

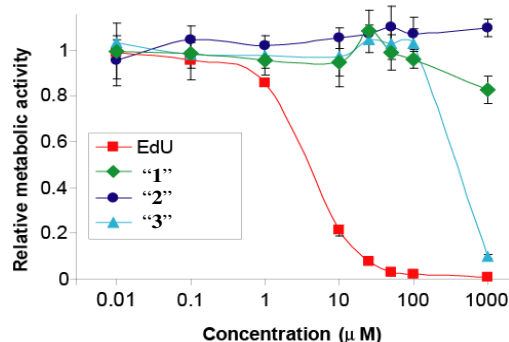


Modified Nucleosides and Nucleotides for Labeling DNA and RNA in Living Cells

New compounds for metabolic labelling of DNA have been identified which have superior properties than 5-ethynyl-2'-deoxyuridine (EdU).

Background Metabolic labeling of DNA has traditionally been performed using [3H]thymidine or 5-bromo-2'-deoxyuridine (BrdU). These methods are limited in terms of subsequent imaging steps, requiring either autoradiography or DNA denaturation followed by antibody staining. An improved metabolic labeling strategy was recently reported using the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU). After being incorporated into newly synthesized DNA in vivo, the ethynyl functionalities were sensitively detected using fluorescent azides and copper(I)-catalyzed "click" cycloadditions. This EdU-based labeling strategy was commercialized by Invitrogen. One critical limitation of EdU is its potent toxicity and ability to cause cell-cycle arrest at or below the concentration required for efficient DNA labeling.

Invention We have synthesized new compounds that overcome the deficiencies of EdU:
Toxicity: After 24 – 72 hours of incubation, the parent compound EdU was a potent inhibitor of cellular respiration with IC₅₀ values ranging from 1 μ M (3T3) to 10 μ M (Vero). Our compounds, in contrast, exhibited little, if any, inhibition of cellular respiration even at concentrations of 100 μ M and for incubation times of three weeks or more with continuous feeding at 10 μ M.
Incorporation into cellular DNA: Our compounds labeled virtually all cells over a concentration range of 0.1 – 100 μ M at incubation times ranging from 1 – 20 days.
Impact on DNA function: Unlike EdU, our compounds have very little impact on cell cycle progression and are therefore compatible with multiple-labeling strategies over extended experimental times.



Viability of HeLa cells as a function of EdU and three of our new compounds according to the AlamarBlue assay.

Fields of Use Proliferation studies, Measuring nucleotide excision repair activity, analysis of nuclear architecture, tissue regeneration studies, cell cycle analysis, live-cell imaging of metabolically-labeled DNA and viruses.

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