

From Nucleosides to NTPs in One Pot

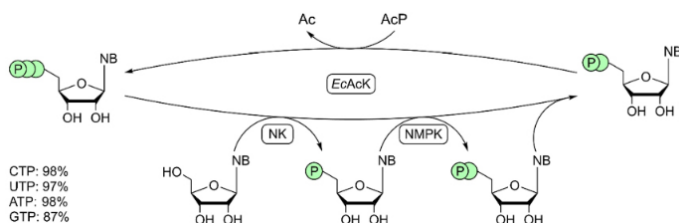
Did you know that nucleotide triphosphates are key building blocks for RNA technologies, diagnostics and synthetic biology? acib offers a modular one-pot kinase cascade for the synthesis of all four canonical ribonucleoside triphosphates.

BACKGROUND

The demand for nucleoside 5-triphosphates (NTPs) is increasing due to their central role in RNA synthesis, sequencing, transcription, synthetic biology and RNA-based therapeutics. However, established chemical synthesis routes can suffer from low atom economy, complex reagent use and substantial waste formation. Enzymatic phosphorylation offers an attractive alternative, but many reported cascades face practical limitations such as incompatible reaction conditions, incomplete conversion, product dephosphorylation, intermediate accumulation, long reaction times or inefficient phosphate donor use.

TECHNOLOGY

acib has developed standardized one-pot enzymatic cascades for the production of ATP, CTP, GTP and UTP from the corresponding ribonucleosides. The platform combines substrate-specific nucleoside kinases with flexible yeast monophosphate kinases and acetate kinase-driven phosphate transfer. ScURA6 enables phosphorylation of AMP, CMP and UMP, while ScGMPK is used for GMP. E. coli acetate kinase drives the final phosphorylation step using acetyl phosphate as phosphate donor, with acetate as the by-product. The cascades were optimized to balance flux through all phosphorylation steps and suppress accumulation of NMP and NDP intermediates. Reactions reached full or near-full conversion ($\geq 93\%$) at 50–100 mM substrate loading within 60–240 minutes. Preparative 10 mL syntheses delivered isolated NTP yields up to 89% and purities up to 99%. The platform can also be adapted toward noncanonical nucleosides and nucleoside analogues, offering a flexible starting point for tailored nucleotide synthesis.



OFFER

acib offers to develop and optimize enzymatic NTP synthesis routes together with companies. Possible collaborations include feasibility studies, substrate screening, enzyme and reaction optimization, preparative synthesis, purification strategy development and adaptation to company-specific nucleotide targets. IP developed in such projects can be fully transferred to the industrial partner.

EXPERTS

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DEVELOPMENT STATUS:

Status of the project proposal – Technology
Readiness Level 4
(technology validated in lab)

KEYWORDS

- Biocatalysis
- NTP synthesis
- Kinase cascade
- Ribonucleotides
- One-pot synthesis
- RNA therapeutics
- mRNA manufacturing
- Modified nucleotides

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