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The Cu(I)-catalyzed Huisgen azide-alkyne 1,3-dipolar cycloaddition reaction in nucleoside, nucleotide and oligonucleotide chemistry

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1. Introduction

Pioneered by Huisgen in the 1960's[1], the 1,3-dipolar cycloaddition reaction between acetylenes and azides was brought back into focus by Sharpless and others[2] when they developed the concept of "click chemistry". This approach, based on the joining of smaller units mimics the approach used by nature to generate substances. This concept takes advantage of reactions that are modular, wide in scope, stereospecific, high yielding, and generate only non-offensive by-products to efficiently access new useful compounds. Moreover, to be completely "click", the process must involve simple reaction conditions, readily available starting materials and reagents, the use of no solvent, or a benign or easily removable solvent.[3] At first, the classical Huisgen 1,3-dipolar cycloaddition did not fall into the above definition, but the discovery of copper (I) salts catalyzing the reaction first by Medal and then by Sharpless[4] allowed chemists to evolve from harsh reaction conditions that lead to a mixture of 1,4- and 1,5- regio-isomers to a regioselective reaction which can be performed at room temperature in very short reaction times (Scheme 1). The Cu alkyne-azide cycloaddition (CuAAC) fit so well into the above definition that it has become almost synonymous of "click chemistry" itself.

Indeed, CuAAC proceeds in a variety of solvents including aqueous media which, combined with the relative innocuousness of the reactants, render it bio-compatible. Compared to other metal-catalyzed reactions, the use of Cu(I) presents the major advantages of being inexpensive and easy to handle (Most of the protocols involve the reduction of stable sources of Cu(II) such as $CuSO_4$ with sodium salts or the comproportionation of Cu(II)/Cu(0) species). In addition, the fact that both alkyne and azide functional groups can be incorporated into a wide range of compounds by several very general methods might also help explain the widespread use of this reaction (Scheme 2 and Scheme 3).[5] All these attributes, combined with the potentially favorable physicochemical properties of the resulting triazoles, has propelled the Cu(I)-catalyzed Huisgen cycloaddition to be one of the most popular and efficient reactions within the concept of click chemistry; and as a result, a burst in the number of publications on the topic has occurred in a last few years.

Over the last 40 years, the development of nucleic acids and nucleoside analogs for medicinal uses have had a marked impact on clinical chemotherapy as applied to antiviral and anticancer treatment. Numerous nucleoside analogs, for instance, were successfully developed for the treatment of human immunodeficiency viruses (HIV), hepatitis B virus (HBV), hepatitis C

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virus (HCV), herpes simplex virus (HSV), cytomegalovirus (CMV) or varicella zoster virus (VZV) (Figure 1) and various cancers (Figure 2).[6]

In parallel, studies of the properties of modified oligonucleotides led to the development of unnatural oligomers with huge therapeutic potential, especially for the treatment of diseases characterized by the expression of unwanted genes.[7] The structural diversity of active nucleosides as shown in Figure 2 and Figure 3 is the evidence that nucleosides analogs do not need to be close to their natural counterpart to be interesting and that any new structure is worth exploring. Therefore it seems logical that researchers turned their attention toward the possible benefits of innovative and new synthetic approaches such as the CuAAC for the synthesis of base- or sugar-modified nucleosides, nucleosides bioconjugates, and modified oligonucleotides. This review covers the literature up to March 2009 and has deliberately excluded postsynthetic DNA modifications since a review on this topic has recently been published.[8]

2. Nucleosides

2.1. Base Modified Nucleosides

The discovery of clinically useful nucleoside analogs, containing a five-membered heterocyclic base such as Ribavirin,[9] Bredinin,[10] or compound **1**[11] (Figure 3), provided a catalyst for creative applications of the CuAAC reaction toward medicinally relevant base modified nucleoside analogs.

Based on these compounds, the most common application of the Huisgen 1,3-cycloaddition has been the reaction of β-1'-azido sugar moieties **2** with various alkynes in order to form modified nucleosides **3** bearing a substituted 1,2,3-triazole base (Scheme 4). Thus, although some cases required a stoichiometric amount of Cu(I), the cycloaddition has been efficiently applied to different alkyne and sugar azide substrates leading to a variety of new nucleosides analogs.[12] Among all the compounds synthesized to date in this series by various laboratories, compound **3B** (R = $CONH_2$) exhibits a potent antiviral activity against vaccinia virus with high selectivity index [EC_{50} = 0.4 μM, selectivity index (SI) > 750].

The use of microwave irradiation has been investigated to enhance reaction yields and to accelerate cycloaddition rates. For example, Guezguez *et al.*[13] showed that, starting from compound **4**, a stoichiometric amount of CuI and not less that 24h was required to achieve complete reaction (Scheme 5). However, when the reaction was performed under microwave irradiation and in the presence of DIEA and CuI adsorbed on silica gel (1 g/mmol of azide), the dipolar cycloaddition proceeds cleanly in near quantitative yield in 1.5 to 3 min.

In the same manner, Ermolat'ev *et al.*[14] (Scheme 6) and Broggi *et al.*[15] (Scheme 7) showed that the Huisgen 1,3-cycloaddition can be completed in high yield in only few minutes using microwave irradiation.

In the search for novel cyclic adenosine diphosphate-ribose (cADPR **12**) mimics, Li *et al.*[16] synthesized compounds **11** in 4 steps, through the construction of a 4-amido-1,2,3-triazole nucleobase (Scheme 8). It is noteworthy that like endogenous cADPR, the targeted cyclo-pyrophosphates **11** appeared to induce Ca^{2+} release in intact human Jurkat T cells.

Interestingly, as part of the same project, Li and coworkers[17] developed an efficient method for the preparation of 5-iodo-1,4-disubsituted-1,2,3-triazole by a multicomponent one pot reaction of azide **13** and phenyl acetylene in presence of CuI and NBS (Scheme 9), opening new perspectives for further interesting modifications. According to the authors, the catalytic

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system used for the reaction provides both I^+ and Cu^+ *in situ* which allows the one pot trapping of the carbon anion intermediate **14** generated during the cycloaddition process.

Novel bis-triazolyl nucleosides were synthesized as anti-tobacco mosaic virus (TMV) agents by Xia *et al.*[18] using the azide/alkyne Huisgen reaction. Thus, compound **17** and analogs were obtained in good to excellent yields from **16** in the presence of $CuSO_4$ and sodium ascorbate in a mixture of water and THF, regardless of the nature of the alkyne (Scheme 10).

However, in the case of 5-azidotriazoles **18**, Cu(I)-promoted 1,2,3-triazole formation was not straightforward (Scheme 11). For instance, when compound **18A** was reacted with phenylacetylene, two completely unexpected products were formed, namely, amine **19A** and amide derivative **21A**. The formation of the anticipated triazole **20A** was never observed in presence or absence of copper catalyst under conventional heating or under microwave irradiation. In the case of the acyclo azido derivative **18B**, the triazolo compound was formed in moderate yield, but again the reduced product **19B** was observed. To explain this unusual reactivity, the authors presumed that the electron-deficient heterocycle that bears the azido group and the steric hindrance induced by the presence of the sugar moiety make 5-azidotriazole compounds **18** rather unsuitable partners for the 1,3-dipolar cycloaddition reaction. Thus, under mild Cu(II)-ascorbate conditions, compound **18A** can be reduced to **19B** and based on the work of Chang *et al.*[19] on the Cu-catalyzed multiple component reactions, the formation of amide **21A** could be explained by the mechanism outlined in Scheme 12.

However, despite the previous result, O'Mahony *et al.*[20] demonstrated that this type of reaction was possible with electron deficient rings such as purines by preparing adenosine dimers **24** linked by a 1,2,3-triazole ring (Figure 4).

Using the same kind of substrates, Cosyn *et al.*[21] synthesized two series of 2-(1,2,3-triazolyl) adenosine derivatives **27** and **29** using the CuAAC starting from the common intermediate **25** (Scheme 13). Thus, compounds **29** can be prepared in 2 steps first, by introduction of an azido group at the 2-position using a Cu(I)-catalyzed nucleophilic substitution with NaN_3 followed by Cu(I)-catalyzed 1,3-dipolar cycloaddition involving various alkynes. Similarly, the 1,2,3-triazol-4-yl analogs **27** were prepared from alkyne derivative **26** by reaction with appropriate azide in presence of $CuSO_4$ and sodium ascorbate at room temperature in a mixture of water and *t*-BuOH. Among all the compounds prepared, several 2-(1,2,3-triazol-1-yl)-N^6-methyl-subsituted adenosine derivatives displayed A_3 adenosine receptor affinities in the low nanomolar range with very high A_3/A_{2A} and moderate to high A_3/A_1 selectivity.

From these results, several observations have been made by the authors. First of all, despite very similar conditions for the Cu(I)-catalyzed nucleophilic substitution of **25** with NaN_3 and the conditions necessary for the CuAAC reaction, the one-pot two-step conversion of **25** to **29** was observed in disappointingly low yield. Secondly, during the preparation of **28**, the authors observed the formation of the tautomeric fused tetrazole form **30** (17%). Indeed, azide substituted π-deficient nitrogen heterocycles are known to spontaneously cyclizes to the corresponding fused tetrazole (Scheme 14).[22] In this case, despite that possible equilibrium, the cycloaddition proceeded smoothly however, some of the lower observed yields for the formation of compounds **29** could possibly be due to a shift of this equilibrium toward compound **30**.

Finally, the same team showed that the copper source used during the Sonogashira coupling could also induce the Huisgen cycloaddition in the presence of an azide group on the molecule (Scheme 15).[23]

As part of their efforts to find new drugs against tuberculosis (TB), Gupte *et al.*[24] extensively studied the importance of substitutions on their lead compounds, 5'-*O*-[*N*-(salicyl)sulfamoyl]

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adenosine (Sal-AMS, **33**) and its analog 2-Ph-Sal-AMS **34** (Scheme 16). Interestingly, modification of the C-2 position of the purine moiety with 4-substituted 1,2,3-triazoles appeared to be well tolerated and a majority of the compounds **36** possessed subnanomolar apparent inhibition constant (K_i^{app}) against aryl acid adenylating enzymes (AAAE) and submicromolar to micromolar antitubercular activities under iron deficient conditions (minimal inhibitory concentration, MIC_{99}).

2.2. Sugar Modified Nucleosides

In order to discover new derivatives potentially endowed with biological activity, the copper catalyzed azide/alkyne 1,3-dipolar cycloaddition has also been applied to the functionalization of nucleoside's sugar moieties. With this in mind, efficient regioselective synthesis of various pyrimidines[25] and adenosine[26] analogs were achieved by different teams (Figure 5). This strategy allowed Lee *et al.*[22d] to identified compound **39** as a new a-2,3-sialyltransferase inhibitor.

Over the past few years, locked nucleic acid (LNA) has received significant attention as nucleic acid analogs, displaying unprecended recognition of complementary nucleic acids.[27] LNA have promising antisense properties and were recognized for their potential in nanoscale engineering and microarray construction. Enderlin *et al.*[28] used the CuAAC for the synthesis of a double-headed nucleoside with a triazole linked to an additional thymine to the 6'-position of a locked nucleic acid -nucleoside monomer (Scheme 17).

As part of their anti-tuberculosis research program and based on the lead compound **46**, Somu *et al.*[29] also studied the potential replacement of the labile acyl phosphate function in compound **47** by a disubstituted triazole (Figure 6).

Recently, nucleoside analogues in which the furanose ring has been replaced by heterocyclic moieties have attracted special attention since some of them were reported to show antiviral and anticancer activities. Thus, Cao *et al.*[30] successfully developed an efficient solid phase parallel synthetic route to a bis–heterocycle library of uracil analogs, tethered to triazoles, using a polymer-supported seleno resin and the CuAAC as the key reaction (Scheme 18).

2.3. Nucleosides Bioconjugates

The Cu-catalyzed azide alkyne 1,3-dipolar cycloaddition has also been applied to the synthesis of new nucleoside bioconjugates. Indeed, its efficiency and simplicity rendered this reaction attractive for the covalent linkage of two molecular entities to provide biomolecules with novel properties such as biological activity, altered hydrophobicity, increased bioaffinity, or the ability to carry metal ions. For instance, a boronic acid-labeled thymidine-5'-triphosphate linked through a 14-atom tether using the CuAAC as the key reaction (Figure 7) was synthesized by Lin *et al.*[31] Compound **52** was recognized by a DNA polymerase and has been incorporated into a growing primer strand.

Working also on boron-bearing nucleic acids, Wojtczak *et al.*[32] developed a methodology involving the CuAAC for the synthesis of pyrimidine as well as purines nucleosides conjugates containing carborane and metallocarborane complexes (Figure 8). The behavior of compounds **53–56** designed mainly as potential boron carrier for boron neutron capture therapy (BNCT) of tumors is still under evaluation.

As a part of their study of modified DNA, Seela and coworkers got interested in the use of the CuAAC as an efficient way to label DNA. In order to evaluate the potential of their strategy they first worked at the nucleoside level and have been able to introduce coumarin dyes[33] and other azido derivative such as AZT[34] on different part of the nucleobase generating notably new fluorescent nucleoside bioconjugates **57a–59a** (Figure 9).

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In the same manner, Kosiova *et al*[35]. reported the preparation of fluorescent triazole linked coumarin nucleoside conjugates **60–62**, the linkage being this time on the sugar moiety (Figure 10).

Taking advantage of the versatility of the Cu(I) catalyzed 1,3-cycloaddition, Jin *et al.*[36] prepared a library of novel 1,2,3-triazole-fused oligonucleosides analogs (Figure 11) and interestingly, compound **64** derived from AZT showed a fairly good antibiotic activity against *E. coli* DH5 α.

Compound **68**[37] represents an attempt to synthesize a dual drug by click chemistry combining AZT and HIV-active compound **67** (Scheme 19). Interestingly, this "chimera" showed antiviral activity against wild type HIV-1 and mutant strains comparable to those observed for **67**.

Pleuromutilin **69** is a naturally occurring antibacterial agent (Figure 12) known to bind to bacterial ribosome in the peptidyl transferase center. Due to the presence of a permissible area near this center, numerous modifications of **69** have been investigated including the attachment of nucleoside derivatives in order to induce better binding through their inherent H-bonding properties and stacking abilities. Thus, the CuAAC has been used in a parallel synthetic strategy by Lolk *et al.*[38] to attach a wide range of nucleoside derivatives to Pleuromutilin **69** and it is noteworthy that the bioconjugates **70** kept their antibacterial activity and in some cases showed better affinity to the peptidyl transferase center in the ribosome than the natural Pleuromutilin.

Lee *et al.*[39] investigated the synthesis of potential inhibitors of fucosyl–transferases (Fuc-T). Fuc-T catalyzes the transfer of an L-fucose sugar from a guanosine diphosphate fucose to an acceptor substrate and is involved in several biological processes. Thus, the inhibition of this enzyme may provide a useful therapy for the control of inflammation or for combating tumor growth. Using the CuAAC as the key step of their strategy, the authors prepared a library of guanosine-5'-diphosphate (GDP) triazole. The direct synthesis in a microtiter plate and the absence of protective groups (even for the dianionic phosphate linkage) allowed *in situ* bio-evaluation (Scheme 20). Among the 85 compounds synthesized, **74** was a nanomolar inhibitor of human α-1,3-fucosyl–transferase.

As part of their project to develop anti-varicela-zoster virus (VZV) drugs, Jin *et al.*[40] prepared a set of a new type of carbohydrate conjugated thymidine analogs in order to potentially improve solubility and molecular recognition of active bicyclic furo[2,3-*d*]pyrimidine nucleosides (Scheme 21). Compound **76**, prepared from **75** in 5 steps, was reacted with various propargylic carbohydrates derivatives in presence of $CuSO_4$ and sodium ascorbate to afford the corresponding 1,4-disubstituted 1,2,3-triazoles **77**. The subsequent deprotection of **77** with catalytic sodium methoxide in methanol gave the opened ketone-type structure **78** whereas the use of methanolic ammonia only produced the expected compounds **79**. The biological study of compounds **78** and **79** is actually underway.

Another application of the Cu(I)-promoted 1,2,3-triazole formation was the synthesis of oligothiophene-nucleoside conjugates **80** and **81** by Jatsh *et al.*[41] (Figure 13). The authors showed that complementary thymidine- and adenosine-functionalized quaterthiophenes form recognition-driven superstructures *via* hydrogen bonding and other competing intermolecular forces, allowing them to characterize self aggregated fibers up to 30 μm in length (Figure 14).

Mindt *et al.*[42] developed the "click to chelate" approach which allowed them to synthesize the metal labeled-nucleoside conjugate **83** in a one pot procedure (Figure 22). They showed that the 1,4-disubstituted triazole forms an integral part of the metal chelating system and facilitates the incorporation of labeled complexes into biomolecules.

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3. Oligonucleotides

3.1- 1,2,3-Triazole as Replacement of the Phosphodiester Linkage

Oligonucleotide chemistry has also benefited from the development of the CuAAC. Thus, given the importance of non-natural oligodeoxyribonucleotide antisense agents acting as post-transcriptional gene silencing agents, several approaches based on repetitions of the Cu(I) catalyzed 1,3-dipolar cycloaddition as a key ligation process have been successfully developed (Figure 15) to replace the phosphodiester linkage in oligonucleotides by a 1,2,3-triazole.[43]

Interestingly, Isobe *et al.*[44] designed and synthesized the new 10-mer triazole-linked analog of DNA (10-mer ^{TL}DNA) **90** (Scheme 23). Their approach, optimized on solid phase, used microwave irradiated a CuAAC reaction to realize the chain elongation. Of significance, the artificial 10-mer ^{TL}DNA **90** was able to form a stable double strand with the complementary strand of natural DNA.

3.2- 1,2,3-Triazole as Linker for Solid Supported Synthesis

Solid phase synthesis is now the most common method used for the preparation of macromolecules such as peptides or nucleic acids. However the conditions necessary for the covalent attachment of the first monomer can be tricky. For instance, in the case of the use of an aminated solid support the loading can be a slow process and must be accomplished under rigorous exclusion of moisture. Potential partial loading, due these constraints, can result in unwanted side reactions and loss of purity for the final product. To circumvent these problems, Oyelere *et al.*[45] developed the azide-coated support **92** and used the versatility offered by the CuAAC to easily load different alkyne-functionalized nucleosides monomers (Scheme 24). The nucleoside-functionalized support **93** was shown to be suitable for solid phase synthesis of 15-mer and 30-mer oligonucleosides.

3.3- Post- and Presynthetic DNA Modifications

The efficiency and simplicity of azide-alkyne dipolar cycloaddition for coupling organic fragments proved to be an attractive way to “decorate” oligonucleotides strands. In this domain, two main strategies co-exist called pre- and post-synthetic labeling. The post labeling term is employed when the modification occurred on the already formed DNA strand in opposition to the pre-synthetic strategy where the labeling is introduced before the formation of the oligonucleotide (Scheme 25).

An excellent article[9] was recently published summarizing the recent applications of the CuAAC for postsynthetic DNA modifications. This application has been successfully used for the preparation of surface immobilized DNA, DNA-protein conjugates, cyclic, and branched DNA structures, but also for analytical purposes, labeling of DNA and for DNA metallization. In light of the recent review, we decided to limit this section to the only pre-synthetic strategy approach.

In order to increase the intracellular delivery of nucleotides, Godeau *et al.*[46] used a triazole linker to prepare some lipid-conjugated oligonucleotides **97** (Scheme 25). These compounds appeared to efficiently inhibit HCV internal ribosome entry site (IRES)-mediated translation in human hepatic cells.

The duplex stability of modified oligonucleotides has also been studied by Kocalka *et al.*[47] who used a one pot azidation procedure under microwave irradiation to form different 2’-deoxyuridines substituted on their 5-position by a 1,2,3-triazole ring. The nucleoside analogs **99** were then introduced into nonamer oligonucleotides by phosphoramidite chemistry (Scheme 27). Interestingly, while single modifications led to decreased duplex stability, the

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stacking of four consecutive modifications led to enhanced duplex stability, especially for DNA-RNA duplexes.

The "fleximers" are a special class of modified nucleosides where the nucleobase is splinted, but still retain the key recognition of DNA bases. Thus, in order to study these unusual nucleoside analogs, Chittepu *et al.*[48] reported the synthesis of new 1,2,3-triazole nucleosides analogs **102** and incorporated their phosphoramidite building blocks into DNA (Scheme 28). In this particular case, these flexible nucleosides appeared to behave as an abasic site with a destabilizing effect on the DNA duplexes.

4. Conclusion

So far, one might think that the concept of "click chemistry" looks more or less like a big menu proposing a single dish but, as a matter of fact, this unique dish appeared mouth-watering and inviting for a lot of chemists. Because of its modularity, its high yields, and its simple conditions and purification procedures the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction between alkynes and azides has been suitable for the synthesis of a large number of modified nucleosides and oligonucleotides with a broad range of applications. As we have seen, the possibilities opened by this reaction have not been only attractive for the formation of 1,2,3-triazoles as bioisosteres, or active moieties, but also for the use of 1,2,3-triazoles as a linker to a solid support or to form probes and bioconjugates. In brief, the CuAAC has a huge potential especially if researcher start to really exploit the relative innocuousness of the reactants used during this reaction to bridge the gap between the chemistry and the biology of nucleosides and nucleotides, allowing direct evaluation in specialized bioassays.

