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New orthogonally trifunctionalized morpholine nucleosides

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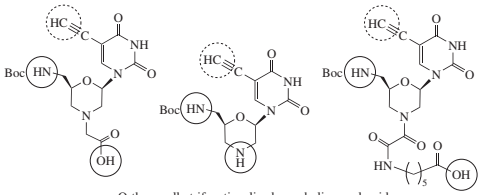
Three novel uracil-based morpholine nucleosides each containing three different orthogonally reactive functional groups, namely, amino, carboxy and acetylene ones, were synthesized. The obtained monomers are intended for the synthesis of labeled nucleotide, nucleic acid or peptide mimics.



Orthogonally trifunctionalized morpholine nucleosides

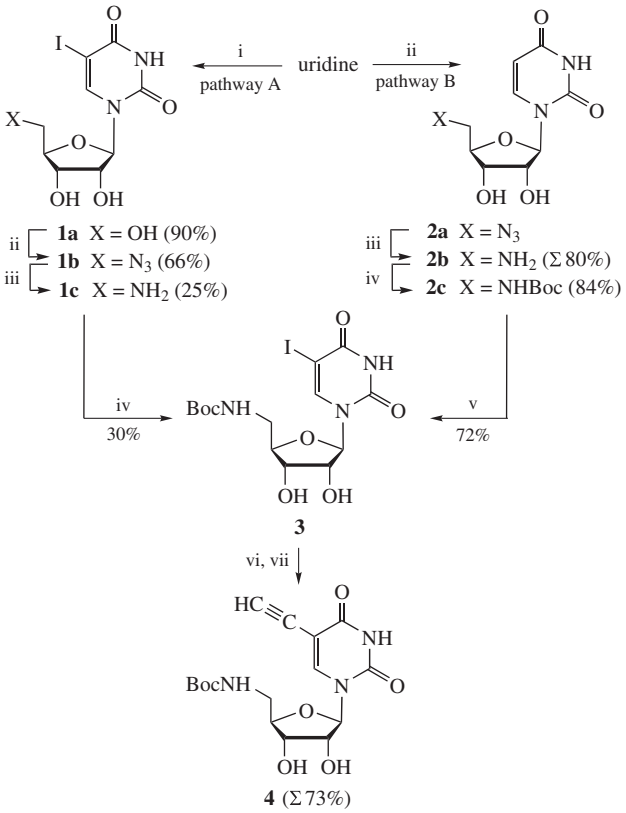
Modified nucleosides,[1] oligonucleotides and nucleic acids are widely used in molecular biology, biochemistry and medicine. Among a huge variety of oligonucleotide analogues and their mimetics available nowadays, morpholine-type oligomers in which sugar residue is replaced by sugar-like morpholine-derived moiety are the most successful ones.[2] Recently, two morpholine oligonucleotides were approved in USA for therapeutic application in the treatment of Duchenne muscular dystrophy and Spinal muscular atrophy.[3,4]

Previously,[5] we have developed the solid phase-supported synthesis of morpholinoglycine oligonucleotide mimics (gMOM)[5] which were shown to be promising for the antisense-based applications.[6] *N-tert*-Butyloxycarbonyl-protected amino acids were the synthons for their solid-phase assembling. These monomers were also used in the synthesis of NAD+ mimics; uracil- and thymine-containing conjugates have turned out to be modest inhibitors of poly(ADP-ribose)polymerase (PARP) **1**.[7] Two different functional groups, amino and carboxy ones, are present in monomer molecule. The solid phase-supported synthesis of gMOM needs application of 2′-aminomethylmorpholine nucleosides[5] whose NH group serves as an orthogonal functional group for binding the first monomer to the solid support through a labile under basic conditions oxalate tether.

