Bioorganic & Medicinal Chemistry Letters 28 (2018) 1248–1251

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

2′,3′-Dideoxyuridine triphosphate conjugated to SiO_2 nanoparticles: Synthesis and evaluation of antiproliferative activity

Svetlana V. Vasilyeva [a,*], Inga R. Grin [a,b], Boris P. Chelobanov [a,b], Dmitry A. Stetsenko [a]

[a] Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, 8 Lavrentiev Avenue, Novosibirsk 630090, Russia
[b] Novosibirsk State University, Novosibirsk 630090, Russia

ARTICLE INFO

Article history:
Received 6 December 2017
Revised 1 February 2018
Accepted 6 February 2018
Available online 7 February 2018

Keywords:
SiO_2 nanoparticles
CuAAC
2′, 3′-Dideoxyuridine triphosphate
MCF7 cells
Cytotoxicity

ABSTRACT

A conjugate of triphosphorylated 2′,3′-dideoxyuridine (ddU) with SiO_2 nanoparticles was obtained via the CuAAC click chemistry between a γ-alkynyl ddU triphosphate and azido-modified SiO_2 nanoparticles. Assessment of cytotoxicity in human breast adenocarcinoma MCF7 cells demonstrated that ddU triphosphate conjugated to SiO_2 nanoparticles exhibited a 50% decrease in cancer cell growth at a concentration of 183 ± 57 μg/mL, which corresponds to 22 ± 7 μM of the parent nucleotide, whereas the parent nucleoside, nucleotide and alkynyl triphosphate precursor do not show any cytotoxicity. The data provide an example of remarkable potential of novel conjugates of SiO_2 nanoparticles with phosphorylated nucleoside analogues, even those, which have not been used previously as therapeutics, for application as new anticancer agents.

Nucleoside analogues are commonly used in medicine for anticancer or antiviral chemotherapy. Amongst these, 2′,3′-dideoxynucleosides (ddN) have been extensively investigated in the last two decades. Such compounds as 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-dideoxycytidine (ddC) or lamivudine are the examples that have undergone thorough clinical investigation.[1] Usually, ddNs act as DNA chain elongation terminators. In cells they have to be metabolized by virus-encoded or, in most cases, by host cell kinases into active 5′-mono-, di-, and triphosphates.[2,3]

In contrast to some other dideoxynucleosides, 2′,3′-dideoxyuridine is not currently used for either anticancer or antiviral therapy. It is a poor substrate for cellular nucleoside kinases.[4] In addition, it was shown that ddU diphosphate is a particularly bad substrate for nucleoside diphosphate kinase (NDP-K). Thus, the conversion of NDP to NTP could be in fact the rate-limiting step in the eventual activation of other nucleoside analogues as well.[5]

To overcome the phosphorylation bottleneck, in a recent work by Meyer et al.[6] some membrane-permeable NTP prodrugs have been obtained by masking the γ-phosphate group by two biodegradable benzyl groups. However, this approach, while successful, requires a complicated multi-step synthesis. In a work by Zelphati et al.[4] the anti-HIV effects of 2′,3′-dideoxyuridine 5′-mono and triphosphate encapsulated in immunoliposomes were studied. Unfortunately, the preparation of liposomes loaded with triphosphates is also quite laborious. In addition, they are unstable during storage.

An alternative to complex synthetic procedures is the use of recent advances in nanotechnology, which have provided a number of biocompatible nanomaterials for cellular delivery of nucleotides such as SiO_2 nanoparticles. In our previous studies[7–9] we have proposed a system for the efficient delivery of nucleosides triphosphates into cells using their covalently linked conjugates with SiO_2 nanoparticles. We studied anticancer and antiviral activity of such conjugates of nucleoside analogues commonly used in therapy, such as AZT, lamivudine, ddC and showed that in most cases conjugates of nucleotides with SiO_2 nanoparticles were more active than the parent nucleoside. They were also stable during long-term storage at −20 °C.

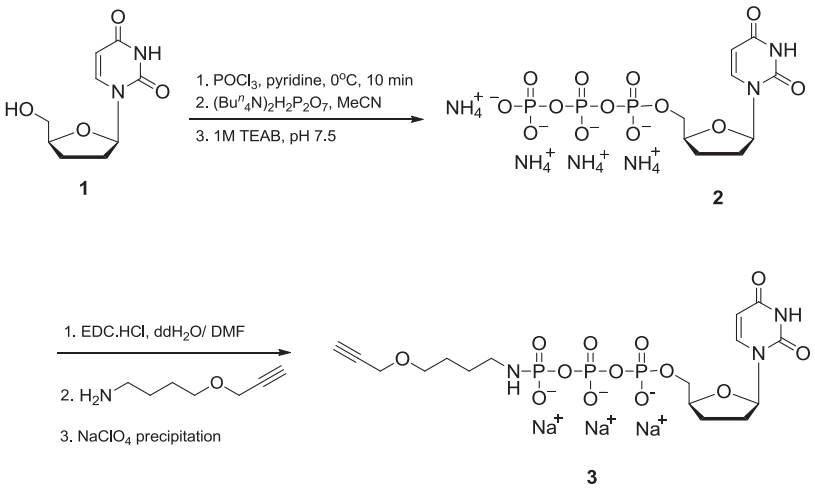
We report herein the synthesis of a conjugate of 5′-phosphorylated ddU with SiO_2 nanoparticles of, analysis of its stability and preliminary assessment of its cytotoxicity to human breast adenocarcinoma MCF7 cells. Copper(I)-catalyzed azide-alkyne cycloaddition reaction was used to conjugate a γ-alkynyl ddU triphosphate with azido-modified SiO_2 nanoparticles.

Synthesis of a γ-alkynyl dideoxyuridine triphosphate (3, l-ppddU) was carried out according to Scheme 1.