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Biographies

Franck Amblard was born in Châteauroux, France. He studied chemistry at the University of Orléans (France), where he received his Ph.D. in 2004 under the guidance of Professor Luigi A. Agrofoglio working on the synthesis of new nucleosides analogs using metathesis and palladium-catalyzed reactions. In 2005, he moved to the USA to join Professor Raymond F. Schinazi's research group at Emory University (Atlanta, GA) as a postdoctoral fellow. In 2008 he was appointed Instructor at the Department of Pediatrics, Emory University School of Medicine. His main research interests involve the design, the synthesis and the study of nucleosides analogs as potential antiviral agents.

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Jong Hyun Cho was born in Gim-hae, South Korea, in 1967. In 2002, he received his Ph.D. in Organic Chemistry from Seoul National University (South Korea) working on biologically active peptide mimetics under the direction of Professor B. M. Kim. He then joined Professor Chung K. Chu's group at the Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia where he studied nucleoside chemistry and medicinal chemistry from 2003 to 2006. He is currently working as a postdoctoral research fellow for Professor Raymond F. Schinazi at Emory University. His research interests include synthetic approaches to nucleosides and peptidomimetics presenting antiviral activities against DNA and RNA viruses.

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Raymond F. Schinazi was born in Alexandria, Egypt in 1950. In 1976, he received his Ph.D. in Organic Chemistry from Bath University on ellipticine, a DNA intercallator, under the direction of Professor Malcolm Sainsbury. He then joined Professor William H. Prusoff in the Department of Pharmacology at Yale University. He is currently the Frances Winship Walter Professor of Pediatrics at Emory University and a Senior Research Career Scientist at the Atlanta Department of Veterans Affairs. Professor Schinazi is the recipient of numerous awards, including an Honorary DSc from the University of Bath, the Georgia Biomedical Industry Growth Award, the Bruce Witte Award, and the 2006 Distinguished Scientist Award from the Hepatitis B Foundation. He is coinventor of two of the most widely used anti-HIV and HBV drugs namely, Lamivudine and Emtricitabine. His research interests include the discovery and the development of antiviral and anticancer agents, focusing on nucleoside analogs.

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References and notes

1. Huisgen R, Szeimies G, Möbius L. Chem.Ber 1967;100:2494.
2. For reviews see: a)Kolb HC, Sharpless KB. Drug Discovery Today 2003;8:1128. [PubMed: 14678739] b)Bock VD, Hiemstra H, van Maarseveen JH. Eur. J. Org. Chem 2006;51 c)Binder WH, Kluger C. Curr. Org. Chem 2006;10:1791. d)Binder WH, Sachsenhofer R. Macromol. Rapid Commun 2007;28:15. e)Moorhouse AD, Moses JE. Chem. Med. Chem 2008;3:715. [PubMed: 18214878] f) Binder WH, Sachsenhofer R. Macromol. Rapid Commun 2008;29:952. g)Lutz J-F, Zarafshani Z. Adv.Drug. Deliv. Rev 2008;60:958. [PubMed: 18406491]
3. Kolb HC, Finn MG, Sharpless KB. Angew.Chem 2001;40:2004. [PubMed: 11433435]
4. Tornφe, CW.; Meldal, M. Proceedings of the Second International and the Seventeenth American Peptide Symposium; 2001. p. 263 b) Tornφe CW, Christensen C, Meldal M. J.Org. Chem 2002;67:3057. [PubMed: 11975567] c) Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. Angew. Chem. Int. Ed 2002;41:2565.
5. For Pd-catalyzed Sonogashira reaction on nucleosides see: a)Agrofoglio LA, Gillaizeau I, Saito Y. Chem. Rev 2003;103:1875. [PubMed: 12744695] For the preparation of alkynes from aldehydes: b) Roth GJ, Liepold B, Mömllller SG, Bestmann HJ. Synthesis 2004;59 For a review on the preparation of azidonucleosides see: c)Pathak T. Chem. Rev 2002;102:1623. [PubMed: 11996546]
6. a) Nucleosides and nucleotides as antitumor and antiviral agents; Chu CK, Baker DC. 1993New YorkPlenum Press For a general review on antiviral drugs see: b)De Clercq E. J. Clin. Virol 2004;30:115. [PubMed: 15125867] For a recent review on the anti-HCV compounds in development including nucleosides analogs see: c)Liu-Young G, Kozal M. J. AIDS Patient Care STDS 2008;22:449. For a recent review on the current antiviral therapy of chronic hepatitis B see: d)Ayoub WS, Keefe EB. Aliment. Pharmacol. Ther 2008;28:167. [PubMed: 18466358] For a review on HIV-1 reverse transcriptase inhibitors see: e)Jochmans D. Virus Res 2008;134:157. [PubMed: 18313784] f) For reviews on nucleosides as antitumor agents see: i)Blagosklonny MV. Cell Cycle 2004;3:1035. [PubMed: 15254418] ii)Robak T, Korycka A, Kasznicki M, Wrzesien-Kus A, Smolewski P. Curr.Cancer Drug Targets 2005;5:421. [PubMed: 16178817] iii)Miura S, Izuta S. Curr.Drug Targets 2004;5:191. [PubMed: 15011952]
7. For reviews on therapeutic applications of nucleic acids see: a)Uhlmann E, Peyman A. A. Chem. Rev 1990;90:543. b)Praseuth D, Guieysse AL, Helene C. Biochim. Biophys. Acta 1999;181 c)Micklefield J. Curr. Med. Chem 2001;8:1157. [PubMed: 11472234]
8. Gramlich PME, Wirges CT, Manetto A, Carell T. Angew. Chem. Int. Ed 2008;47:8350.
9. Lau JY, Tam RC, Liang TJ, Hong Z. Hepatology 2002;35:1002. [PubMed: 11981750]and references cited therein.
10. Mizushina Y, Matsukage A, Sakaguchi K. Biochim. Biophys. Acta 1998;1403:5. [PubMed: 9622583] and references cited therein.
11. Alvarez R, Valazquez S, San-Felix A, Aquaro S, De Clercq E, Perno C-F, Karlsson A, Balzarini J, Camarasa MJ. J. Med. Chem 1994;37:4185. [PubMed: 7527463]
12. a) Perez-Castro I, Caamano O, Fernandez F, Garcia MD, Lopez C, De Clercq E. Org. Biomol. Chem 2007;5:3805. [PubMed: 18004460] b) Cho JH, Bernard DL, Sidwell RW, Kern ER, Chu CK. J. Med. Chem 2006;49:1140. [PubMed: 16451078] c) Goeminne A, McNaughton M, Bal G, Surpateanu G, Van der Veken P, De Prol S, Versees W, Steyaert W, Apers S, Haemers A, Augustyns K. Bioorg. Med. Chem. Lett 2007;17:2523. [PubMed: 17317181] d) Broggi J, Diez-Gonzalez S, Petersen JL, Berteina-Raboin S, Nolan SP, Agrofoglio LA. Synthesis 2008:141. e) Broggi J, Joubert N, Aucagne V, Zevaco T, Berteina-Raboin S, Nolan SP, Agrofoglio LA. Nucleosides Nucleotides 2007;26:779. f) Pradere U, Roy V, McBrayer TR, Schinazi RF, Agrofoglio LA. Tetrahedron 2008;64:9044.
13. Guezguez R, Bougrin K, El Akri K, Benhida R. Tetrahedron Lett 2006;47:4807.
14. Ermolat'ev DS, Metha VP, Van der Eycken EV. QSAR Comb. Sci 2007;26:1266.

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15. a) Broggi J, Joubert N, Aucagne V, Berteina-Raboin S, Diez-Gonzalez S, Nolan SP, Topalis D, Deville-Bonne D, Balzarini J, Neyts J, Andrei G, Snoeck R, Agrofoglio L. Nucleosides Nucleotides 2007;26:1391. b) Broggi J, Joubert N, Diez-Gonzalez S, Berteina-Raboin S, Zevaco T, Nolan SP, Agrofoglio L. Tetrahedron 2009;65:1162.
16. Li L, Lin B, Yang Z, Zhang L, Zhang L. Tetrahedron Lett 2008;49:4491.
17. Li L, Zhang G, Zhu A, Zhang L. J. Org. Chem 2008;73:3630. [PubMed: 18357998]
18. a) Xia Y, Li W, Qu F, Fan Z, Liu X, Berro C, Rauzy E, Peng L. Org. Biomol. Chem 2007;5:1695. [PubMed: 17520136] b) Li W, Xia Y, Fan Z, Qu F, Wu Q, Peng L. Tetrahedron Lett 2008;49:2804.
19. a) Cho SH, Yoo EJ, Bae I, Chang SJ. Am. Chem. Soc 2005;127:16046. b) Yoo EJ, Ahlquist M, Bae I, Sharpless KB, Fokin V, Chang S. J. Org. Chem 2008;73:5520. [PubMed: 18557650]
20. O'Mahony G, Ehrman E, Grotli M. Tetrahedron Lett 2005;46:6745.
21. Cosyn L, Palaniappan KK, Kim S-K, Duong HT, Gao Z-G, Jacobson KA, Van Calenbergh S. J. Med. Chem 2006;49:7373. [PubMed: 17149867]
22. a) Temple C, Kussner CL, Montgommery JA. J. Org. Chem 1966;31:2210. b) Lioux T, Gosselin G, Mathe C. Eur. J. Org. Chem 2003;20:3997.
23. Cosyn L, Gao Z-G, Van Rompaey P, Lu C, Jacobson KA, Van Calenbergh S. Bioorg. Med. Chem 2006;14:1403. [PubMed: 16266807]
24. Gupte A, Boshoff HI, Wilson DJ, Neres J, Labello N, Somu RV, Xing C, Barry CE, Aldrich CC. J. Med. Chem 2008;51:7495. [PubMed: 19053762]
25. a) Zhou L, Amer A, Korn M, Burda R, Balzarini J, De Clercq E, Kern ER, Torrence PF. Antiviral Chem. Chemother 2005;16:375. b) Ding H, Yang R, Song Y, Xiao Q, Wu J. Nucleosides Nucleotides 2008;27:368. c) Byun Y, Vogel SR, Phipps AJ, Carnrot C, Eriksson S, Tiwari R, Tjarks W. Nucleosides Nucleotides 2008;27:244. d) Lee L, Chang K-H, Valiyev F, Liu H-J, Li W-S. J. Chin. Chem. Soc 2006;53:1547.
26. O'Mahony G, Svensson S, Sundgreen A, Grφtli M. Nucleosides Nucleotides 2008;27:449.
27. Kaur H, Babu BR, Maiti S. Chem. Rev 2007;107:4672. [PubMed: 17944519]
28. Enderlin G, Nielsen P. J. Org. Chem 2008;73:6891. [PubMed: 18672932]
29. Somu RV, Boshoff HI, Qiao C, Bennet EM, Barry CE, Aldrich CC. J. Med. Chem 2006;49:31. [PubMed: 16392788]
30. Cao J, Huang X. J. Comb. Chem 2008;10:526. [PubMed: 18553984]
31. a) Lin N, Yan Z, Altier C, Li M, Carrasco N, Suyemoto M, Johnston L, Wang S, Wang Q, Fang H, Caton-Williams J, Wang B. Nucleic Ac. Res 2007;35:1222. b) Li M, Lin N, Huang Z, Du L, Altier C, Fang H, Wang B. J. Am. Chem. Soc 2008;130:12636. [PubMed: 18763762]
32. a) Olejniczak A, Wojtczak BA, Lesnikowski Z. Nucl. Nucl. Nucl. Acids 2007;26:1611. b) Wojtczak BA, Andrysiak A, Gruner B, Lesnikowski Z. J. Chem. Eur. J 2008;14:10675.
33. a) Seela F, Sirivolu VR. Org. Biomol. Chem 2008;6:1674. [PubMed: 18421402] b) Seela F, Sirivolu VR, Chittepu P. Bioconjugate Chem 2008;19:211. c) Seela F, Ming X. Helv. Chim. Acta 2008;91:1181. d) Ming X, Leonard P, Heindl D, Seela F. Nucl. Acid Sym. Ser 2008;52:471.
34. a) Seela F, Sirivolu VR. Chem. Biodiv 2006;3:509. b) Seela F, Sirivolu VR. Helv. Chim. Acta 2007;90:535. c) Seela F, Sirivolu VR. Nucleosides Nucleotides 2007;26:597.
35. Kosiova I, Kovackova S, Kois P. Tetrahedron 2007;63:312.
36. Jin P-Y, Jin P, Ruan Y-A, Ju Y, Zhao Y-F. Synlett 2007:3003.
37. Danel K, Larsen LM, Pedersen EB, Sanna G, La Colla P, Loddo R. Bioorg. Med. Chem 2008;16:511. [PubMed: 17904371]
38. Lolk L, Pφhlsgaard J, Jepsen AS, Hansen LH, Nielsen H, Steffansen SI, Sparving L, Nielsen AB, Vester B, Nielsen P. J. Med. Chem 2008;51:4957. [PubMed: 18680270]
39. Lee LV, Mitchell ML, Huang S-J, Fokin VV, Sharpless B, Wong C-H. J. Am. Chem. Soc 2003;125:9588. [PubMed: 12904015]
40. a) Jin X, Yang R, Jin P, Xiao Q, Ju Y. Synthesis 2007;19:2967. b) Jin X, Ding H, Yang R, Xiao Q, Ju Y. Synthesis 2008:865.
41. Jatsch A, Kopyshev A, Mena-Osteritz E, Bäuerle P. Org. Lett 2008;10:961. [PubMed: 18260670]

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42. Mindt TL, Struthers H, Brans L, Anguelov T, Schweinsberg C, Maes V, Tourwe D, Schibli R. J. Am. Chem. Soc 2006;128:15096. [PubMed: 17117854]

43. a) Nuzzi A, Massi A, Dondoni A. QSAR Comb. Sci 2007;26:1191. b) Lucas R, Neto V, Hadj Bouazza A, Zerrouki R, Granet R, Krausz, Champavier Y. Tetrahedron Lett 2008;49:1004. c) Lucas R, Zerrouki R, Granet R, Krausz, Champavier Y. Tetrahedron 2008;64:5467.

44. Isobe H, Fujino T, Yamazaki N, Guillot-Nieckowski M, Nakamura E. Org. Lett 2008;10:3729. [PubMed: 18656947]

45. Oyelere AK, Chen PC, Yao LP, Boguslavsky N. J. Org, Chem 2006;71:9791. [PubMed: 17168598]

46. Godeau G, Staedel C, Barthelemy P. J. Med. Chem 2008;51:4374. [PubMed: 18605715]

47. Kocalka P, Andersen NK, Jensen F, Nielsen P. Chem. Bio. Chem 2007;8:2106.

48. Chittepu P, Sirivolu VR, Seela F. Bioorg. Med. Chem 2008;16:8427. [PubMed: 18774721]

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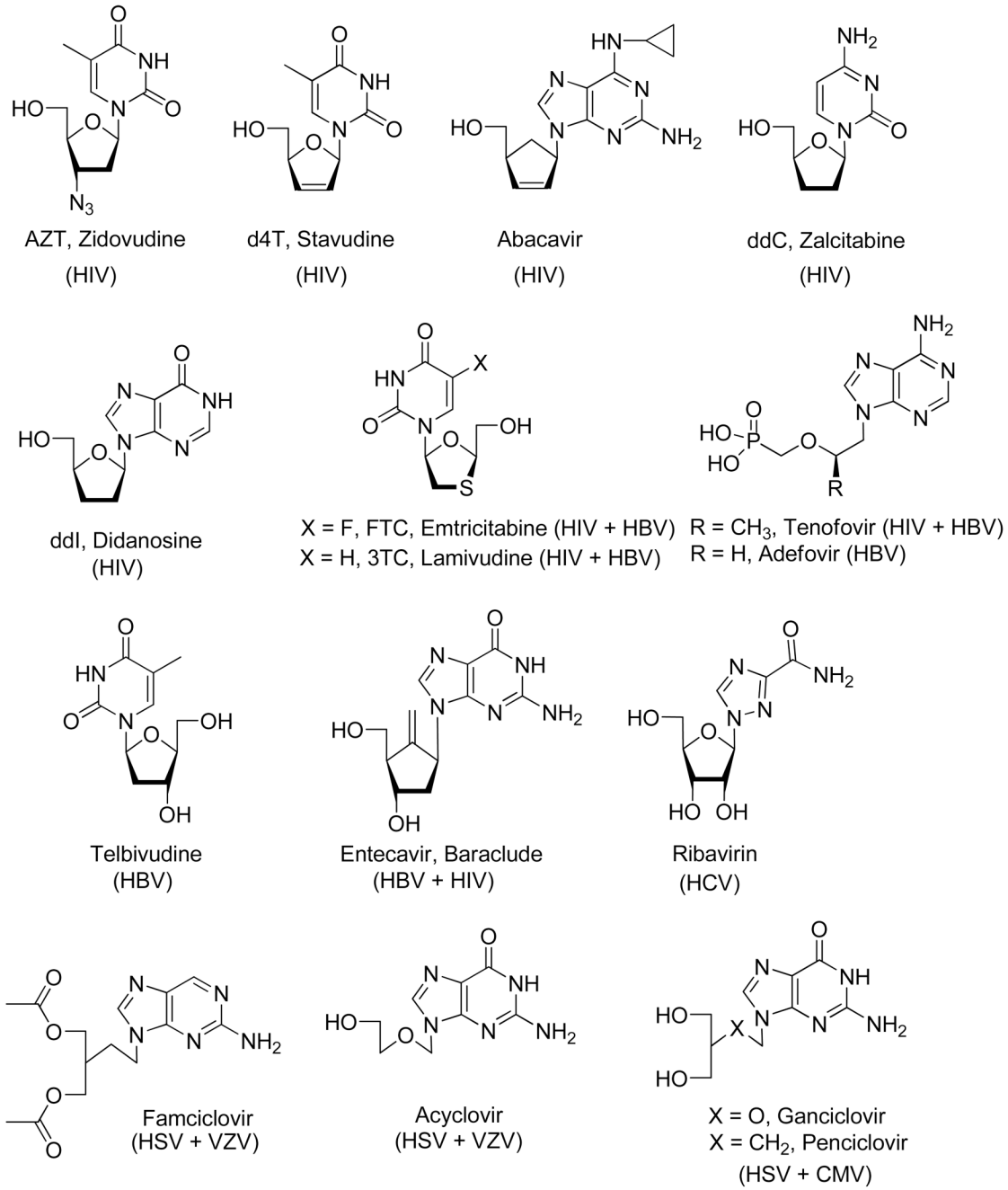

Figure 1.
Nucleoside Analogs Used for the Treatment of HIV, HBV, HCV, HSV, CMV and VZV.

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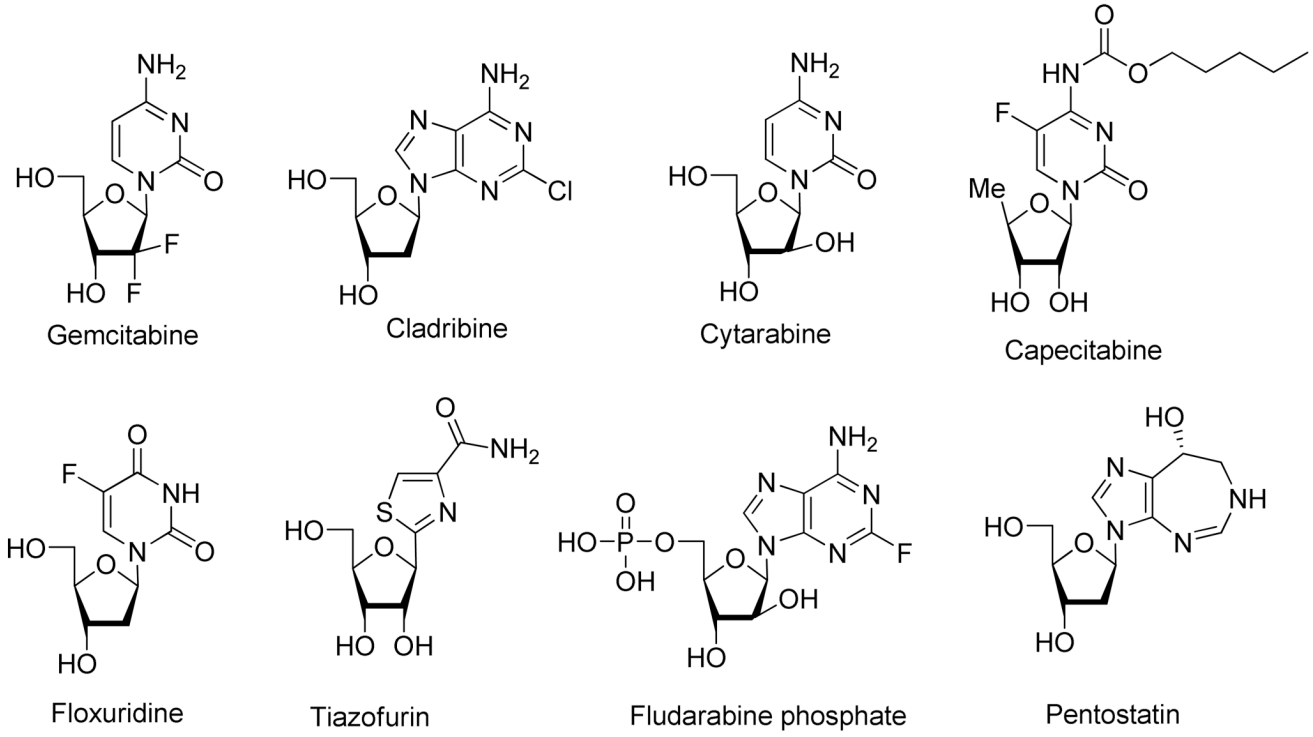

Figure 2.
Nucleoside Analogs Used for the Treatment of Various Cancers.

