *J* 19.3, P_γ), -10.31 (1P, d, *J* 20.3, P_α), -22.64 (1P, dd, P_β). Mass (m/e): 494.2 [M^+-1].

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