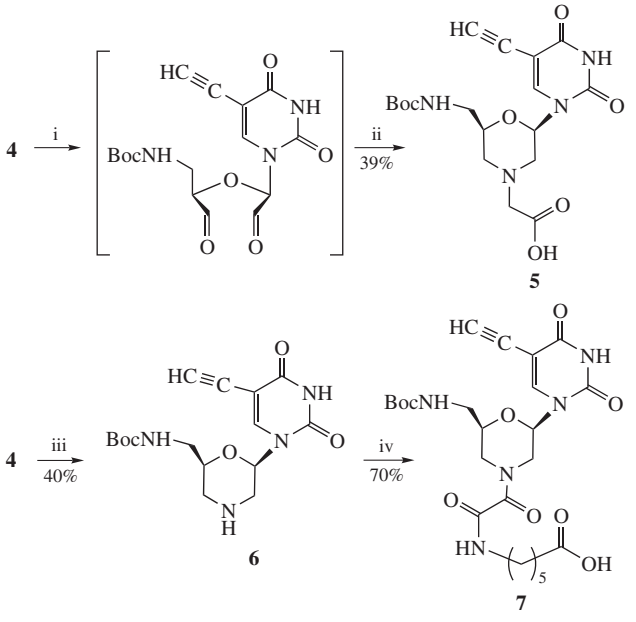
Labeling of biological macromolecules and bioimaging in cell culture and living organisms continue to be a booming area in bioorganic chemistry and molecular biology for decades.[8,9] A variability of functional groups in biomacromolecules suitable for modification mainly includes thiol[10,11] and amino[12,13] ones met in natural biopolymers. Non-natural amino acids and modified nucleotides incorporated in proteins and nucleic acids are used as well.[14–16] For biomolecule modification and cell targeting in bioimaging, the use of multimodal linkers with bioorthogonal functional groups is of vital importance. Recently, a trifunctional biocompatible linker containing a strained alkene for inverse electron demand Diels–Alder reaction, an alkyne for copper catalyzed azide–alkyne cyclization and an electron-deficient olefin for thiol-Michael addition was synthesized.[17]

Along with other approaches, a fruitfulness of biocompatible azide–alkyne coupling for contemporary biochemistry and molecular biology cannot be overestimated.[18,19] Among other compounds, 2′-deoxy-5-ethynyluridine has found notable application in molecular biology and biochemistry.[16,20] Recently, synthesis of 5-iodouracil-containing morpholine nucleosides and the corresponding 5-alkynylated derivatives suitable for the introduction of alkyne functionality into phosphorodiamidate morpholine oligonucleotides was described.[21–23]

Therefore, search for novel means for the introduction of the alkyne functionality into the uracil-containing morpholine synthons is an important task in the synthesis of nucleic acid mimics containing orthogonal functional groups. Herein, we report on the protocol for the synthesis of the target monomers **5**–**7** comprising minimal number of steps (Schemes 1 and 2).



Scheme 1 *Reagents and conditions*: i, ICl, MeOH; ii, $PPh_3/CBrCl_3$, NaN_3, DMF; iii, Ph_3P, pyridine, then NH_3/H_2O; iv, $(Boc)_2O$, Pr^iOH, 1 M NaOH; v, ICl, MeOH, then NH_3/H_2O, $Na_2S_2O_3$; vi, $Me_3SiC{\equiv}CH$, $Pd(PPh_3)_4/CuI/Et_3N$, DMF; vii, NH_3/H_2O.



Scheme 2 *Reagents and conditions*: i, $NaIO_4$, $MeOH/H_2O$; ii, $H_2NCH_2C(O)OH$, Et_3N, $NaCNBH_3$, then CF_3COOH, Et_3N; iii, $NaIO_4$, $MeOH/H_2O$, then $(NH_4)_2B_4O_7$, Et_3N, $NaCNBH_3$, then CF_3COOH, Et_3N; iv, $MeOC(O)C(O)–NH(CH_2)_5C(O)OH/Et_3N$/pyridine.