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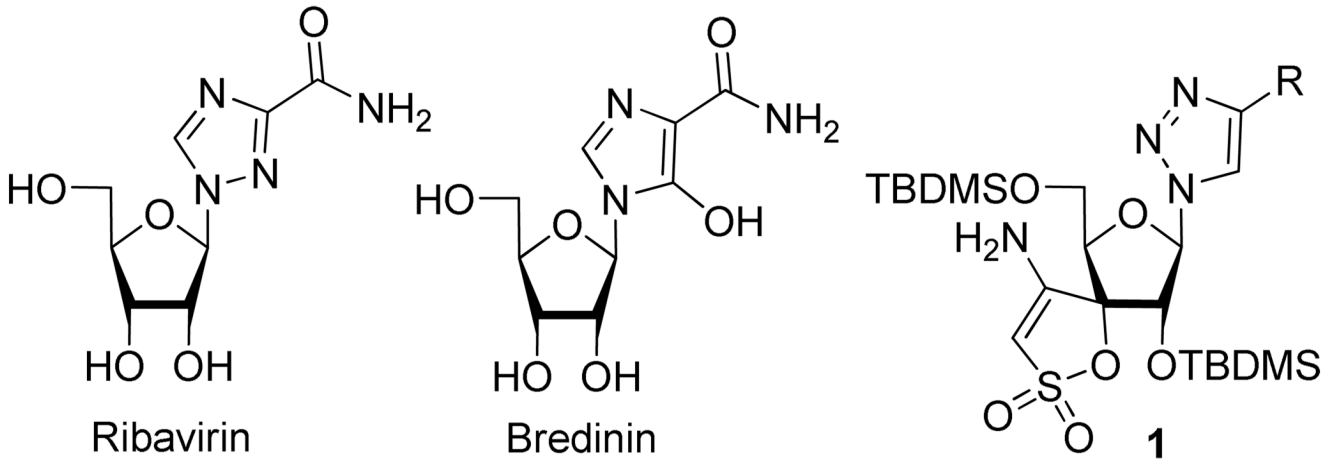

Figure 3.
Actives Five Membered Heterocyclic Based Nucleosides Analogs.

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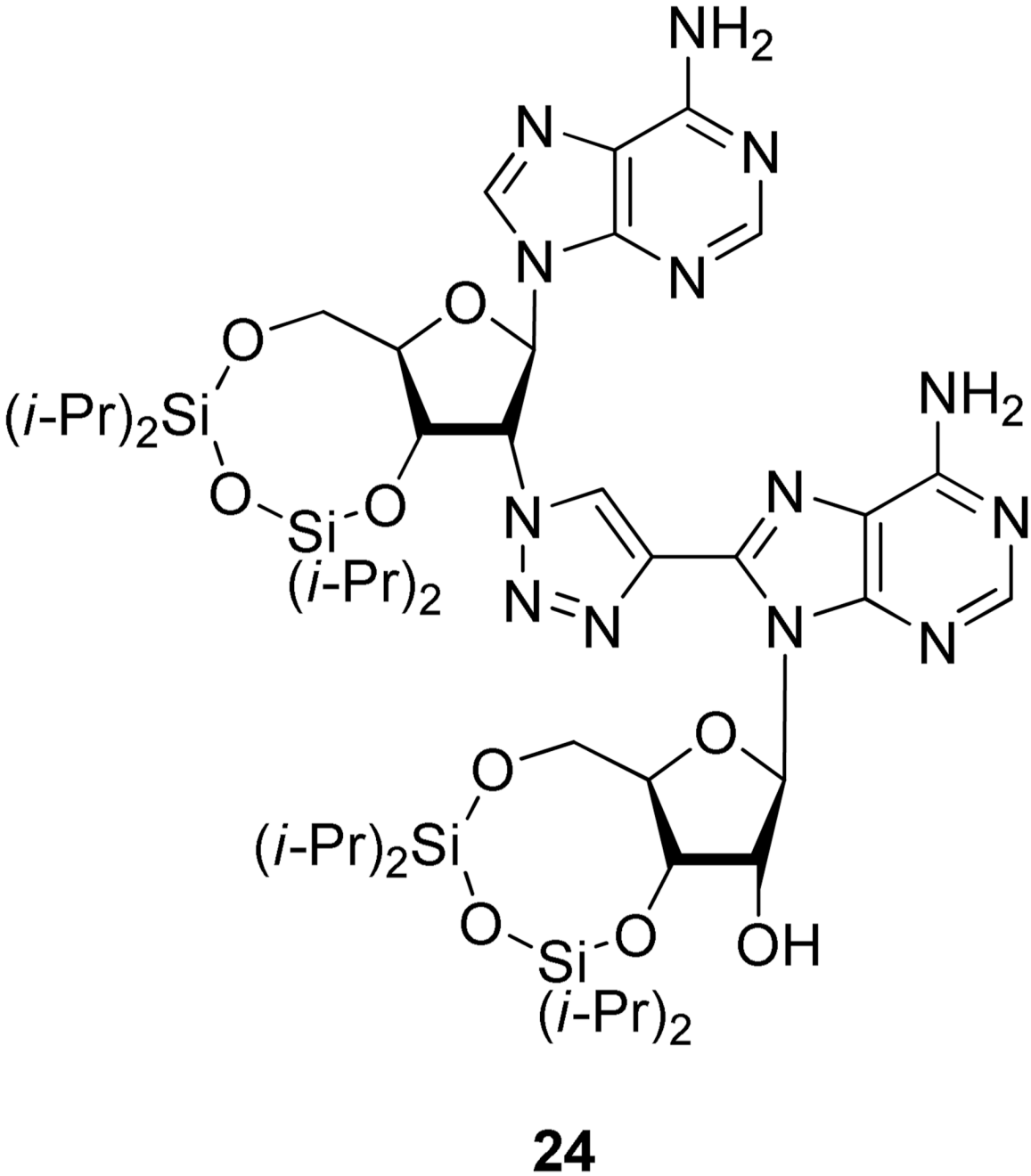

Figure 4.
Adenosine Dimer 24.

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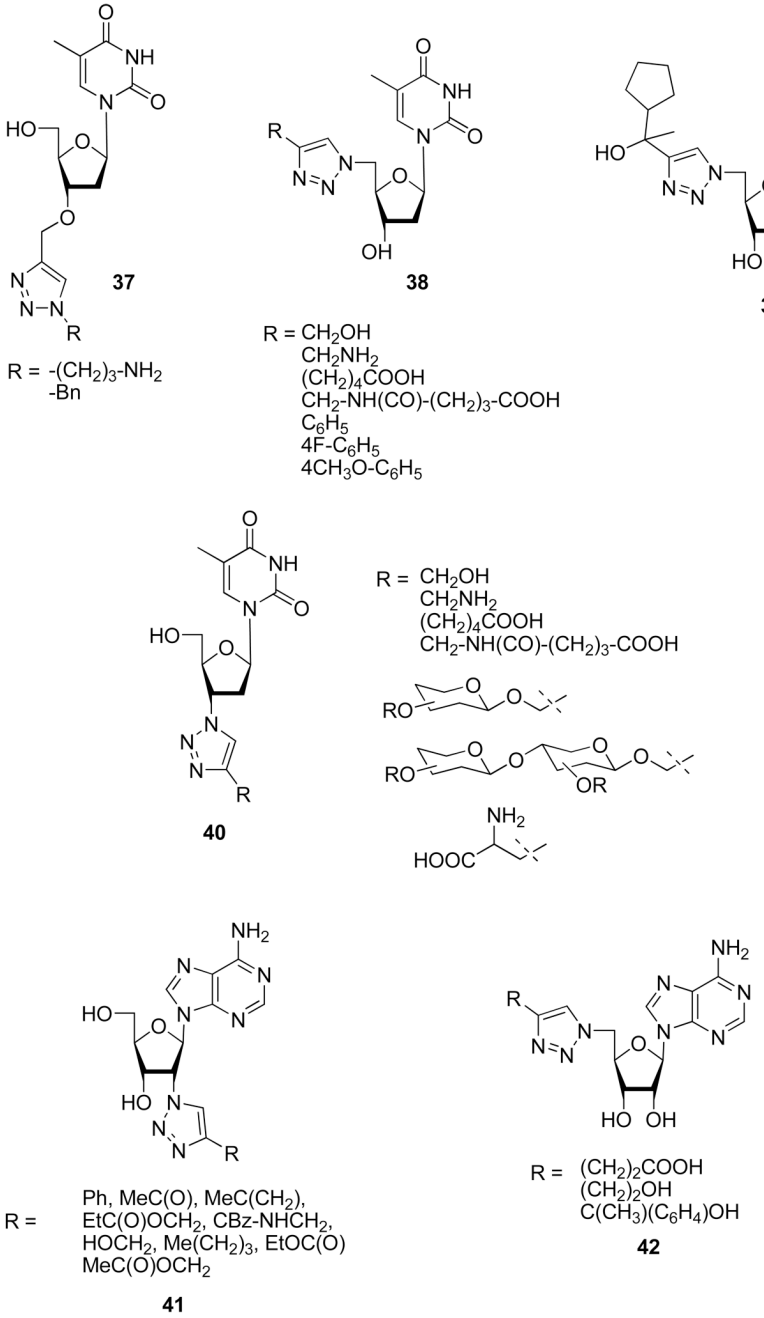

Figure 5.
Structures of Compounds 37–42.

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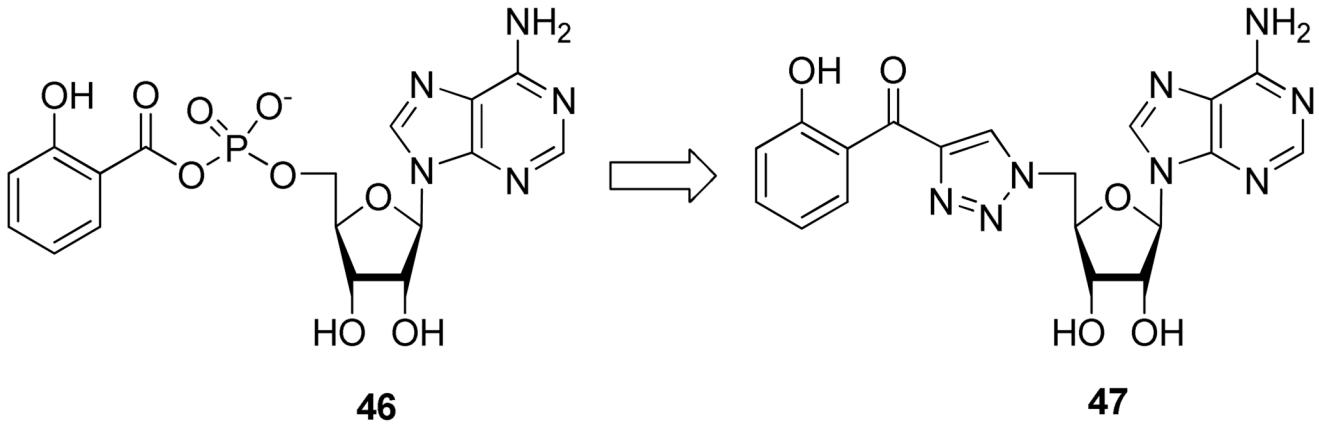

Figure 6.
Rational for the Design of Compound 47.

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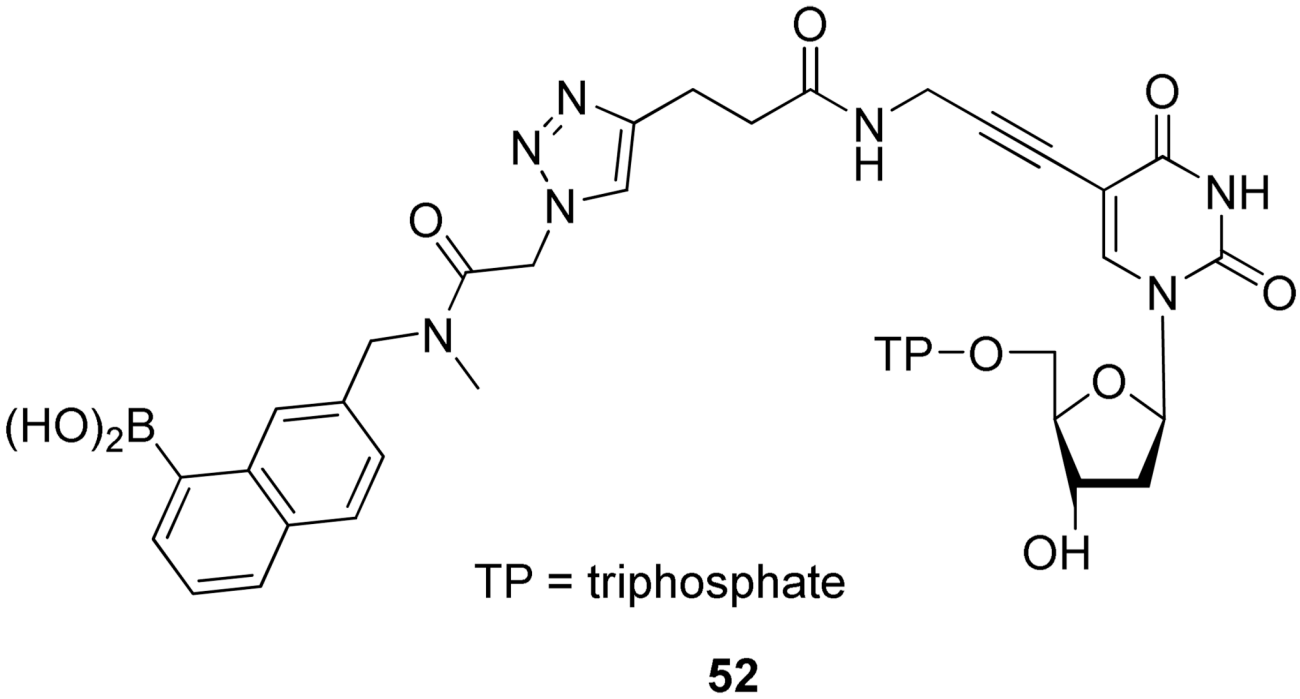

Figure 7.
Chemical Structure of Boronic Acid-Labeled Thymidine-5'-Triphosphate 52.

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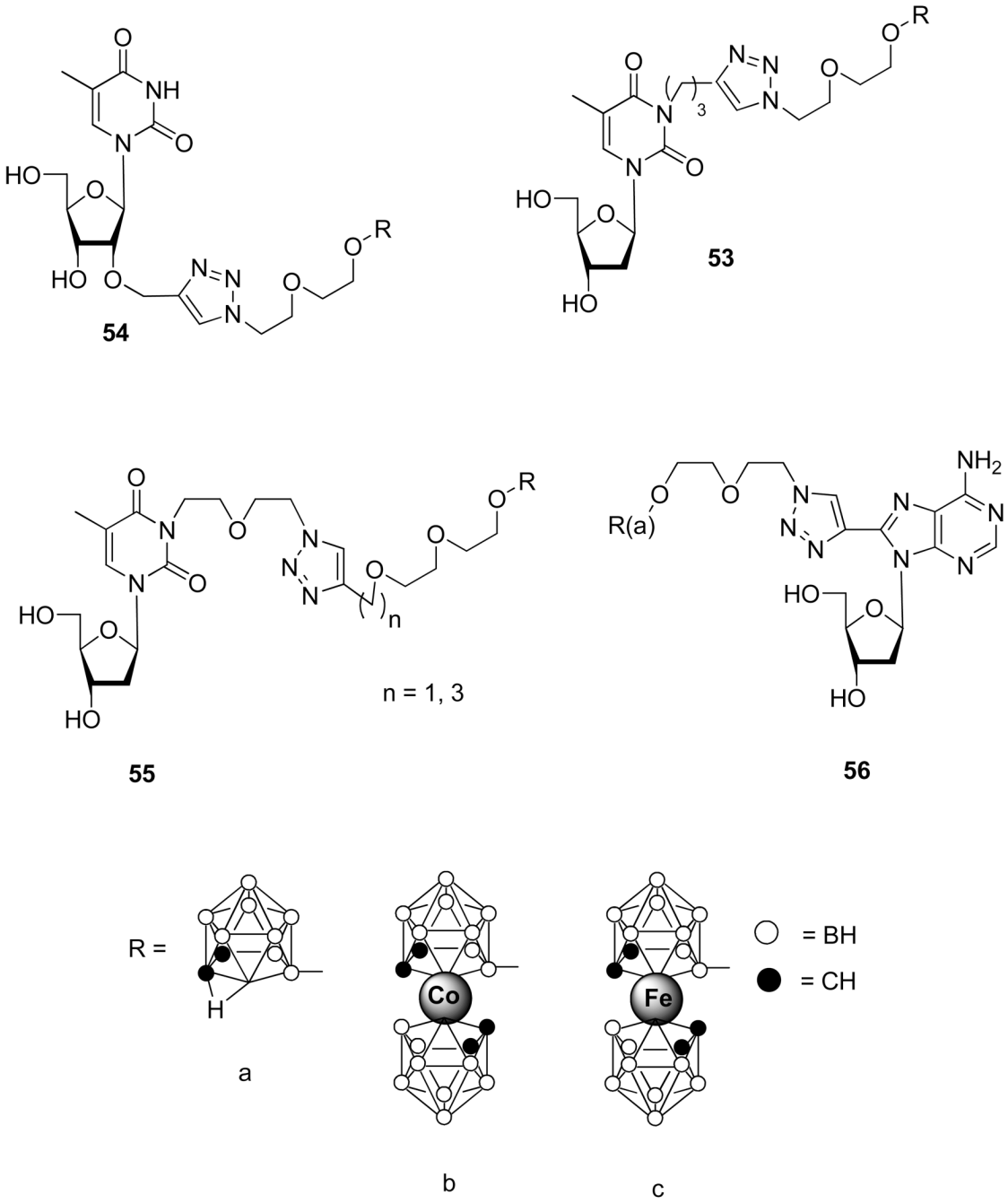

Figure 8.
Structure of Nucleosides Conjugates 53–56.

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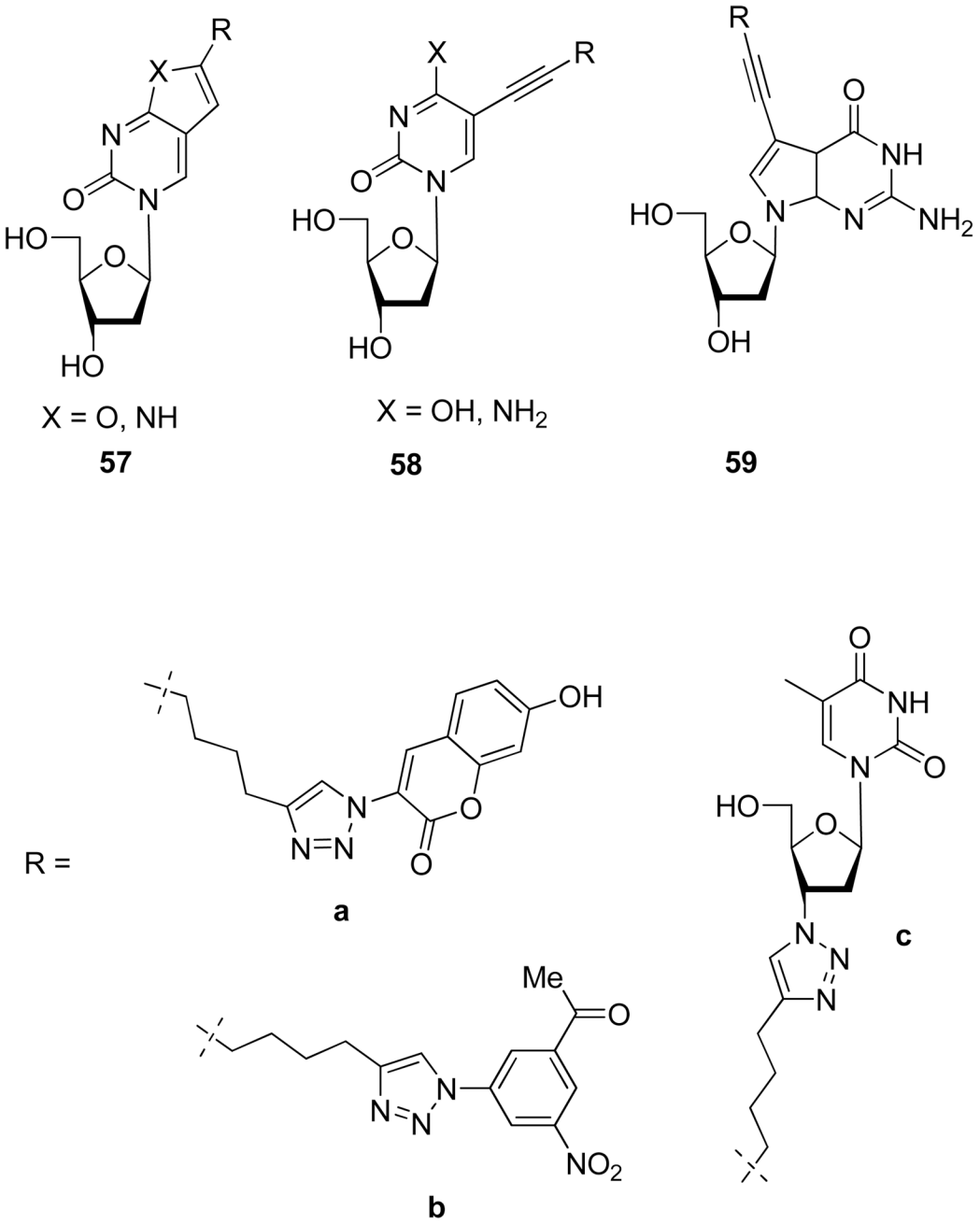

Figure 9.
Structure of Nucleosides Conjugates 57–59.