A bifunctional reagent 4-(prop-2-yn-1-yloxy)butylamine containing both terminal alkynyl group and primary amino group furnished the linker between ddU triphosphate and SiO_2 nanoparticle.[10] Terminal alkynyl group is needed for CuAAC reaction and primary amino group for the formation of phosphoramidate bond

* Corresponding author.
E-mail address: svetlana2001@gmail.com (S.V. Vasilyeva).

https://doi.org/10.1016/j.bmcl.2018.02.007
0960-894X/



Scheme 1. Synthesis of a γ-alkynyl ddU triphosphate (**3**, L-ppddU).

to preserve the acceptance of ddNTP substrate by a range of polymerases. Synthesis starts from commercially available dideoxyuridine. The 5′-triphosphate **2** (pppddU) was obtained by the same way as in our earlier work.[11] The bifunctional linker was introduced onto the γ-phosphate of ddNTP according to the procedure described by Serdjukow et al.[12] Isolated yields of alkynyl triphosphate **3** were in the range of 40–50%. The compounds **2, 3** have been fully characterized.

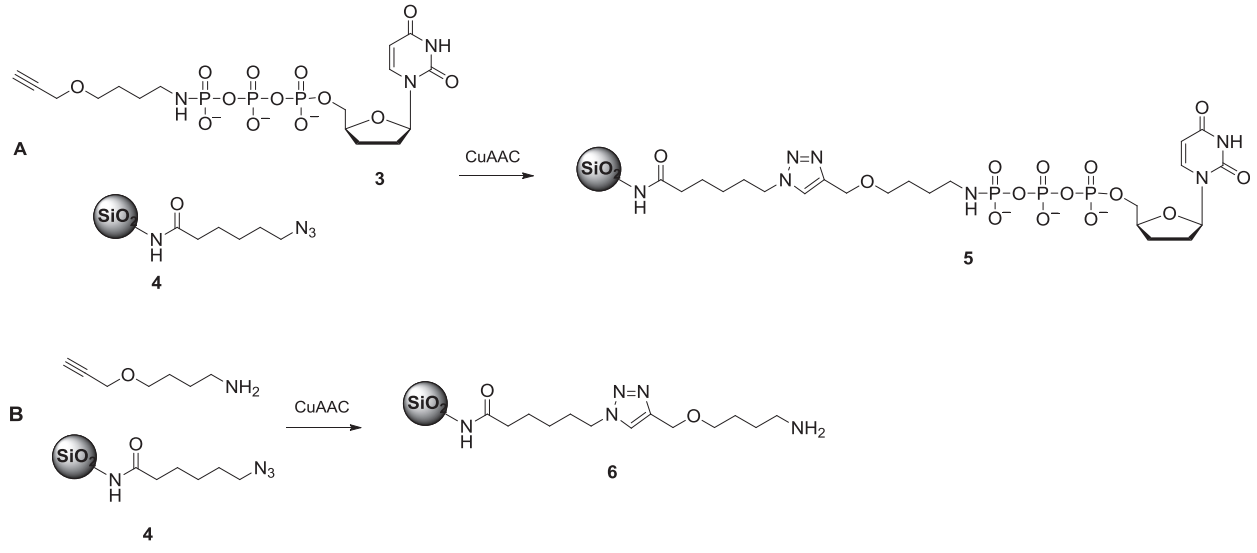
Synthesis of ddU triphosphate conjugate with SiO_2 nanoparticles (**5**, SiO_2∼L6∼pppddU) was carried out using CuAAC click chemistry. The procedure outlined in Scheme 2 was similar to the one we described earlier.[9] Nanoparticles containing alkyl azide groups **4** (SiO_2-L6-N_3, 0.43 μmol N_3 groups/mg nanoparticles) were prepared by treatment of SiO_2-NH_2 (0.5 μmol NH_2 groups/mg nanoparticles) with *N*-hydroxysuccinimide ester of 6-azidohexanoic acid.[7]

The pppddU content in the conjugate was calculated from UV absorbance of **5** and SiO_2∼L6 dissolved in 0.2 M NaOH using the nucleotide extinction coefficient λ max/nm (ε): 260 (1883). It was found to be 0.124 μmol/mg SiO_2 conjugate. It was possible to obtain the drug content of 0.400 μmol/mg by increasing the compound **3** excess to eight equiv.

To remove copper ions, the conjugate was washed carefully with 10% Na_2EDTA in water. The same method was used to purify oligonucleotide conjugates from Cu(II) in earlier works.[13,14] In this case, we observed no blue color in the last washing solution as well as no decomposition of the conjugate. To verify that the SiO_2 nanoparticles loaded with ddU triphosphate are really free of Cu (II), the conjugate was dissolved in 0.2 M NaOH followed by centrifugation. In the absence of Na_2EDTA wash, residual Cu(II) remained and we observed some precipitate of blue $Cu(OH)_2$ at the bottom of a test tube. Usually 3–5 washes were sufficient to remove Cu(II) nearly completely (no precipitate formed).

The particle size and zeta potential values of **5** were similar to the obtained in our preliminary studies of the conjugates of SiO_2 nanoparticles with nucleoside triphosphates.[7–9]

The chemical stability of SiO_2∼L6∼pppddU conjugate (nucleotide content 0.400 μM/mg) was studied in three buffers with different pH values, which corresponded to the pH in blood (pH 7.3), in mouth (pH 6.5) and in stomach (pH 1.5–2.0). Degradation products



Scheme 2. Synthesis of the conjugate of ddU triphosphate with SiO_2 nanoparticles **5** (SiO_2∼L6∼pppddU) (A) and the control conjugate **6** (SiO_2∼L6) without nucleotide (B) via the CuAAC reaction.