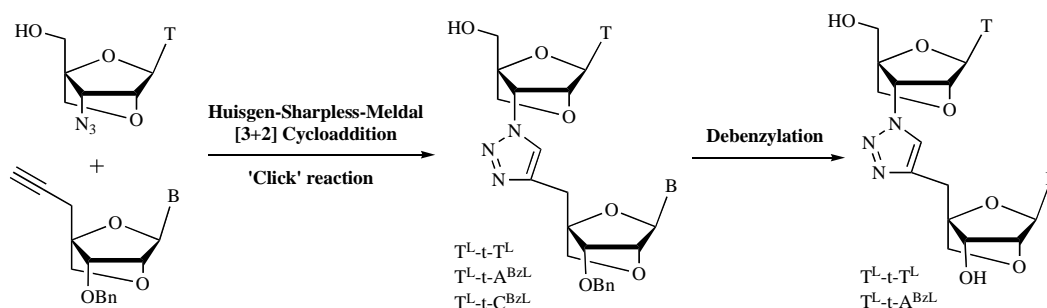


Synthesis of Triazole-linked LNA-based Non-ionic Nucleoside Dimers Using Cu(I)-Catalyzed 'Click' Reaction

Sunil K. Singh^a, Vivek K. Sharma^a, Kapil Bohra^a, Carl E. Olsen^b and Ashok K. Prasad^{a*}

^aBioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110 007, India; ^bUniversity of Copenhagen, Faculty of Life Sciences, Department of Plant and Environmental Sciences, DK- 1871 Frederiksberg C, Denmark

Abstract: Three benzylated triazole-linked nonionic locked nucleoside dimers T^L-t-T^L, T^L-t-A^{BzL} and T^L-t-C^{BzL} have been synthesized by Cu(I) catalyzed Huisgen-Sharppless-Meldal [3+2] cycloaddition reaction of 3'-azido-3'-deoxy-2'-O,4'-C-methylenethymidine with 3'-O-benzyl-5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylenethymidine, 3'-O-benzyl-5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-6-N-benzoyladenine and 3'-O-benzyl-5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-4-N-benzoylcytidine, which on debenzylation afforded the corresponding hydroxy analogs T^L-t-T^L and T^L-t-A^{BzL} in 69% and 63% overall yields. An effort to debenzylate T^L-t-C^{BzL} failed. Hydroxy T^L-t-T^L, T^L-t-A^{BzL} have alternatively been synthesized by the reaction of azidonucleoside with 5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylenethymidine and 5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-6-N-benzoyladenine in 79% and 76% overall yields, respectively. Among the two Cu(I) reagents used for cycloaddition (click) reaction, CuSO₄·5H₂O-sodium ascorbate in THF:^tBuOH:H₂O (1:1:1) was found to be better yielding than CuBr·SMe₂ in THF.



Keywords: Click-chemistry, locked nucleic acid, phosphate backbone modification, triazole-linkage.

INTRODUCTION

Different types of modified nucleosides and oligonucleotides have been synthesized for their use in drug discovery, because natural DNA/RNA based oligonucleotides pose some limitations, such as poor binding affinity, low degree of nuclease resistance, etc. affecting their direct use as antisense therapeutics [1, 2]. To overcome these limitations, modifications in the backbone [3], base [4] and sugar [5] functionalities of oligonucleotides have been carried out resulting in significant progress towards establishing oligonucleotides as viable therapeutic agents. The first antisense oligonucleotide Vitravene [6], approved for the treatment of retinitis contains backbone modification where as Kynamro [7], recently approved second antisense agent for the treatment of hypercholesterolemia contains both backbone and base modifications. Among numerous known modifications, enhancement of conformational rigidity of sugar moiety in nucleosides by the introduction of a 2'-O,4'-C methylene-linkage, such as in locked nucleic acids (LNA **1b**, Fig. 1) [8], has been found to be very useful for antisense applications [9-12].

Varizhuk, *et al.* [13], Lucas, *et al.* [14] and Isobe, *et al.* [15] have synthesized the triazole-linked 3',5'-thymidine dimer and their oligomers (**1c**, **1d** and **1e**, Fig. 1). The internucleoside triazole fragments demonstrated higher melting temperature and increased

resistance to nuclease digestion. Herein, we report the design of three triazole-linked LNA based nucleoside dimers T^L-t-T^L **3**, T^L-t-A^{BzL} **4** and T^L-t-C^{BzL} **5** expecting that they might be ideal antisense oligonucleotide monomers with respect to natural dimer **2** because of their preorganized sugar moiety and neutral triazolyl-linkage (Fig. 2).

RESULTS AND DISCUSSION

It was envisaged to synthesis triazole-linked LNA-based dimers T^L-t-T^L **3**, T^L-t-A^{BzL} **4** and T^L-t-C^{BzL} **5** by [3+2] cycloaddition reaction [16] of 3'-azido-3'-deoxy-2'-O,4'-C-methylenethymidine (**9**) [17,18] with 3'-O-benzyl-5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylenethymidine (**10**), 3'-O-benzyl-5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-6-N-benzoyladenine (**11**) and 3'-O-benzyl-5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-4-N-benzoylcytidine (**12**), respectively followed by debenzylation of the resulted nucleoside dimers **6-8** or alternatively by cycloaddition reaction of azidonucleoside **9** with 5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylenethymidine (**13**), 5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-6-N-benzoyladenine (**14**) and 5'-deoxy-5'-C-ethynyl-2'-O,4'-C-methylene-4-N-benzoylcytidine (**15**), respectively (Scheme 1). The cycloaddition reaction may be carried out using different Cu(I) reagents, *e.g.* CuSO₄·5H₂O-sodium ascorbate in THF:^tBuOH:H₂O (1:1:1) or CuBr·SMe₂ in THF. The synthesis of azido compound **9** and alkynes **10-15** can be accomplished from a common synthon 4-C-hydroxymethyl-1,2-O-isopropylidene-β-L-threo-pentofuranose (**17**), which can easily be obtained from D-glucose following the

*Address correspondence to this author at the Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110 007, India; Tel: 00-91-11-27662486; E-mail: ashokenzym@yahoo.com.