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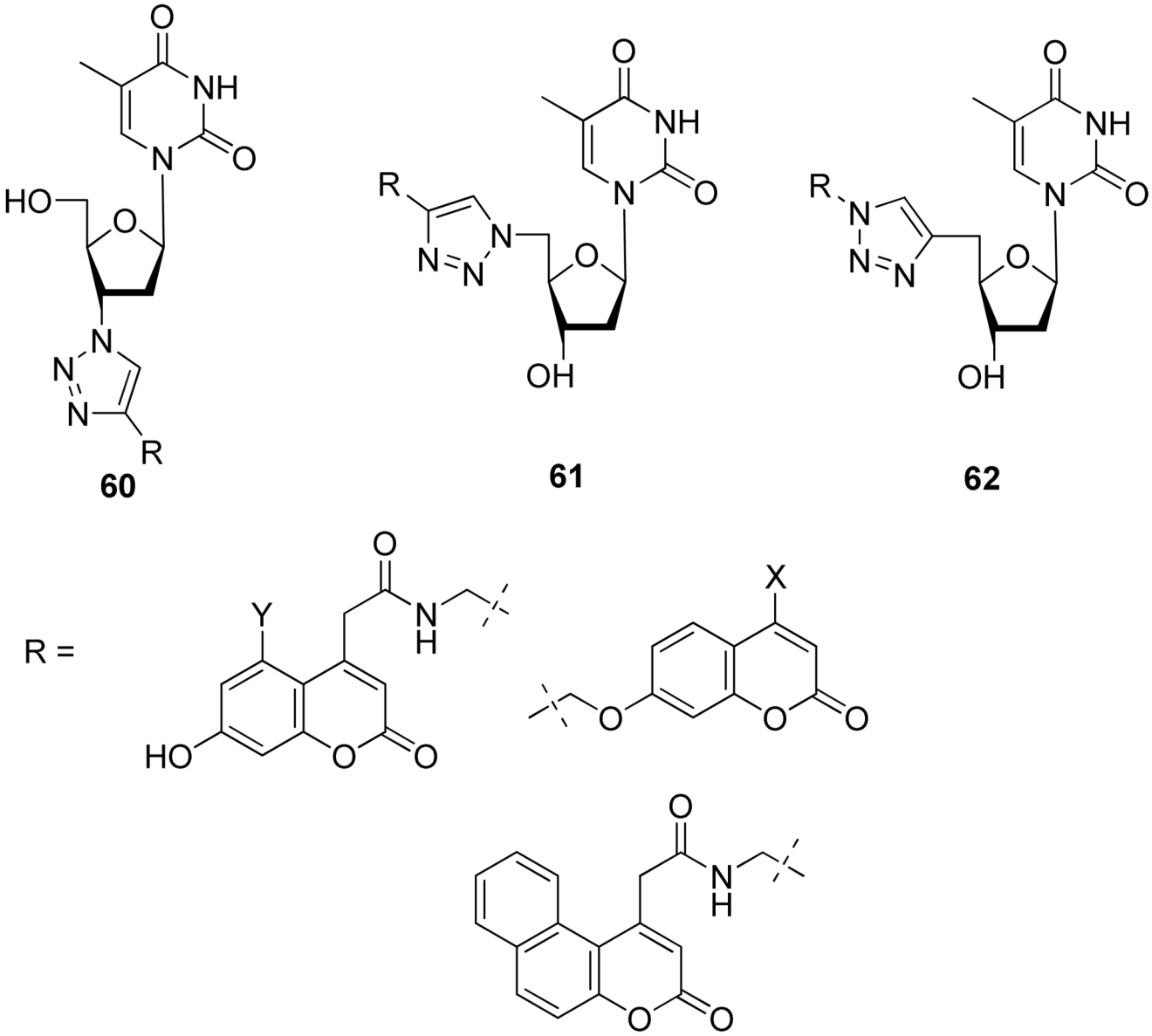

Figure 10.
Structure of Nucleosides Conjugates 60–62.

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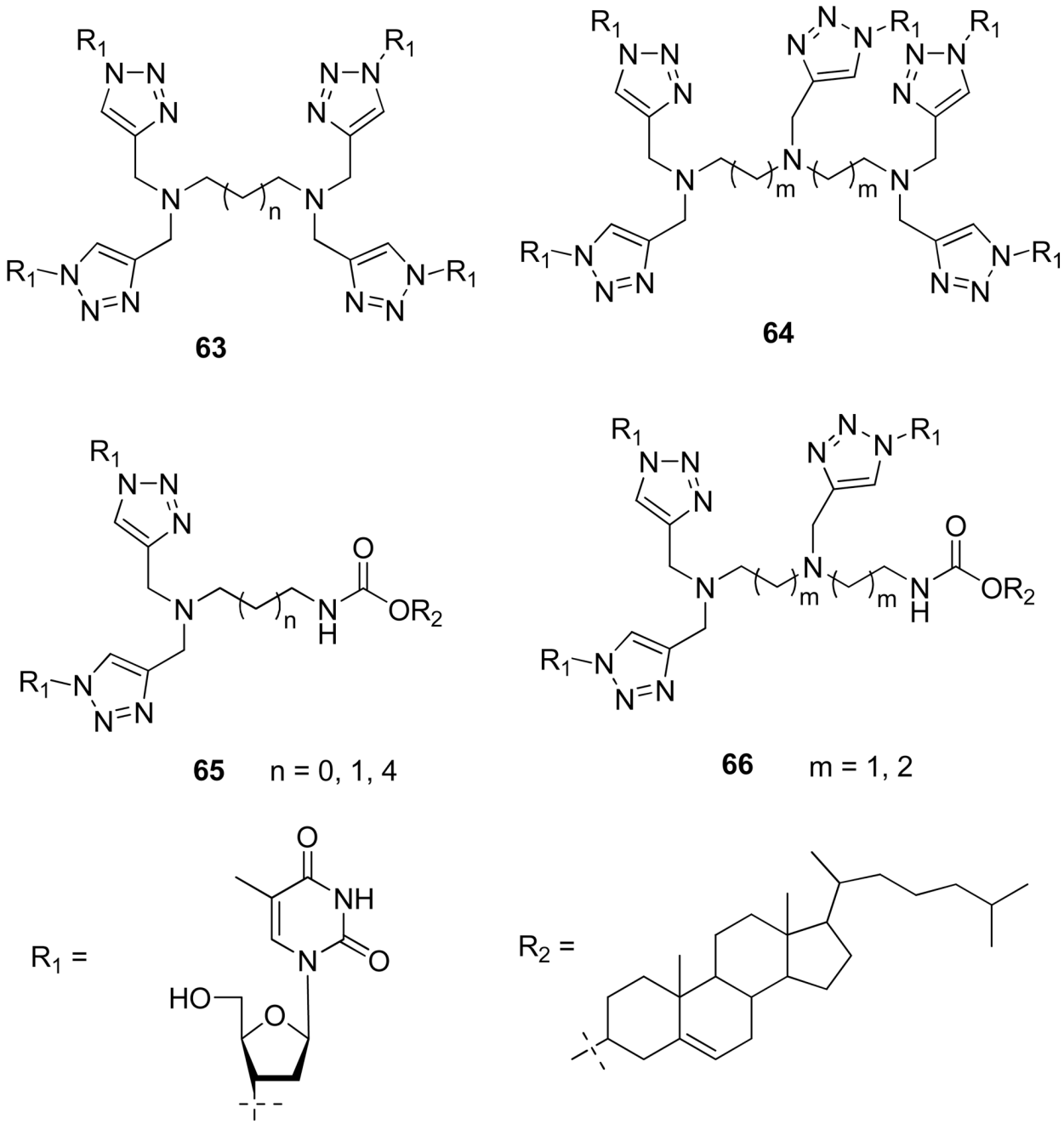

Figure 11.
Structure of 1,2,3-Triazole-Fused Oligonucleosides Analogs 63–66.

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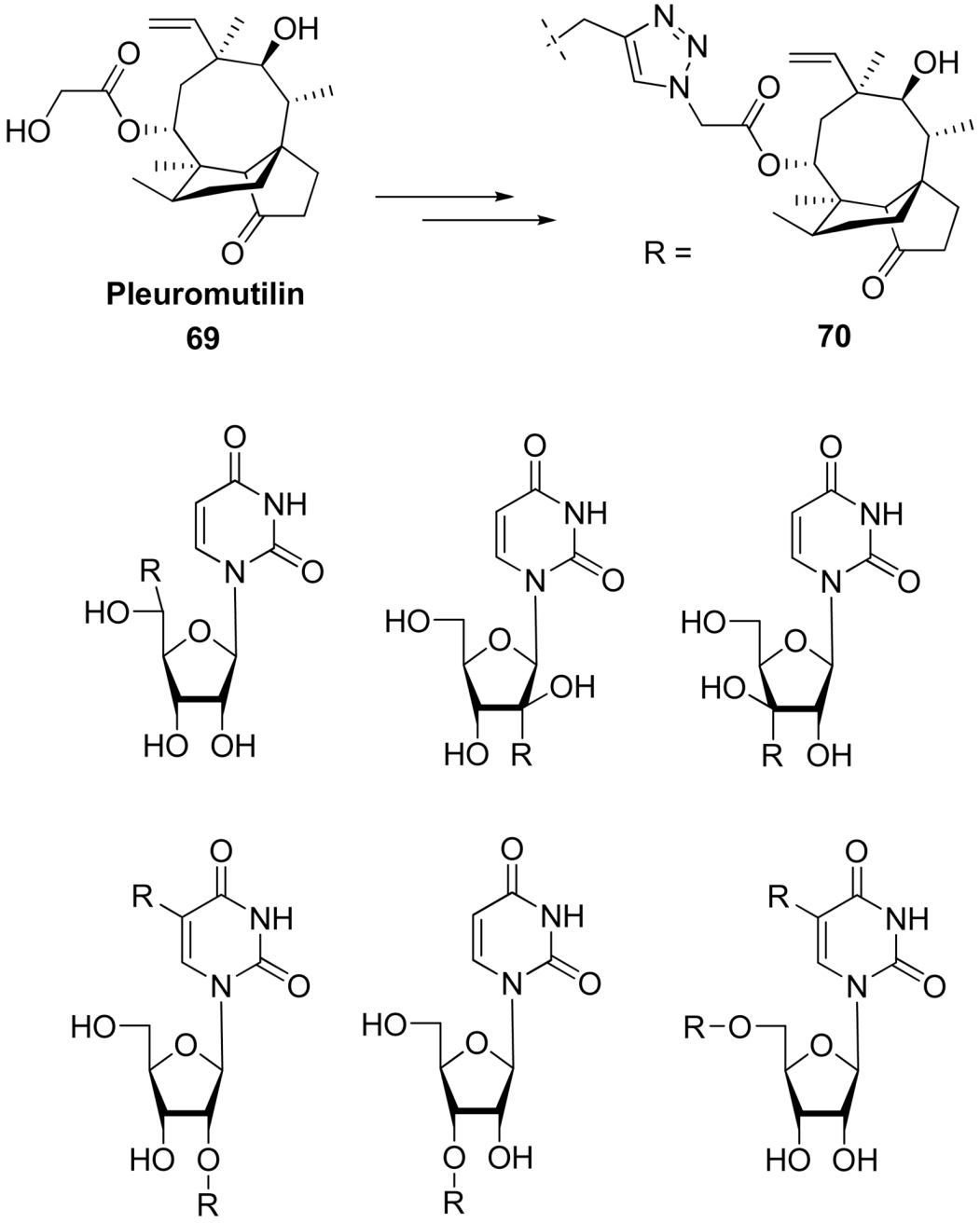

Figure 12.
Structure of Pleuromutilin 69 and Pleuromutilin Bioconjugates 70.

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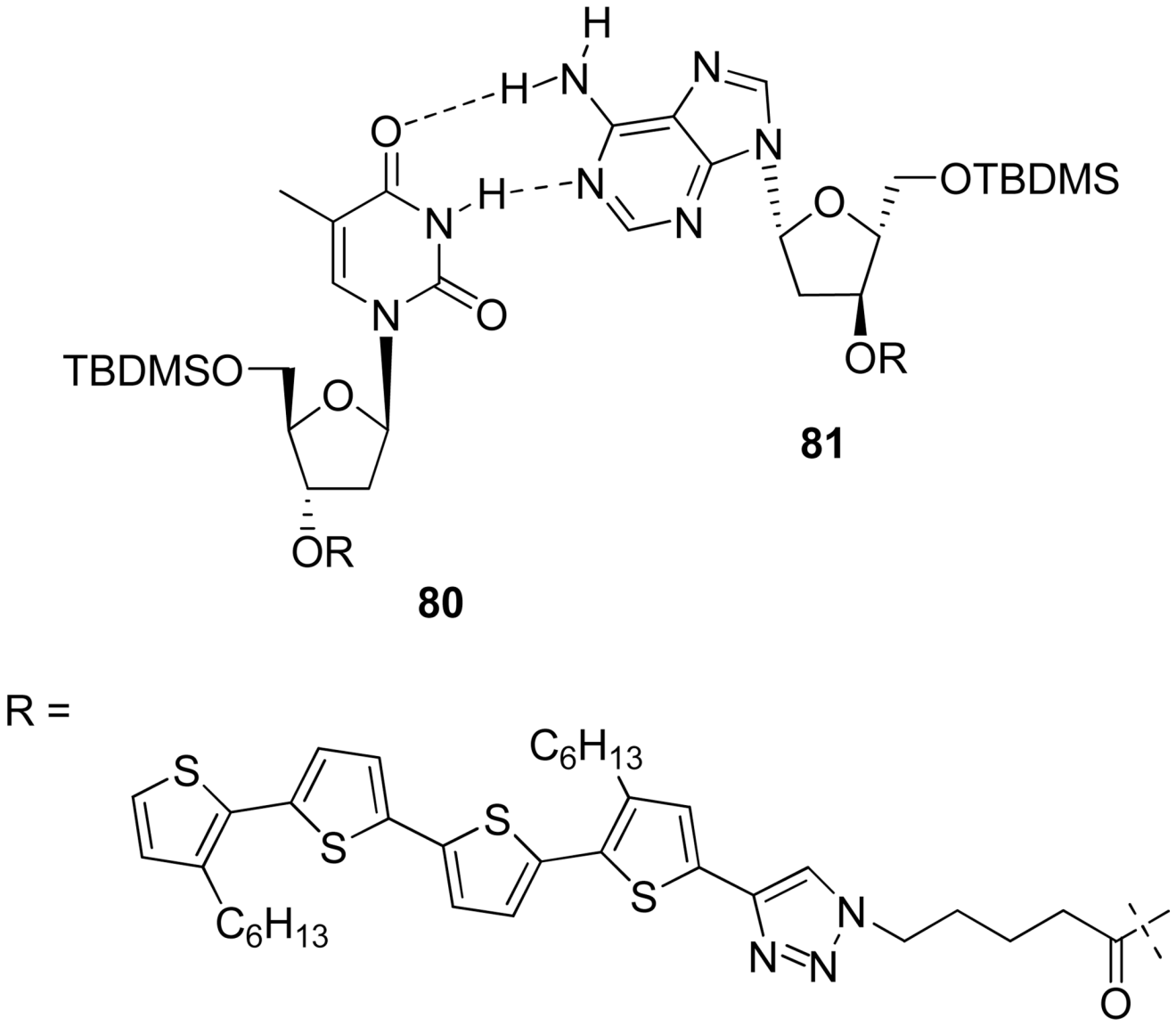

Figure 13.
Structure of Adenosine-Quaterthiophene 81 and Corresponding Thymidine 80.

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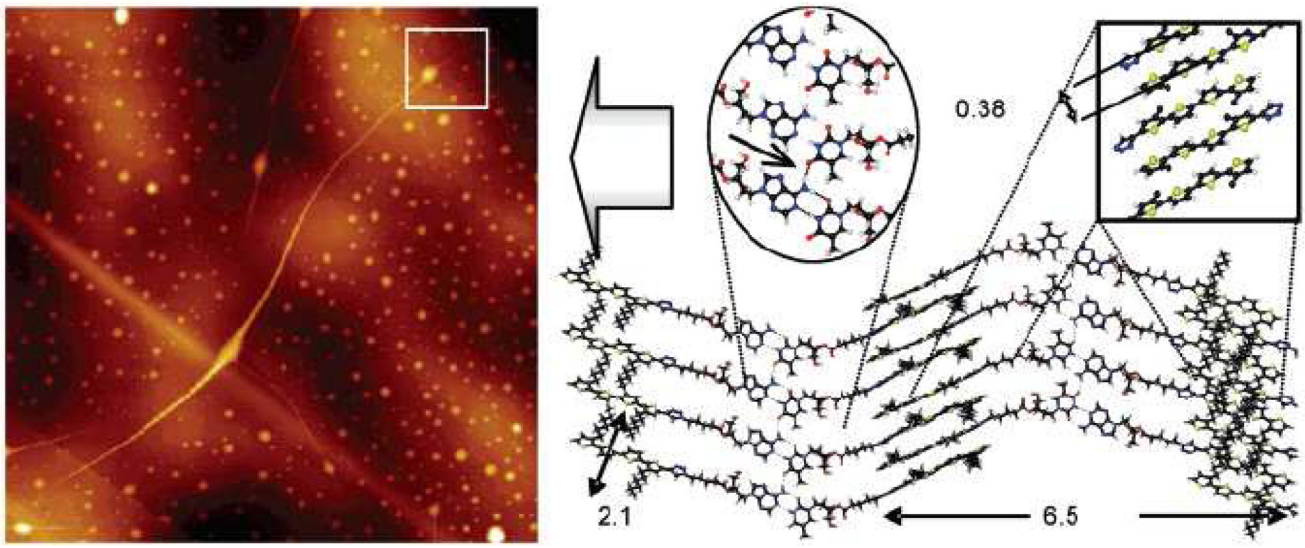

Figure 14.
AFM tapping-mode image of a 1:1 mixture of adenosine-quaterthiophene 81 and corresponding thymidine 80 deposited from toluene on HOPG after annealing: topography representation (17 × 17 μm^2) (left) and detailed amplitude image (2.5 × 2.5 mm^2) (right). In the middle a calculated model for the fiber growth (gray arrow) is shown, including a detail of the molecular interactions involved (oval and square insets) and the molecular dimensions. Reprinted with permission from ref 35. Copyright 2008 American Chemical Society.