In the light of this task, we consider *N*-Boc-protected 5′-amino-5′-deoxy-5-ethynyluridine **4** as a key compound for obtaining morpholine monomer **5** as well as monomers **6** and **7**.

Recently, we have developed the synthesis of the 5-iodouracil containing 2′-(hydroxymethyl)morpholine nucleoside starting from 5-iodouridine **1a**[23] which was synthesized according to the published procedure[24] (see Scheme 1, pathway A). The transformation of the 5′-hydroxyl group into the 5′-amino one by the reduction of the intermediate azido derivative **1b** was carried out as described earlier.[25,26] According to HPLC analysis using multichannel UV detection, the reduction of the azide derivative **1b** followed by the hydrolysis of the intermediate iminophosphorane proceeded smoothly and quantitatively. However, much (up to 20%) of the side dehalogenated nucleoside was formed thus affording the target 5′-amino-5′-deoxy-5-iodouridine **1c** in low yield (25%) and of insufficient purity. A lability of the halogen atom in some 5-halogenated pyrimidine nucleosides under reducing conditions is known.[23,27] It is interesting that 5′-amino-5′-deoxy-5-iodoarabinouridine[28] and 5′-amino-5′,2′-dideoxy-5-iodouridine[29] were obtained previously, whereas preparation of analogous 5′-deoxy-5′-amino-5-iodoribouridine **1c** was not described according to the Reaxys Database. An introduction of Boc-protective group into nucleoside **1c** contaminated by dehalogenated impurities according to procedure[5] gave product **3** in only 30% yield based on compound **1c** (see Scheme 1, pathway A).

In search for more effective synthesis of key compound **4** we performed the reaction sequence depicted in Scheme 1, pathway B. 5′-Azido-5′-deoxyuridine **2a** was subjected to reduction without chromatographic purification in contrast to previously published procedure.[25,26] 5′-Amino-5′-deoxyuridine **2b** was obtained in 80% yield after two steps and purification by a cation exchange chromatography. An introduction of acid-labile Boc-protective group into nucleoside **2b** was performed in a standard manner to afford nucleoside **2c** in 84% yield. For acid-sensitive nucleosides, mild 5-iodination of uridine moiety with iodine chloride was proposed.[30] We modified this procedure varying the excess of ICl and reaction time and obtained nucleoside **3** from nucleoside **2c** in a yield of 75%. Along with the target compound **3**, some (3–5%) 5′-amino-5′-deoxy-5-iodouridine **1c** was formed due to the minor Boc-deprotection. An introduction of the trimethylsilylethynyl grouping by the Sonogashira cross-coupling was carried out similarly to protocol published for 5-ethynyluridine.[31] Trimethylsilyl protection was removed from the ethynyl part with aqueous ammonia thus affording the key nucleoside **4** in 73% yield (2 steps) based on nucleoside **3**.

At the next stage, we obtained trifunctionalized monomers **5** and **6** in 40% yield by subjection nucleoside **4** to an optimized procedure for the creation of morpholine moiety (Scheme 2).[23,32,33] Additional functionalization of *NH*-morpholine derivative **6** by treatment with methyl *N*-(6-hydroxy-6-oxohexyl)oxamate resulted in multifunctional compound **7** equipped with flexible 6-aminohexanoic acid moiety.

In conclusion, we have prepared three orthogonally functionalized morpholine nucleosides necessary for the synthesis of biocompatible functionalized gMOM[6] as well as NAD+ mimics.[7] Both kinds of these biomolecules mimicking native enzyme substrates have a great potential as molecular tools in clarifying the intrinsic mechanisms of PARP-depended DNA repair processes.[16,34,35] Our studies on the synthesis of these promising molecular tools are now in progress.

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Online Supplementary Materials

Supplementary data associated with this article (experimental procedures and characteristics for all compounds) can be found in the online version at doi: 10.1016/j.mencom.2019.03.017.

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