

Development of Novel Solid-Phase Synthesis for Nucleoside Mono-, Di- and Triphosphates and their Use to Detect DNA Replication in Plasmodium Parasites

Anne-Sophie C. Braun, David H. Hilko, Katherine T. Andrews, Sally-Ann Poulsen

Institute for Biomedicine and Glycomics, Griffith University, Nathan, QLD, Australia

Abstract

Analogues of the endogenous nucleoside thymidine are widely used as metabolic chemical probes to investigate DNA replication and dissect features of the cell cycle or other cellular processes. Plasmodium, the parasite that causes malaria, lacks the enzyme thymidine kinase needed for the incorporation of current thymidine-based chemical probes into its DNA. To address these limitations, we designed, synthesised and validated next-generation chemical probes based on the 5-vinyl-2'-deoxyuridine monophosphate (VdU-P1) scaffold in Plasmodium. The VdU-based probes enable the detection of labelled parasites via a catalyst-free inverse-electron-demand Diels-Alder (IEDDA) click-reaction with a fluorescent tetrazine dye and was compatible in live-cell DNA labelling and imaging, reported by us recently.¹ To provide malaria researchers with new and better tools to study the cell cycle and growth of Plasmodium, we designed VdU-based probes that enter the thymidine metabolism pathway further downstream than VdU-P1, skipping other kinases that are potential metabolic roadblocks. The synthesis of nucleoside di- and triphosphate probes using known methods is challenging due to very low product yields, complex mixtures of reaction byproducts, and the highly polar nature of the desired compounds, making purification difficult and laborious.² To address the limitations of current methods we have developed a novel solid-phase synthesis. Yields are high and the synthetic approach is compatible with sensitive functional groups, leading to clean reactions and uncomplicated purification (Figure 1). I will describe this new synthesis approach and the application of mono-, di- and triphosphates of VdU to study DNA synthesis in wild type *P. falciparum* parasites.

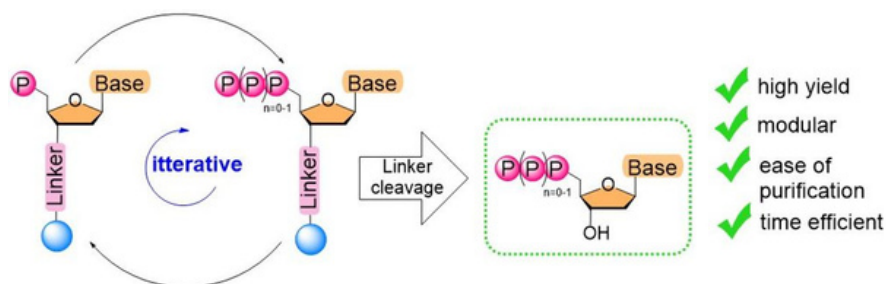


Fig. 1: New solid-phase synthesis method for nucleoside di- and triphosphate chemical probes.

[1] Braun, A.-S. C.; Fisher, G. M.; Hilko, D. H.; Macdonald, J. R.; Thomas, S.; Andrews, K. T.; Poulsen, S.-A. Vinyl-DNA Detect Probes to Assess DNA Proliferation in Plasmodium Parasites using Inverse-Electron-Demand Diels–Alder Click Reactions. ACS Chem. Biol. 2025.

[2] Roy, B.; Depaix, A.; Périgaud, C.; Peyrottes, S. Recent Trends in Nucleotide Synthesis. Chem. Rev. 2016, 116 (14), 7854.

Biography

Anne-Sophie completed her Bachelor's and Master's degree in Chemistry at the University of Freiburg, Germany, with a major in organic chemistry. Anne-Sophie is currently a third-year PhD candidate at Griffith University working in the field of Chemical Biology. Anne-Sophie's research focuses on developing novel synthetic chemistry methods for the synthesis of modified nucleoside analogues and using these compounds to visualise DNA proliferation in *Plasmodium* parasites.