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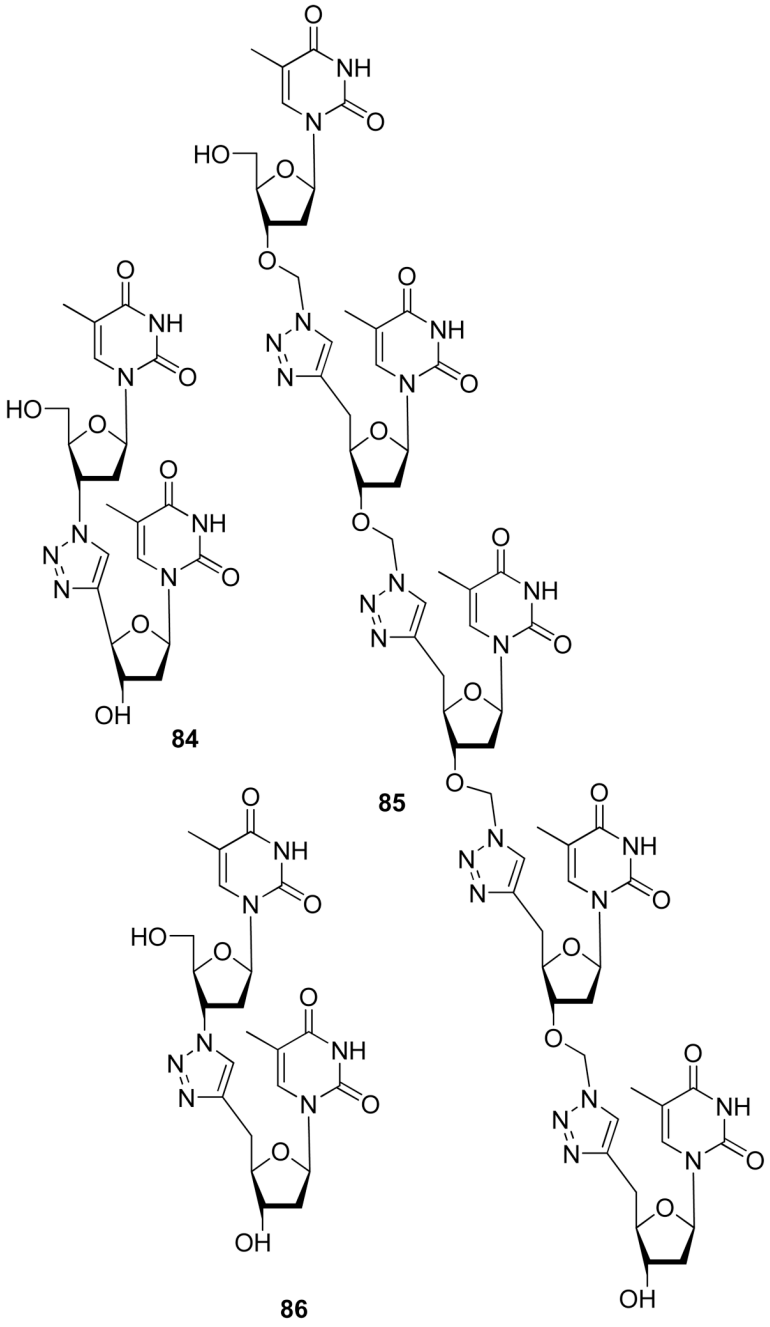

Figure 15.
Structures of oligonucleotides analogs 84–86

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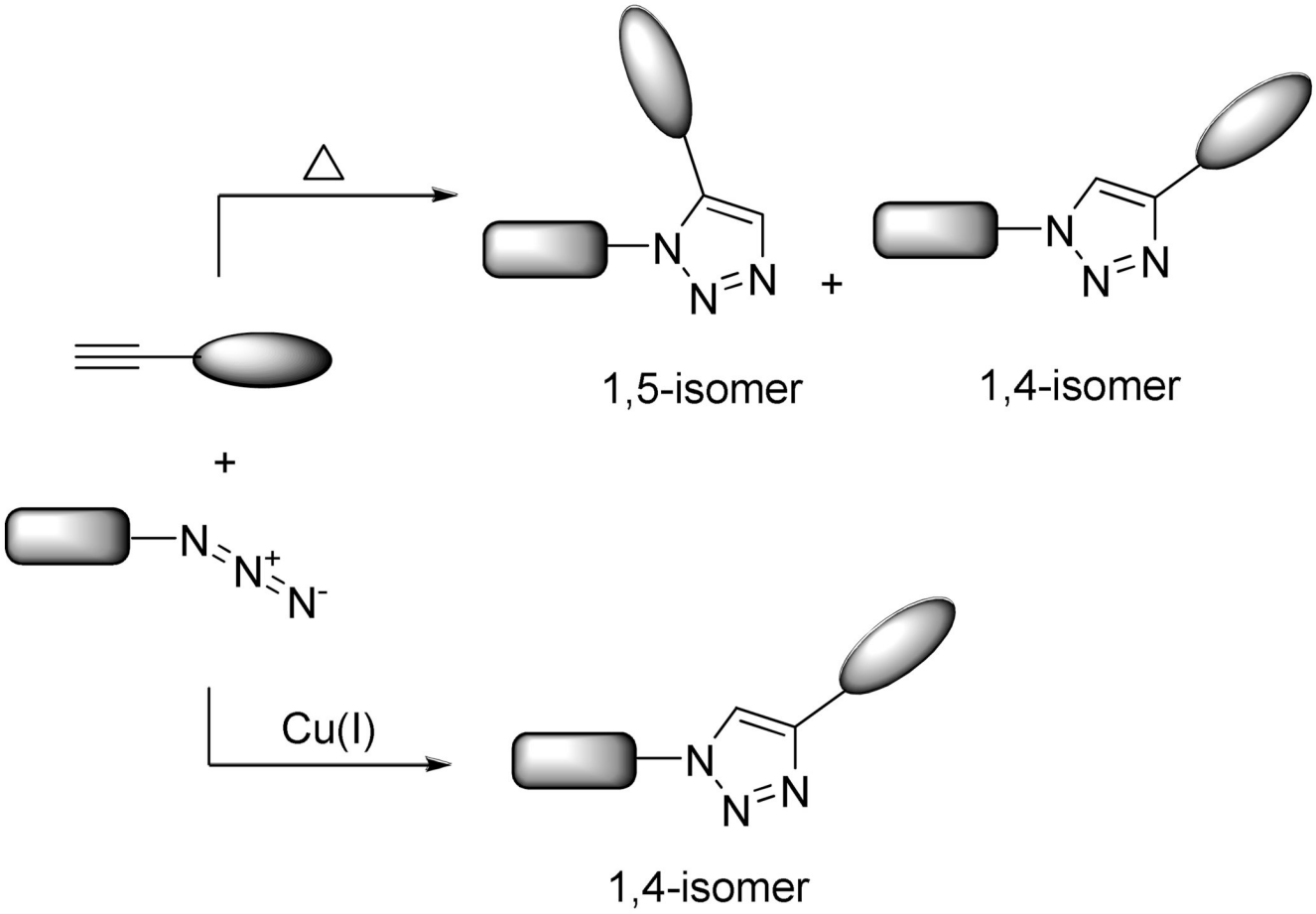

Scheme 1.
1,3-Dipolar Cycloaddition Between Azides and Alkynes

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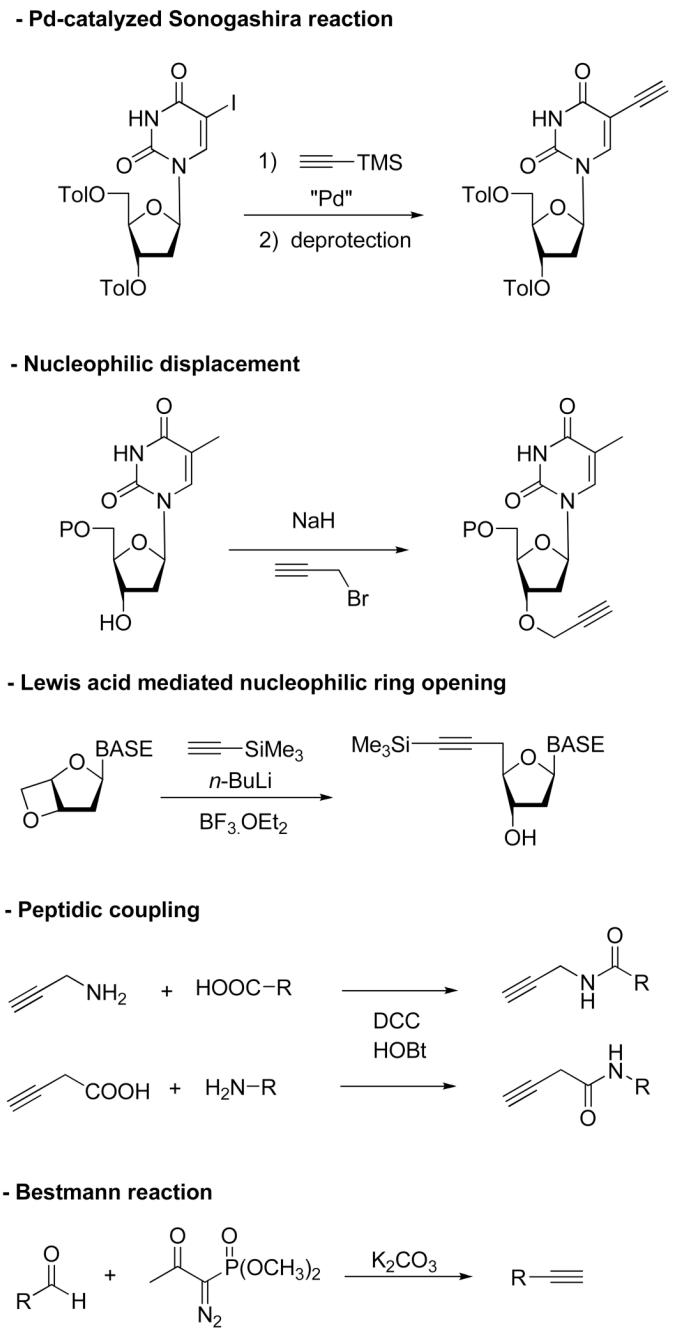

Scheme 2.
Most Common Methods for the Introduction of a Terminal Alkyne

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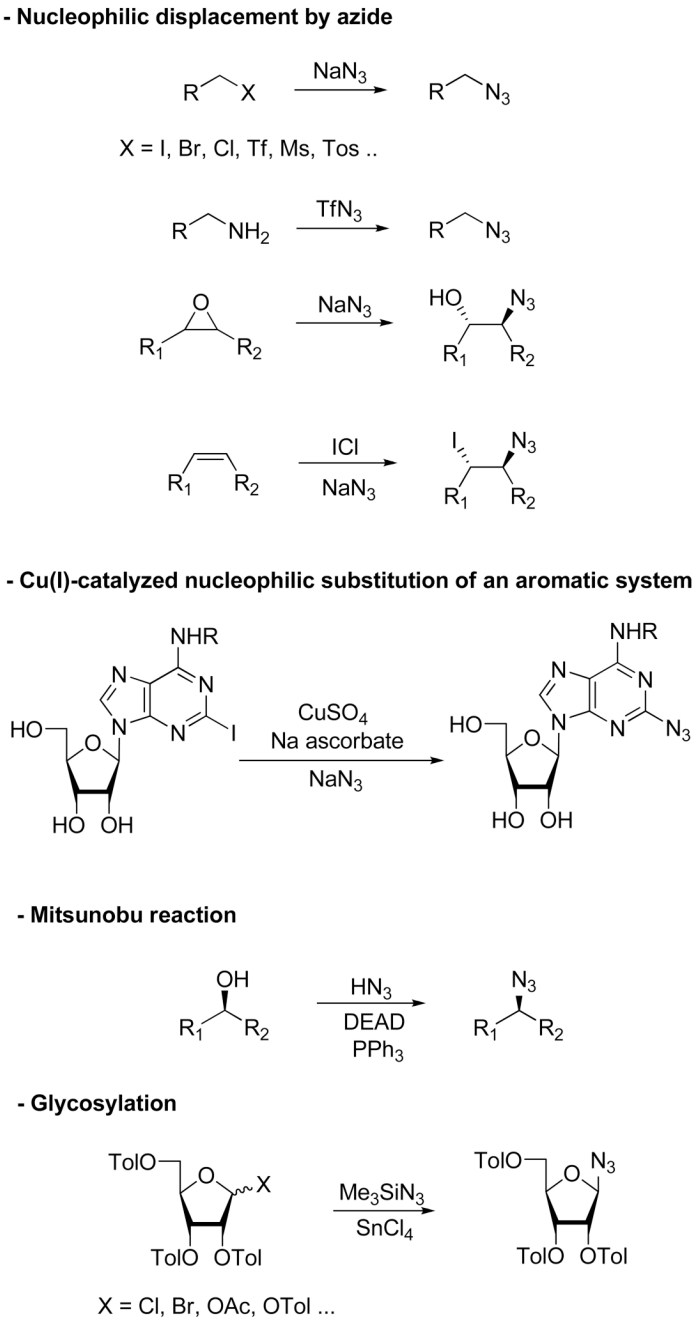

Scheme 3.
Most Common Methods for the Introduction of an Azide Functional Group

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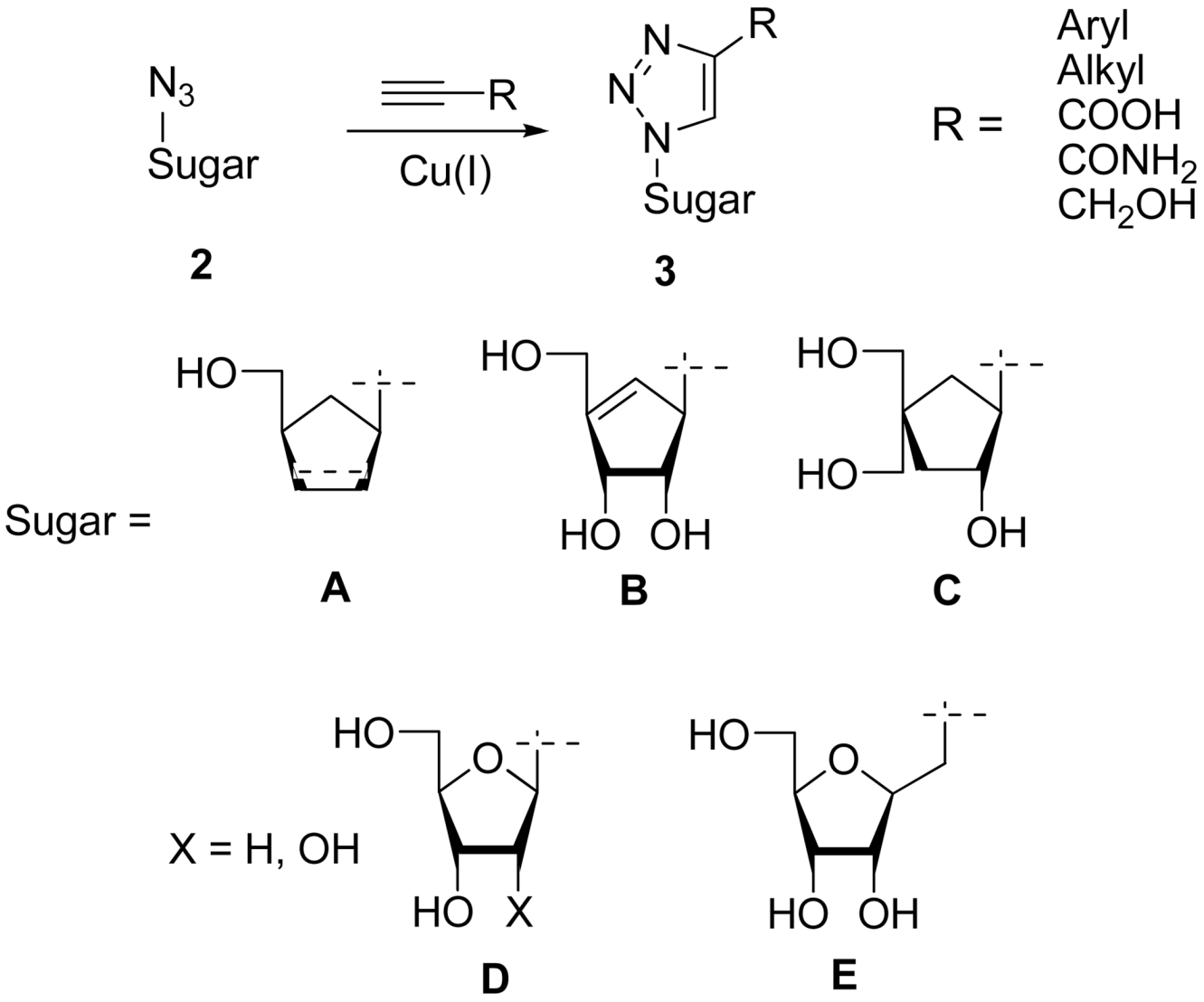

Scheme 4.
Synthesis of 1,2,3-Triazolo Nucleosides Analogs 3.

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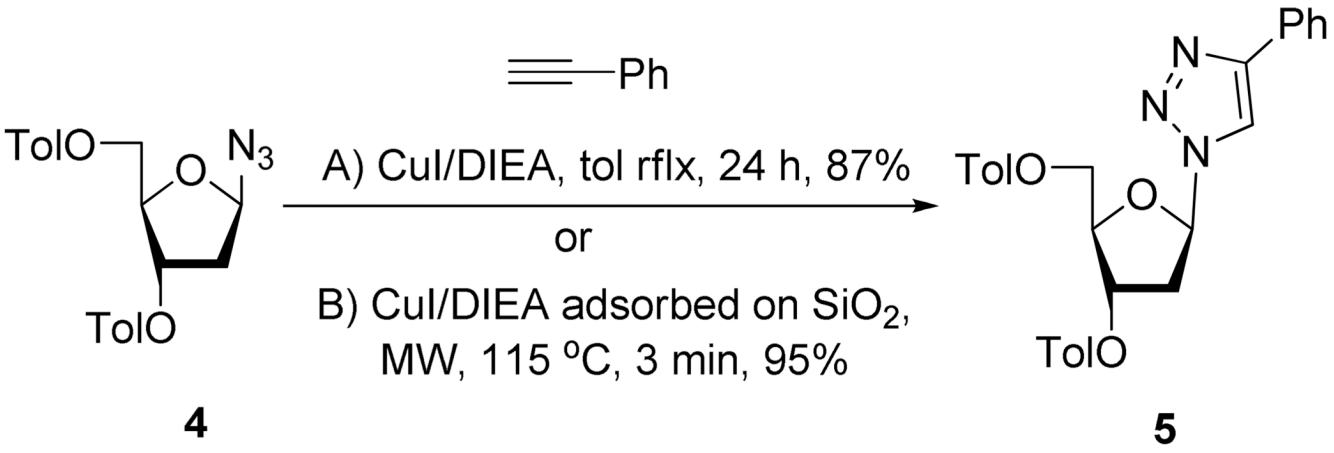

Scheme 5.
Synthesis of 1,2,3-Triazolo Nucleosides Analogs 5.

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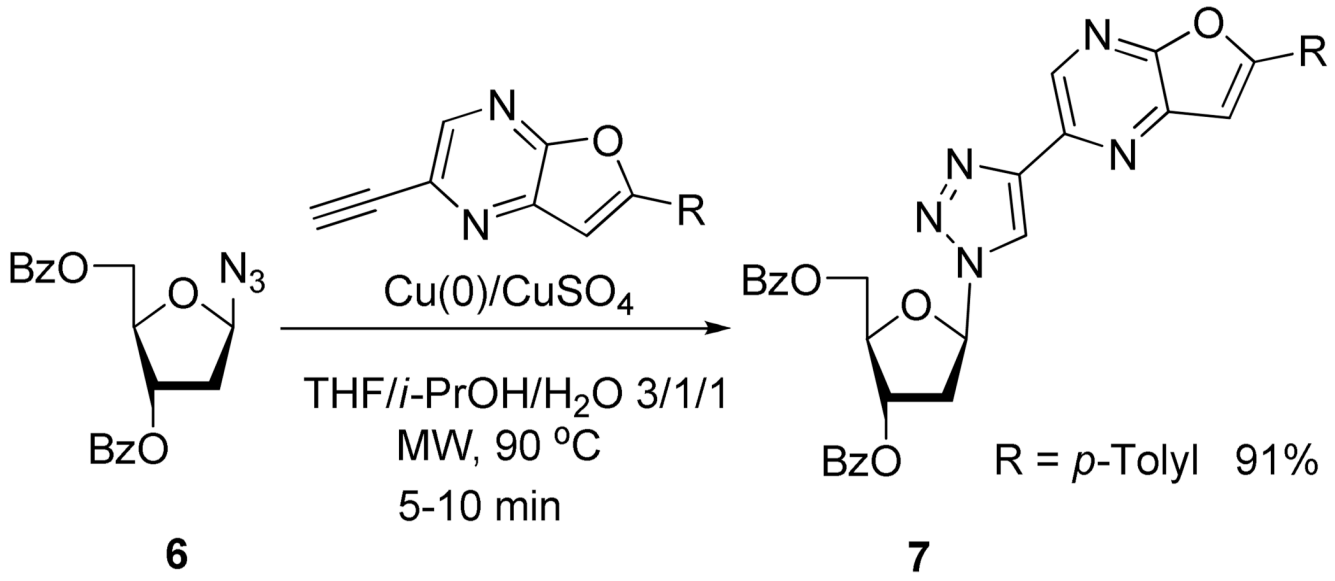

Scheme 6.
Synthesis of 1,2,3-Triazolo Nucleosides Analogs 7 Using Microwave Irradiation.

