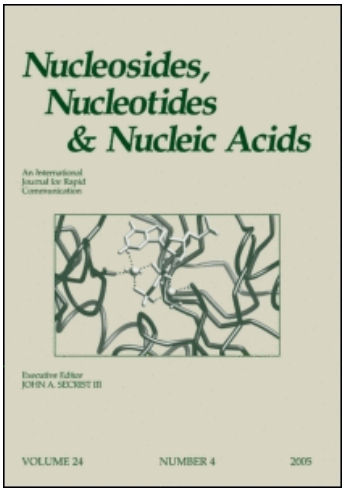

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Synthesis of New Photocross-Linking 5-C-Base-Substituted UTP Analogs and Their Application in Highly Selective Affinity Labelling of the Tick-Borne Encephalitis Virus RNA Replicase Proteins

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SYNTHESIS OF NEW PHOTOCROSS-LINKING 5-C-BASE-SUBSTITUTED UTP ANALOGS AND THEIR APPLICATION IN HIGHLY SELECTIVE AFFINITY LABELLING OF THE TICK-BORNE ENCEPHALITIS VIRUS RNA REPLICASE PROTEINS

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ABSTRACT: A new photocross-linking 5-C-base-substituted UTP analogs, carrying 4-azidoperfluorobenzoyl and 4-azidoaniline residues were synthesized. Two flavivirus proteins NS5 and NS3 are shown to be labelled after RNA synthesis in the presence of the analogs, irradiation ($\lambda > 300$ nm) and subsequent [α-^{32}P]NTP incorporation.

A few years ago nucleoside-5'-triphosphate's derivatives carrying an aryl azido residue attached to heterocyclic moiety were proposed to study of a number of RNA and DNA polymerases. Recently a new version of the identification of viral replicase in the nuclear fraction by CTP photoanalogs was proposed [1]. In this paper we described the synthesis and the use of photoreactive analogs of UTP as affinity labels of the active centers of individual nonstructural proteins of tick-borne encephalitis virus (TBEV) involved in the initiation of viral RNA replication in porcine embryo kidney (PEK) cells.

5-[3-(E)-(4-Azido-2,3,5,6-tetrafluorobenzamido)-propenyl-1]- (I) and 5-{3-(E)-[[2-[2-(4-azidophenyl)aminoethyl]-carbamoyl]-propionamido]-propenyl-1}uridine-5'-triphosphates (II) were obtained as 5-[3-(E)-(2-nitro-5-azidobenzamido)-propenyl-1]-uridine-5'-triphosphate (III) [2] with the use of *N*-hydroxysuccinimide esters of 4-azido-2,3,5,6-tetrafluorobenzoic acid and 3-{[2-(4-azidophenyl)aminoethyl]-carbamoyl}-propionic acid.

Compound (I): UV (H_2O), λ_{max} 258 nm (ε 34 600), λ_{min} 232 nm (ε 19 500); IR (H_2O, ν_{max}, cm^{-1}) 2205 ($-N_3$); ^{31}P NMR (D_2O), J (Hz) δ -4.17 (d, J = 20, P_γ, 1P), -10.24 (d, J = 20, P_α, 1P), -19.56

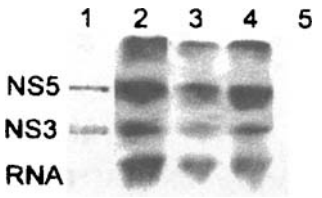

FIG. 1. Affinity labelling of TBEV replicase proteins by the analogs I - III, incorporated into RNA. Autoradiogram of SDS-PAAG of labelled proteins from the nuclear fraction of unifected PEK cells (line *5*) and from the TBEV-infected cells in 48 h postifection (lines *2-4*). Analogs concentration is 10^{-6} M. Analogs: I lane - *3*, II - lane *2*, III - lane *4*. Lane *1* is the immunoblotting of the nuclear fraction from TBEV-infected with the monoclonal antibodies against TBEV NS5 and NS3 proteins.

(t, J = 20, P_β, 1P); ^{1}H NMR (D_2O), J (Hz) δ 4.00 (d, J = 4.5, H9, 2H), 4.24 (m, H2', H3', H4', 3H), 4.62 (d, J = 4.5, , H5', 2H), 6.04 (d, J = 4.5, H1', 1H), 6.41-6.45 (m, H7, H8, 2H), 7.85 (s, H6, 1H). **Compound (II)**: UV (H_2O), λ_{max} 268 nm (ε 20 600), λ_{min} 230 nm (ε 6 400). IR (H_2O, ν_{max}, cm^{-1}) 2117 ($-N_3$);^{31}P NMR (D_2O), J (Hz) δ -4.41 (d, J = 20, P_γ, 1P), -10.40 (d, J = 20, P_α, 1P), -19.70 (t, J = 20, P_β, 1P); ^{1}H NMR (D_2O), J (Hz) δ 3.92 (m, H9, 2H), 4.26 - 4.50 (m, H2', H3', H4', H5', 5H), 6.01 (d, J = 4.0 , H1', 1H), 6.15-6.40 (m, H7, H8, 2H), 7.77 (s, H6, 1H), 2.45 (t, H10, 2H), 2.57 (m, H11, 2H), 3.20-3.40 (m, H12,13, 4H), 6.82-6.94 (m, H14, 15, 4H).

The labelling procedure consists of several consequent steps: 1) incorporation of analogs into growing RNA due to the RNA polymerase activity; (50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 10 mM KCl, 10 μg/ml actinomycin D and 14 μl nuclear fraction of infected cells were incubated for 5 min at 30 ^{0}C, and analogs (I - III) were added; 2) covalent photocross-linking of newly synthesized photoreactive RNA to proteins (after incubation with analog for 15 min at 30 ^{0}C irradiation was carried by filtered light (300 - 365 nm); 3) subsequent labelling of RNA-protein complexes by [α-^{32}P]ATP and RNA replicase activity (10 min at 30 ^{0}C); 4) RNase treatment (30 min at 37 ^{0}C).

The comparison of the autoradiogram (lanes 2-4) and Western immunoblotting with monoclonal antibodies against TBEV NS5 and NS3 proteins (lane 1) revealed that the affinity labelled proteins with molecular masses 100 and 69 kDa are TBEV nonstructural proteins NS5 and NS3, respectively. The TBEV new radioactive labelled RNA strands could interact with viral nonstructural proteins NS5 and NS3 in the nuclear fraction of infected cells at the different postinfection stages (data not shown).

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