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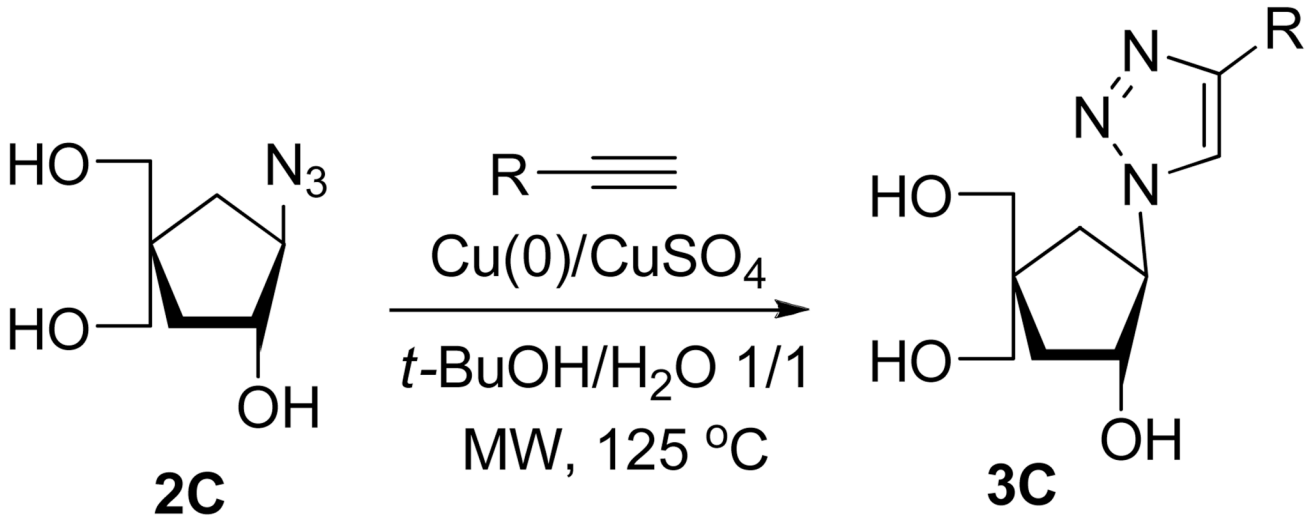

R	Yield	Time (min)
$-C_6H_5$	98%	2
$-CH_2OAc$	95%	< 1
$-(CH_2)_2-OH$	95%	2
$-(CH_2)_5-CH_3$	98%	60

Scheme 7.
Synthesis of 1,2,3-Triazolo Nucleosides Analogs 3C Using Microwave Irradiation.

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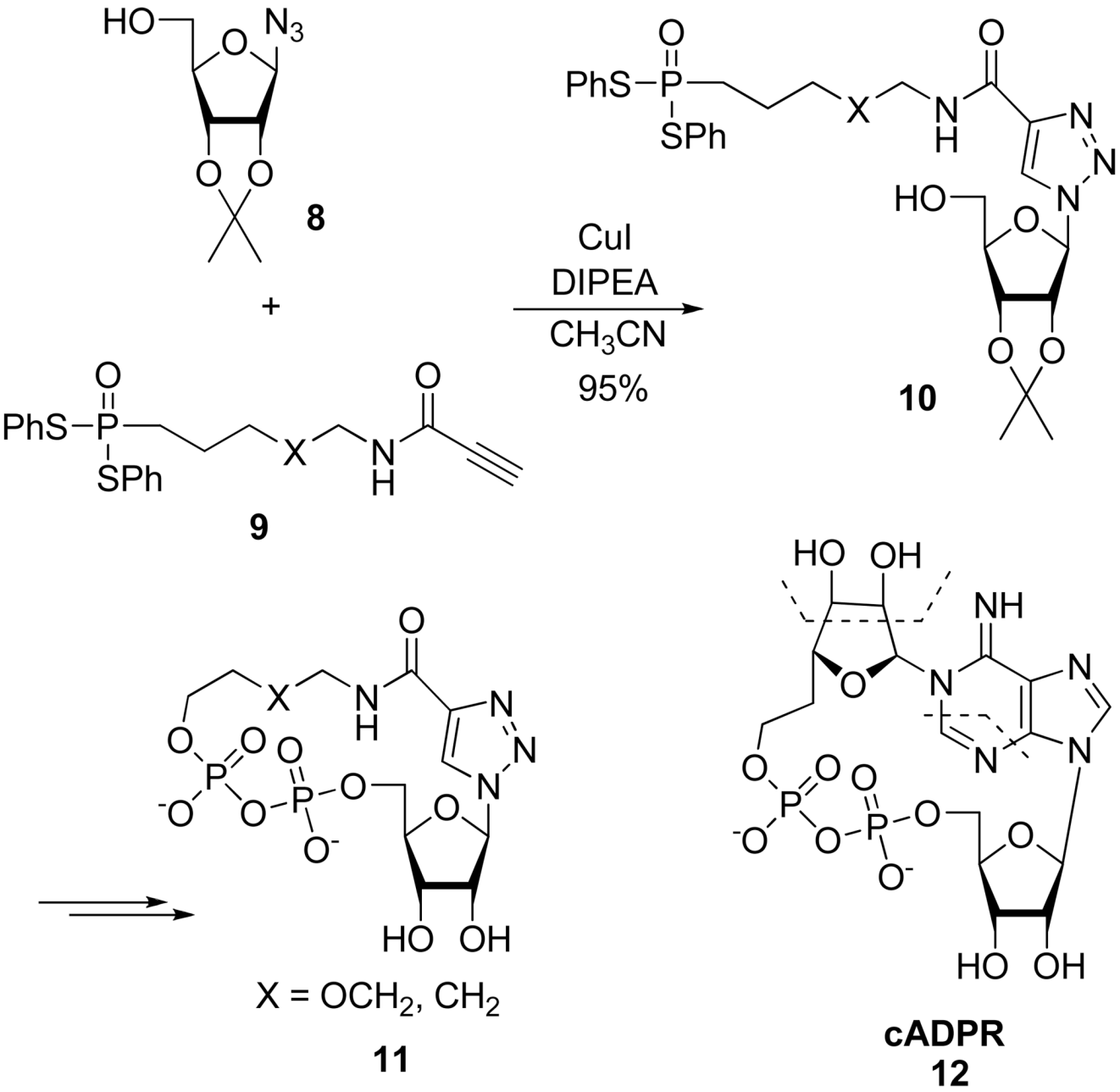

Scheme 8.
Synthesis of Nucleobase-Simplified cADPR Mimics 11.

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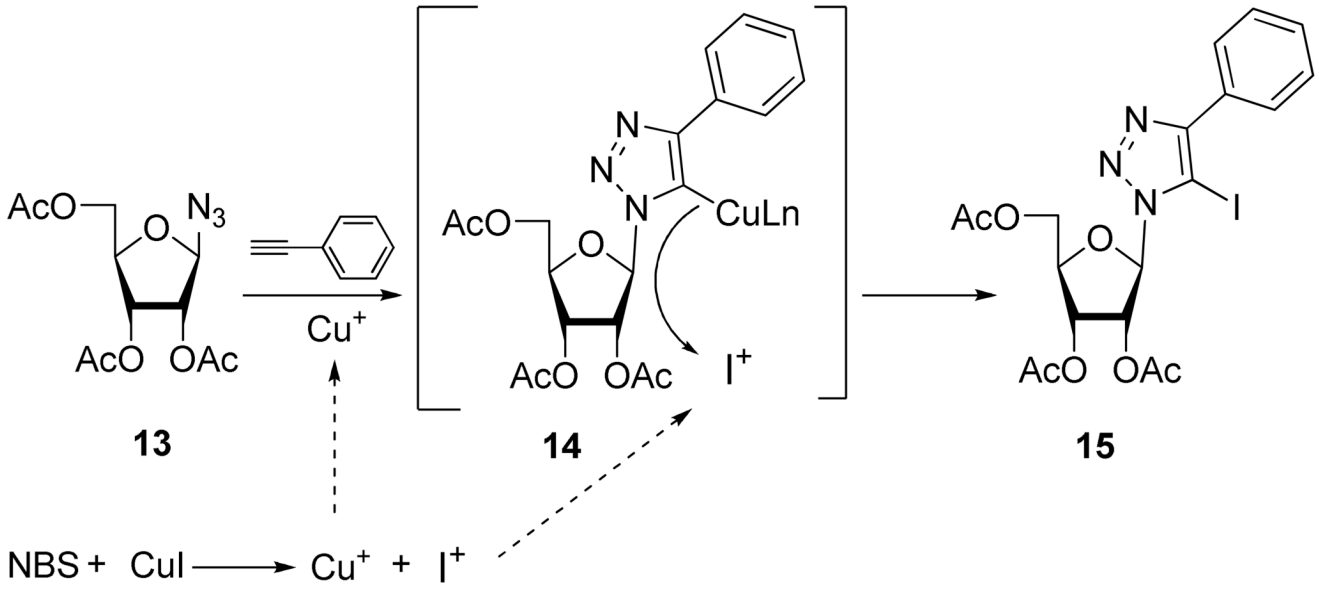

Scheme 9.
Plausible Mechanism of Preparation of Compound 15.

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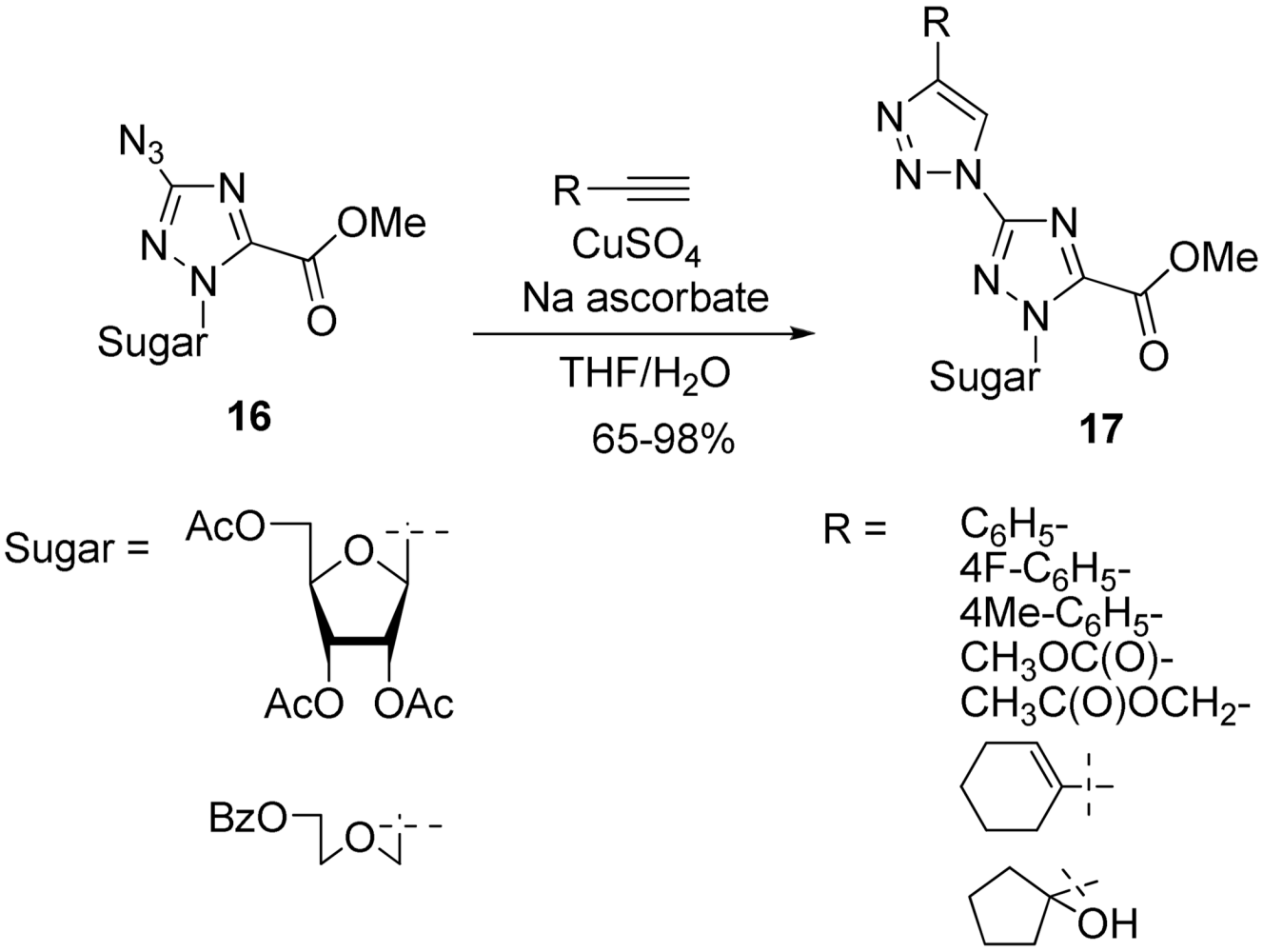

Scheme 10.
Synthesis of Bis-triazolyl Nucleosides 17.

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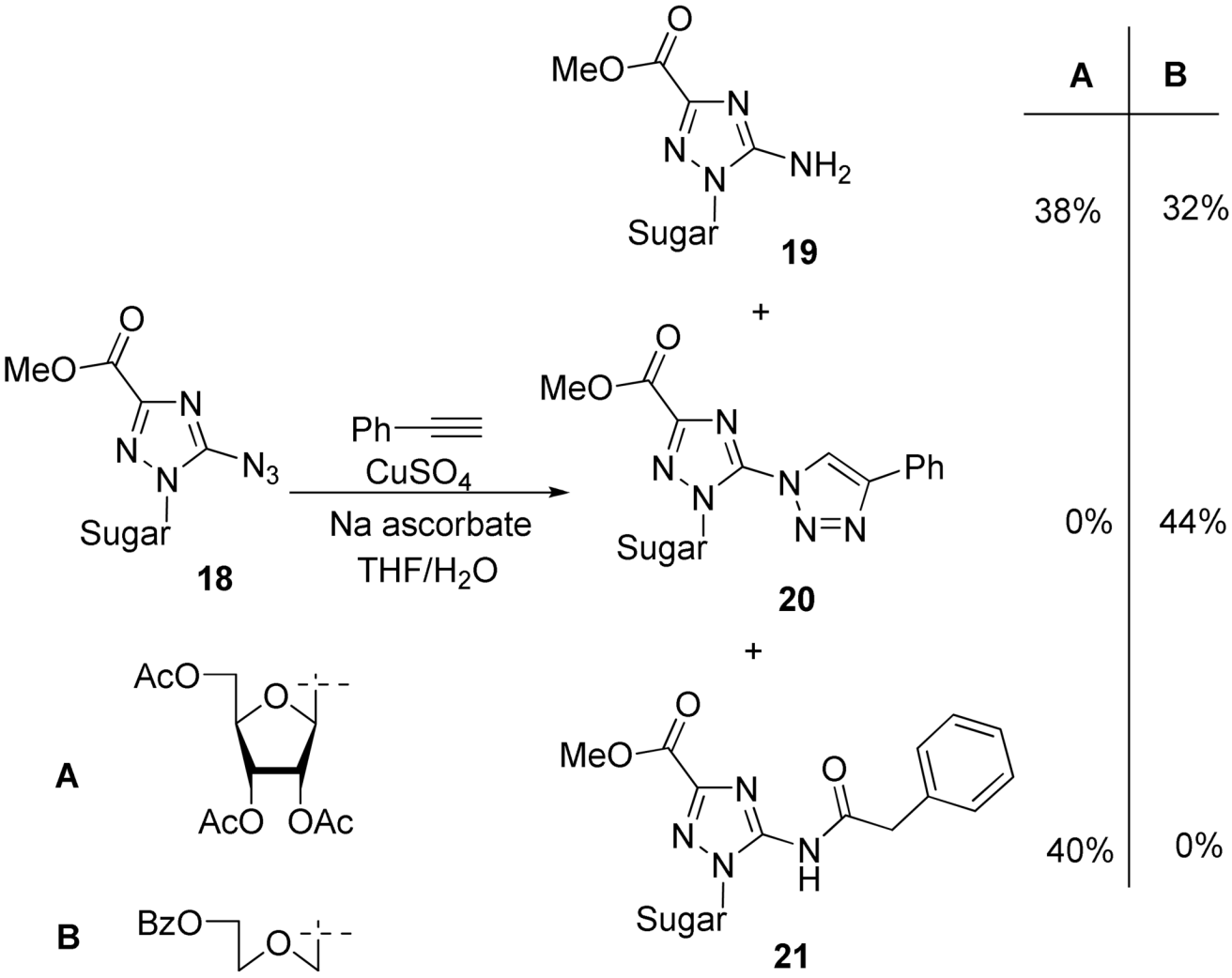

Scheme 11.
Reactivity of Azido Compound 18 Under CuAAC Conditions.

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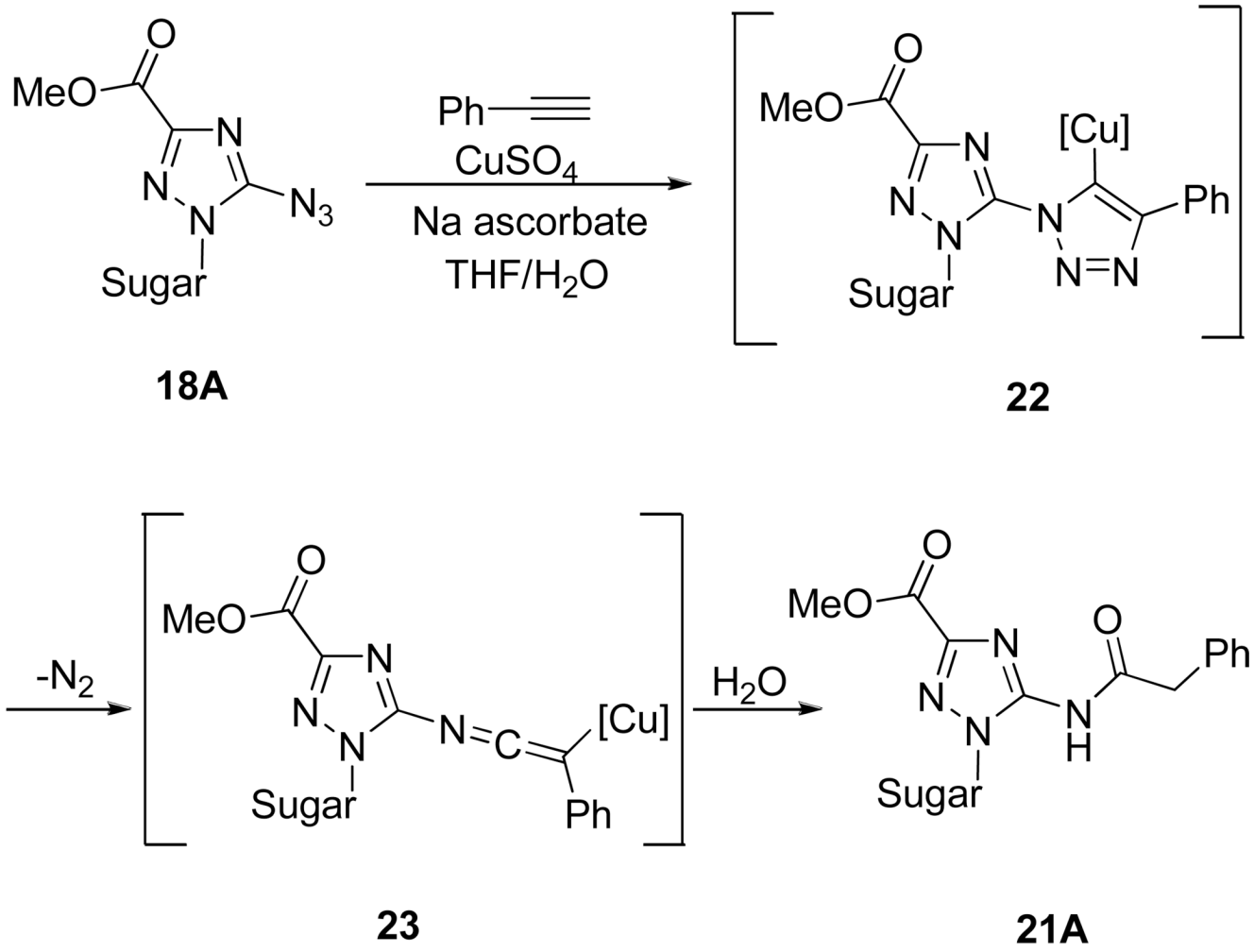

Scheme 12.
Plausible Mechanism for the Formation of Amide 21A.

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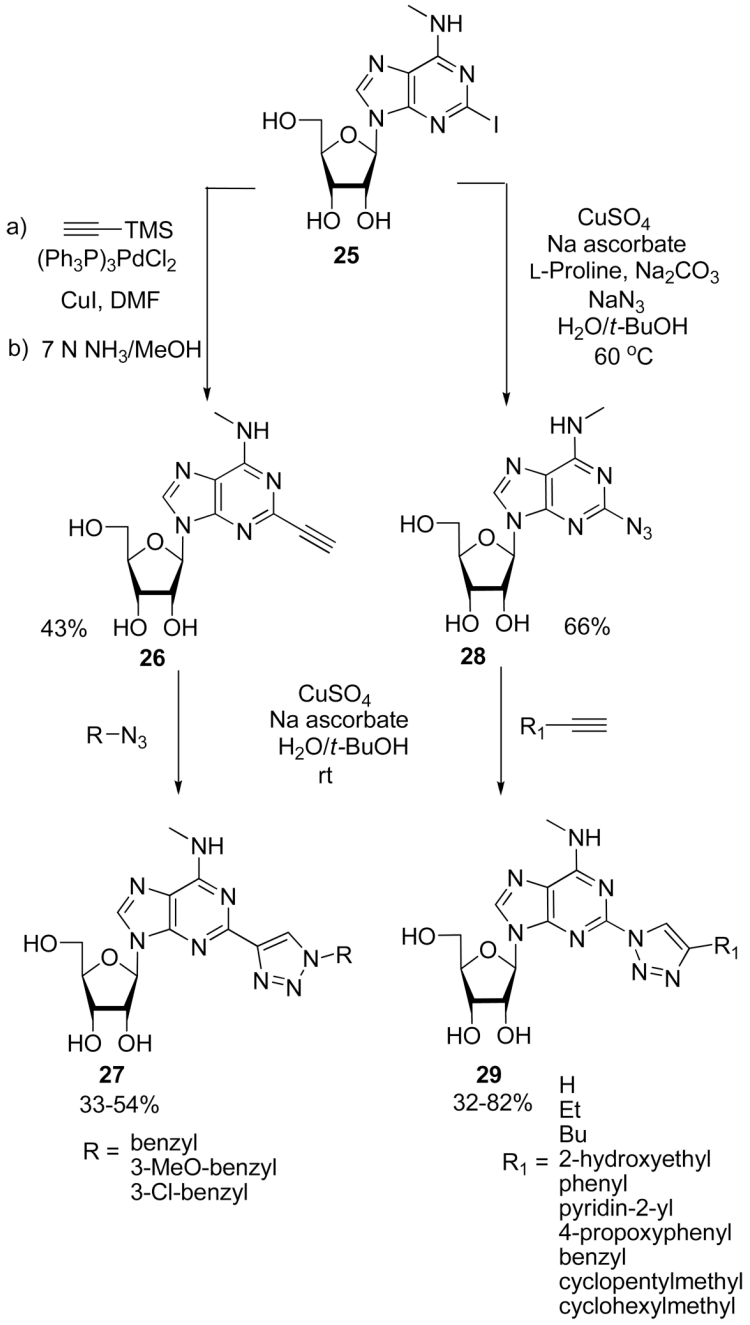

Scheme 13.
Synthesis of 2-(1,2,3-Triazolyl)Adenosine Derivatives 27 and 29.

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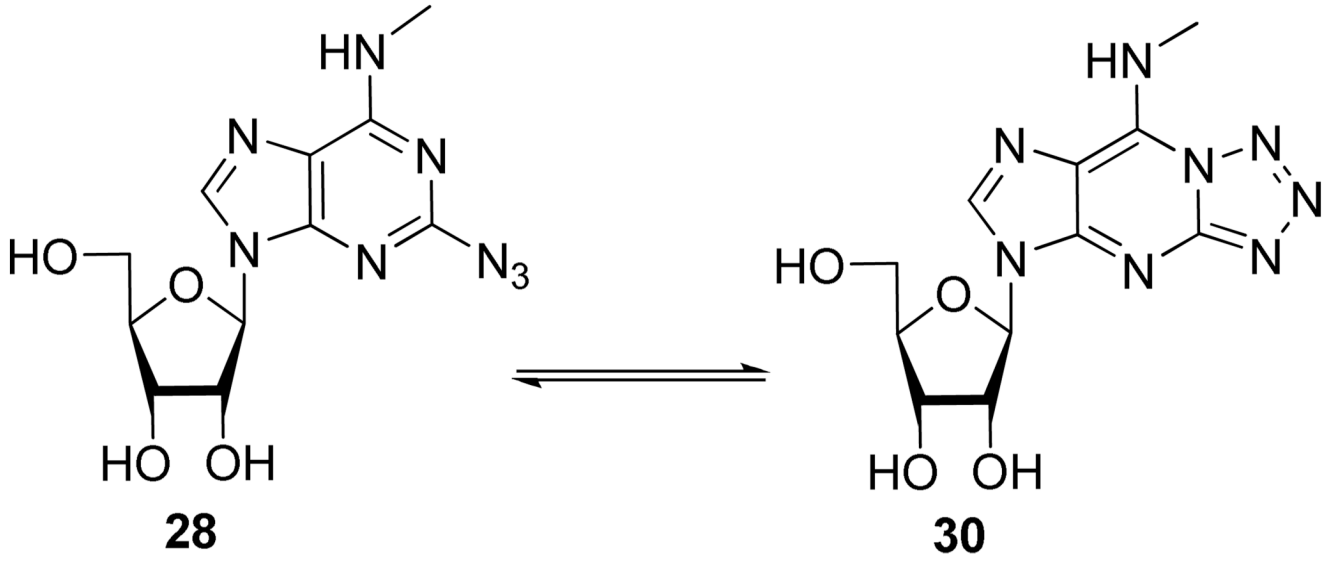

Scheme 14.
Azido/Tetrazole Tautomerism of 2-Substituted Adenosine Derivative 28.

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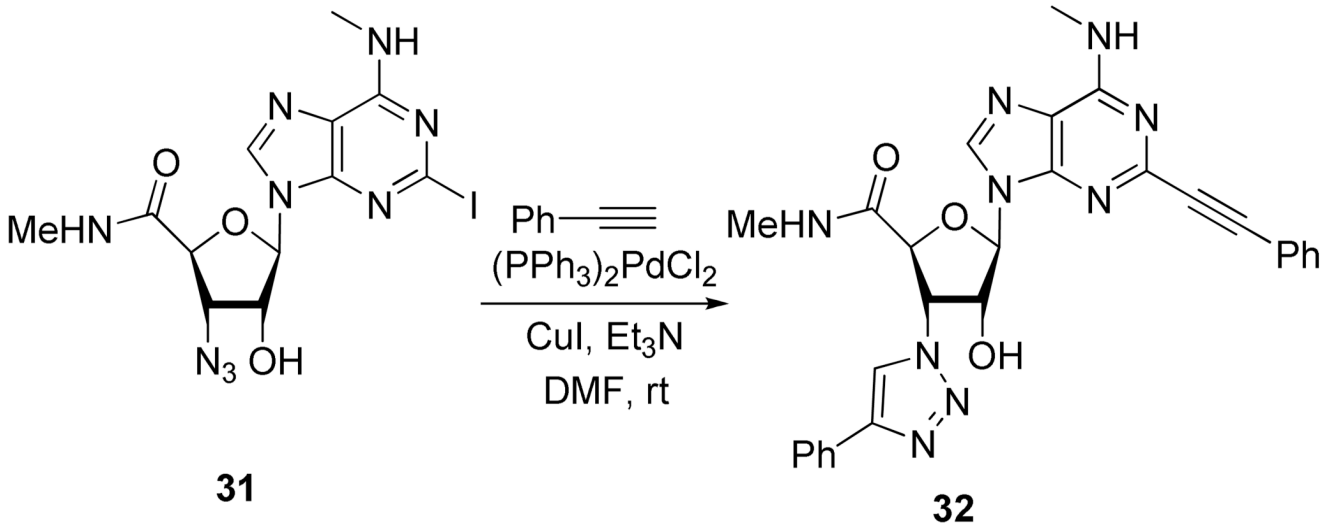

Scheme 15.
Double Effect of the Sonogashira Conditions on Compound 31.

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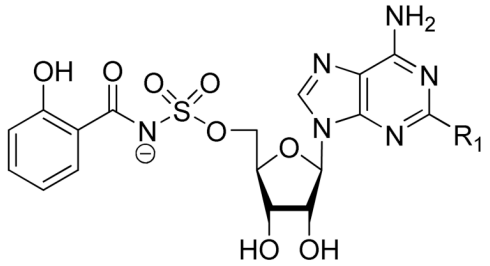
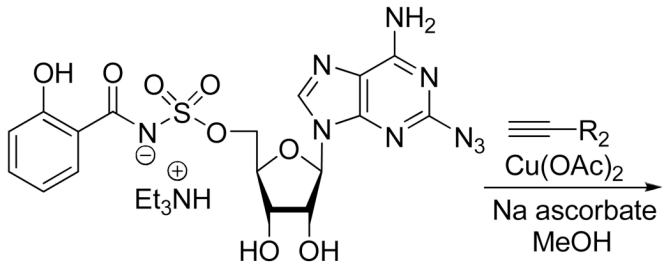


		K_i^{app} (nM)	MIC_{99} (μM)
Sal-AMS **33**	R_1 = H	6.6	0.39
2-Ph-Sal-AMS **34**	R_1 = Ph	0.27	0.049



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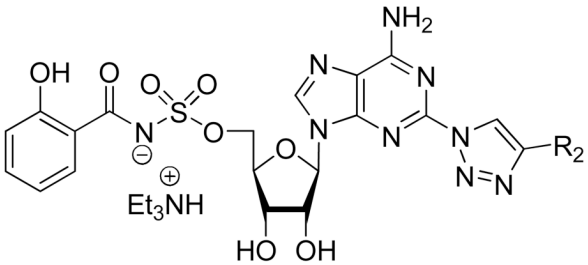

36

30 compounds synthesized
R_2 = alkyl, aryl, heteroaryl

⇒ R_2 = 2-NH_2-C_6H_4 | K_i^{app} = 0.77 nM, MIC_{99} = 0.78 μM

Scheme 16.
Synthesis of 2-Triazole Substituted 36, Analogs of Sal-AMS 33.

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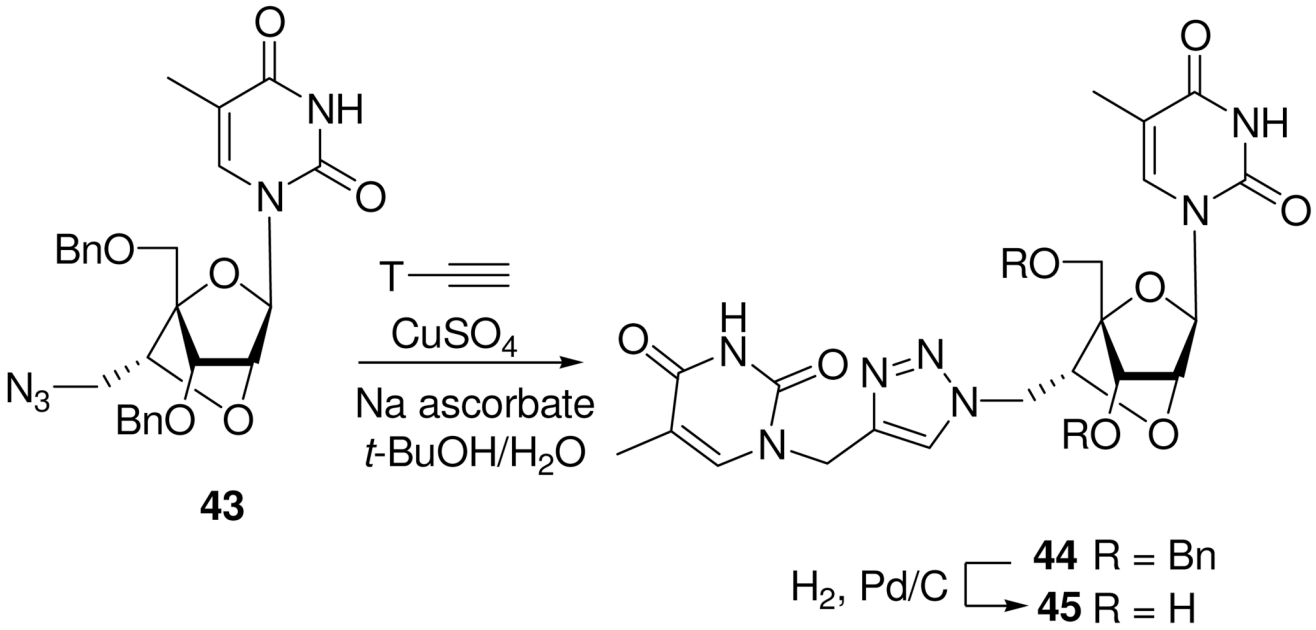

Scheme 17.
Synthesis of 6'-Branched Locked Nucleic Acid 45.

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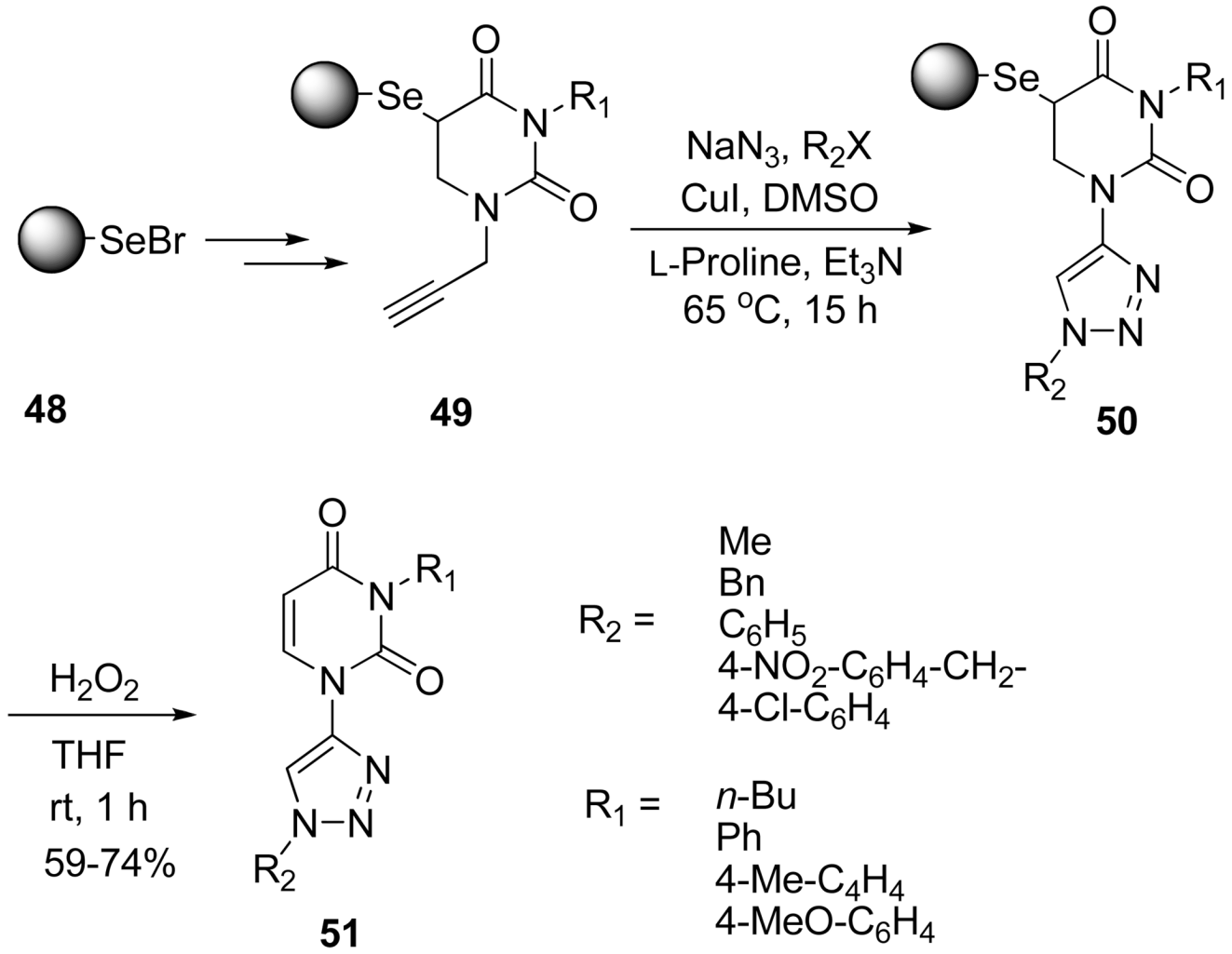

Scheme 18.
Solid-Phase Synthesis of Heterocyclic Nucleosides Analogs 51.

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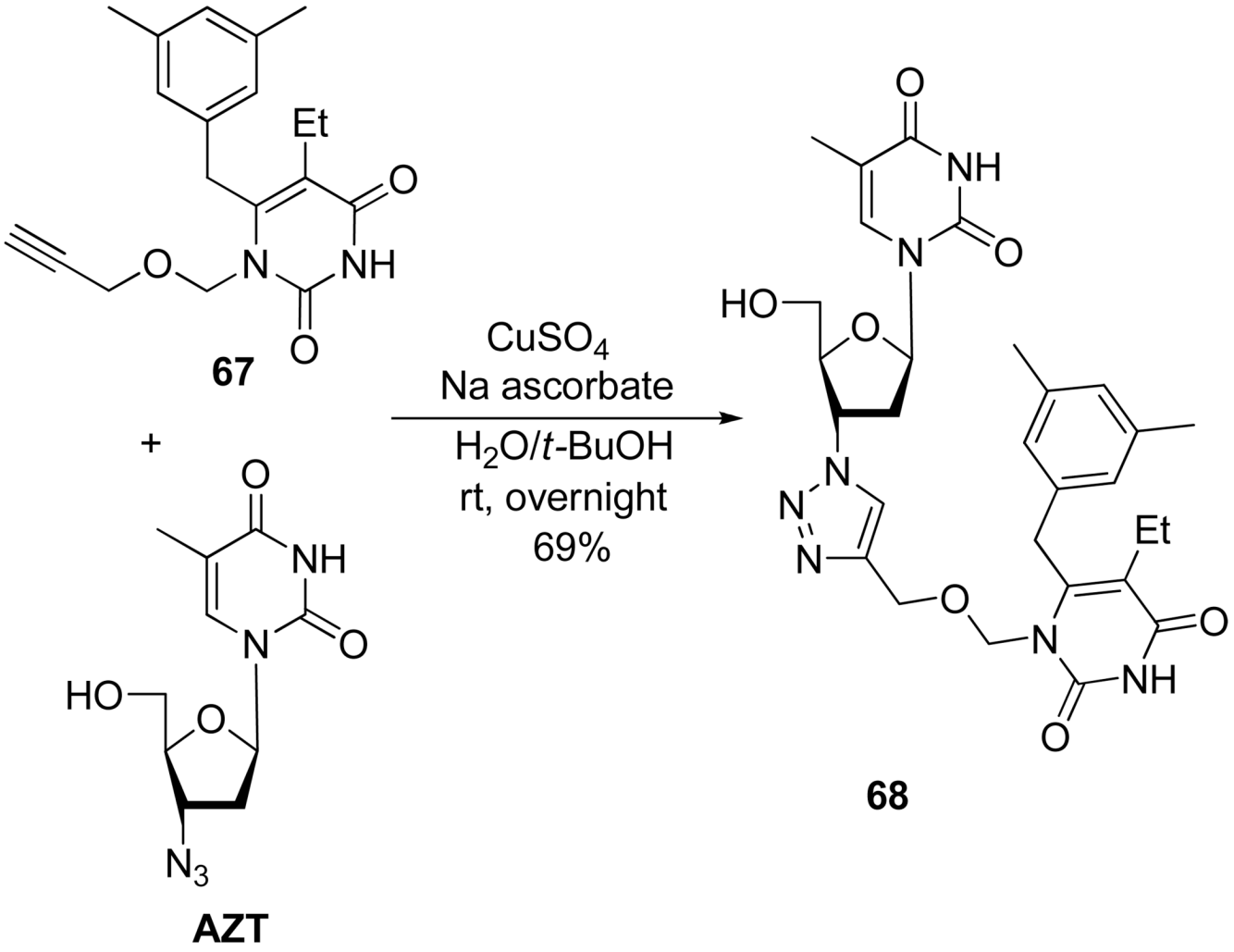

Scheme 19.
Synthesis of Compound 68.

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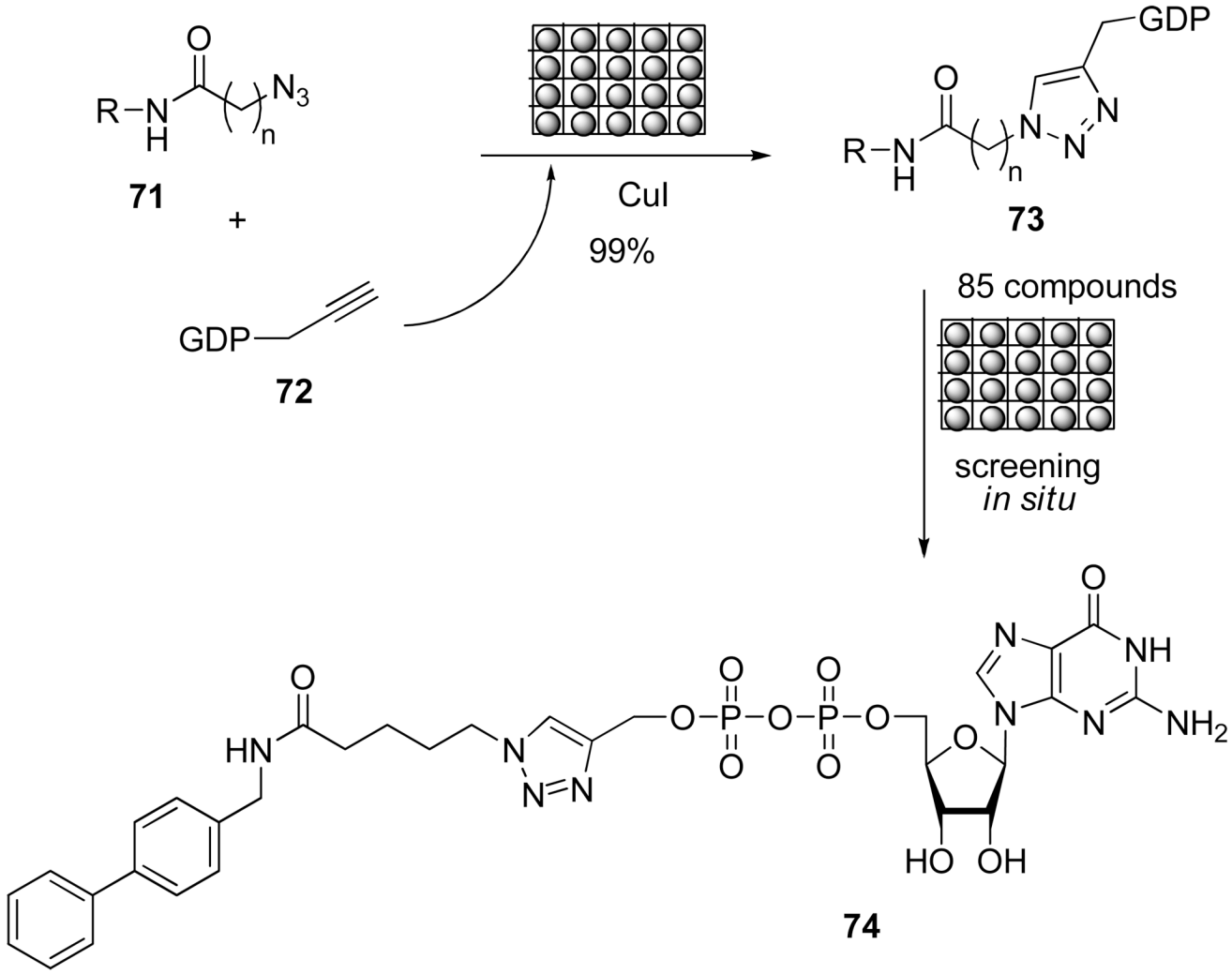

Scheme 20.
Triazole Synthesis in Microliter Plate for Screening *in situ*.

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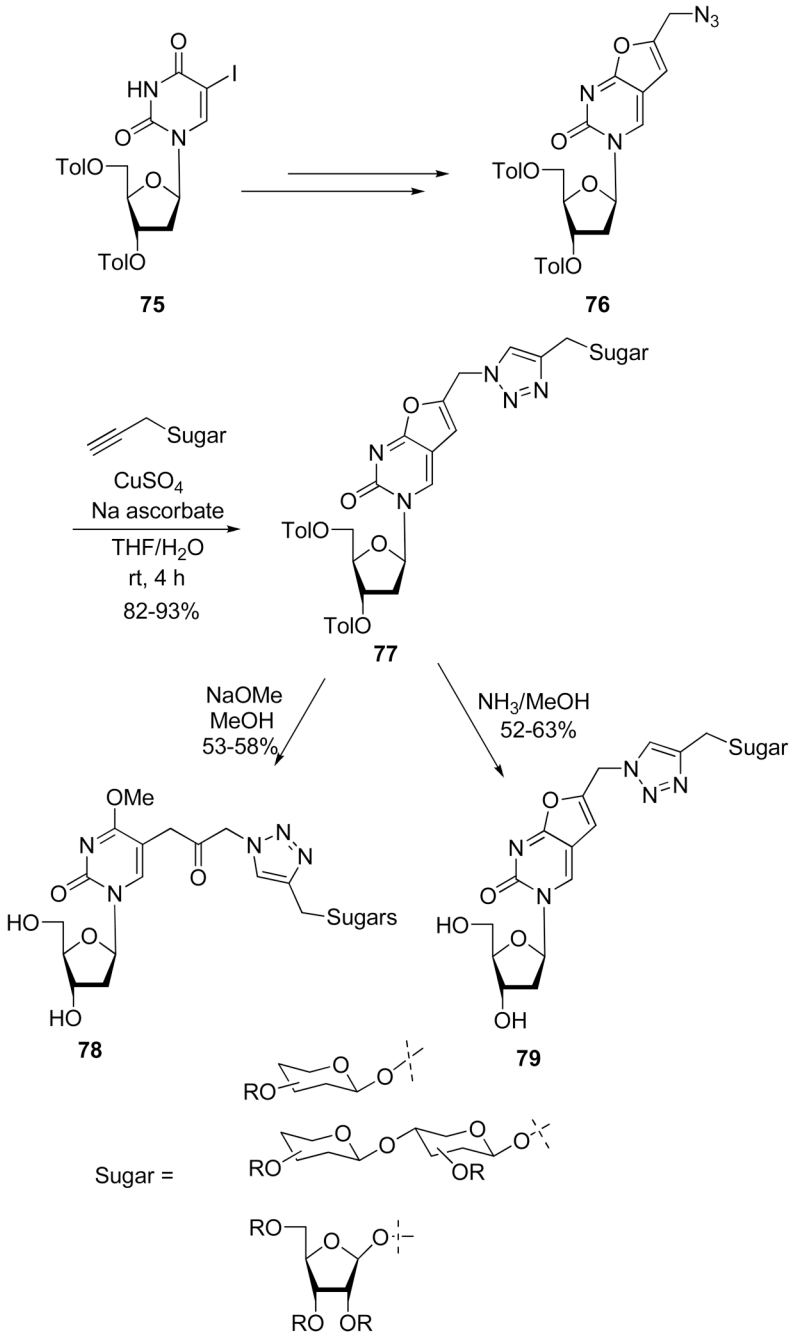

Scheme 21.
Synthesis of Compounds 78 and 79.

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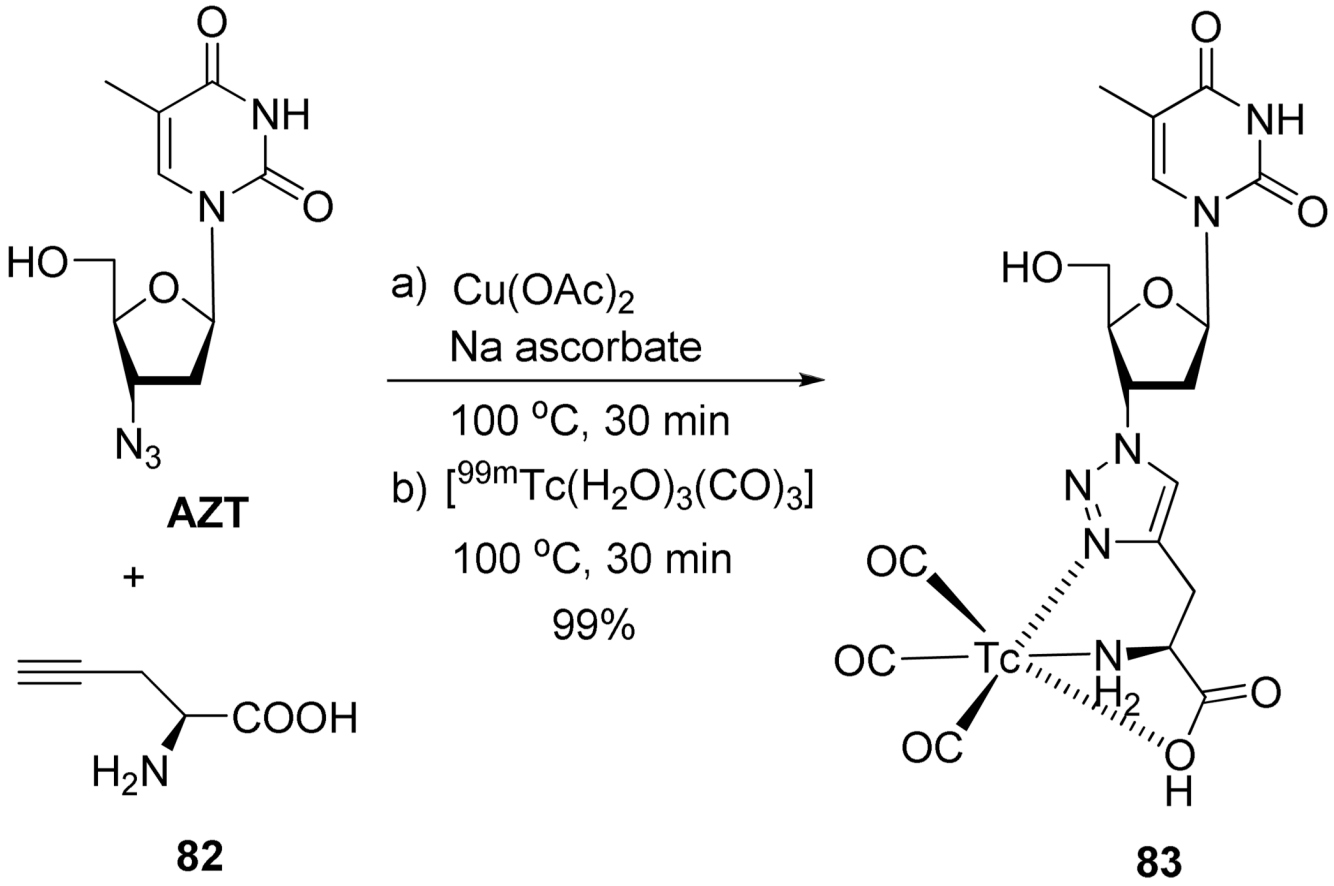

Scheme 22.
One-pot Synthesis of Radiolabeled Conjugate 83.

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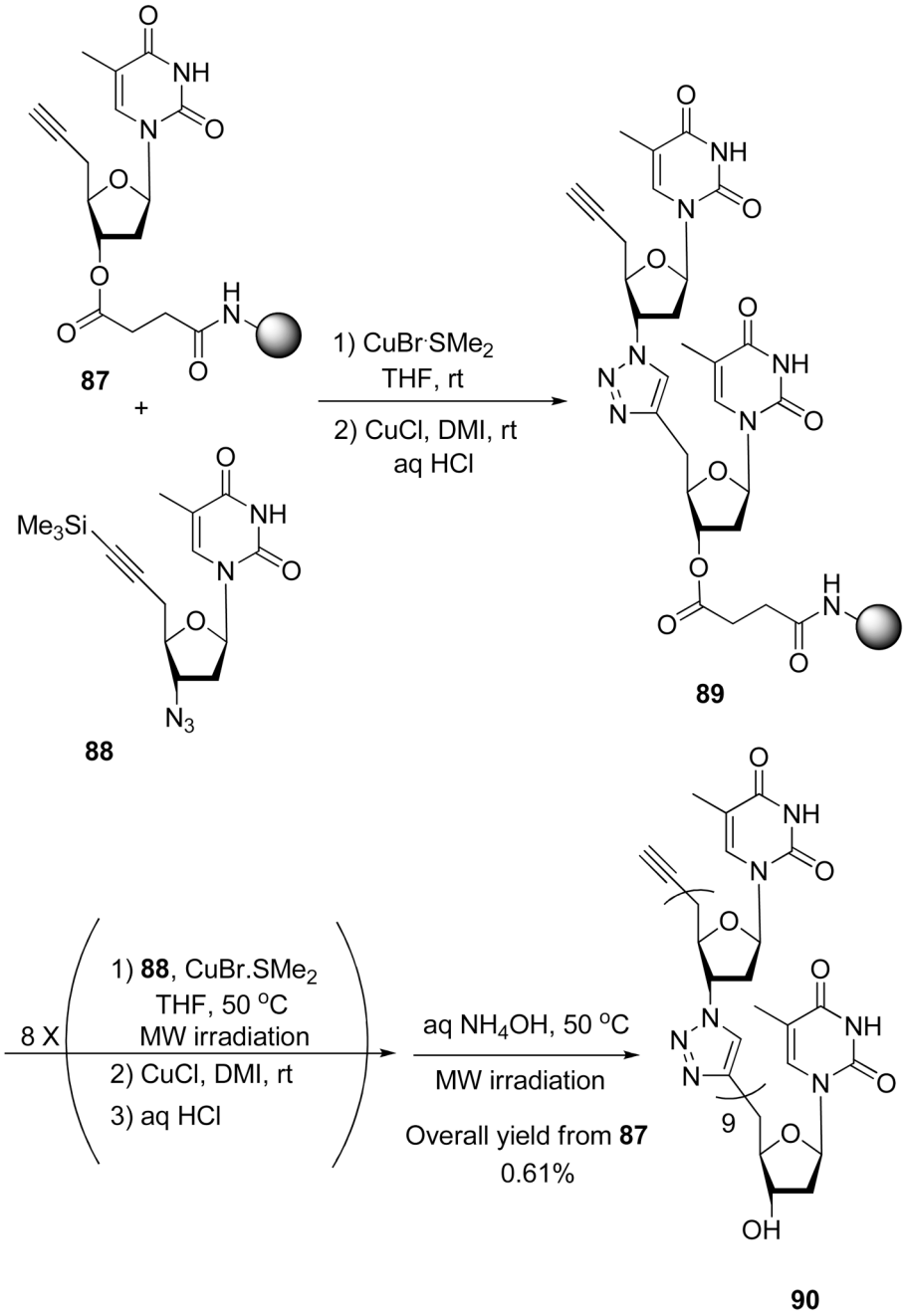

Scheme 23.
Synthesis of 10-Mer ^{TL}DNA 90.

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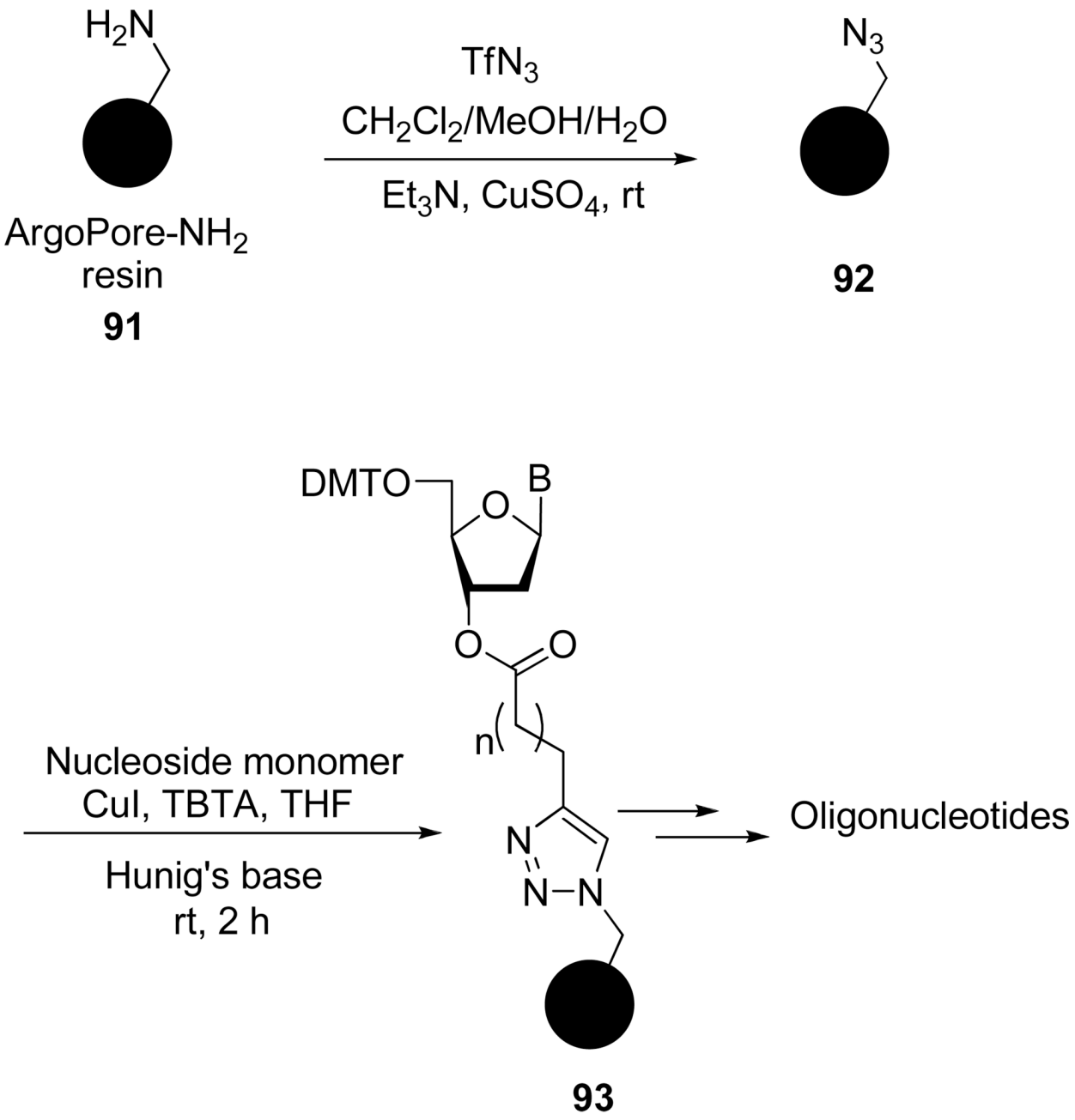

Scheme 24.
Azido Resin Derivatization with a Nucleoside Monomer using CuAAC.

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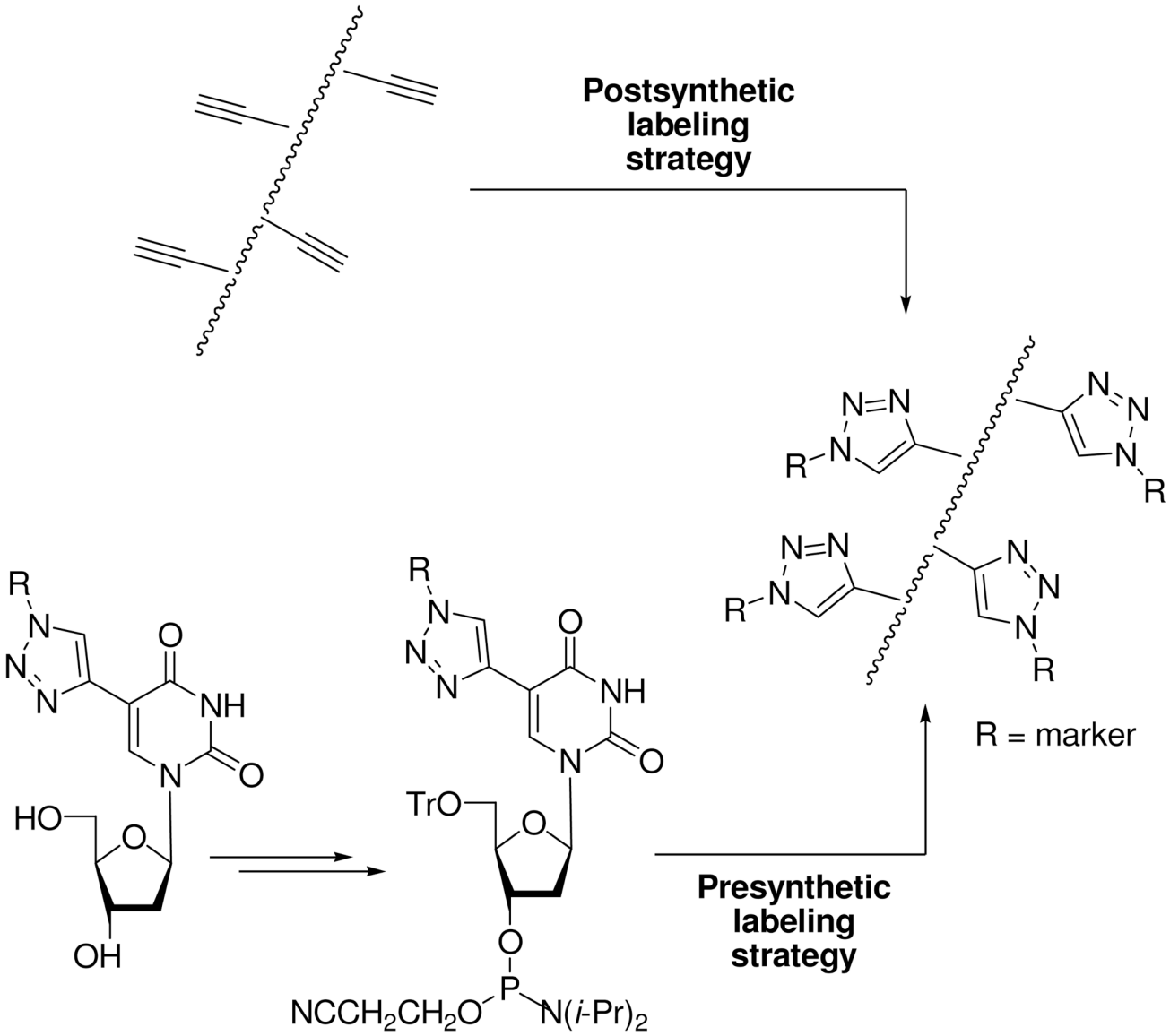

Scheme 25.
Postsynthetic and Presynthetic Strategies for DNA Labeling.

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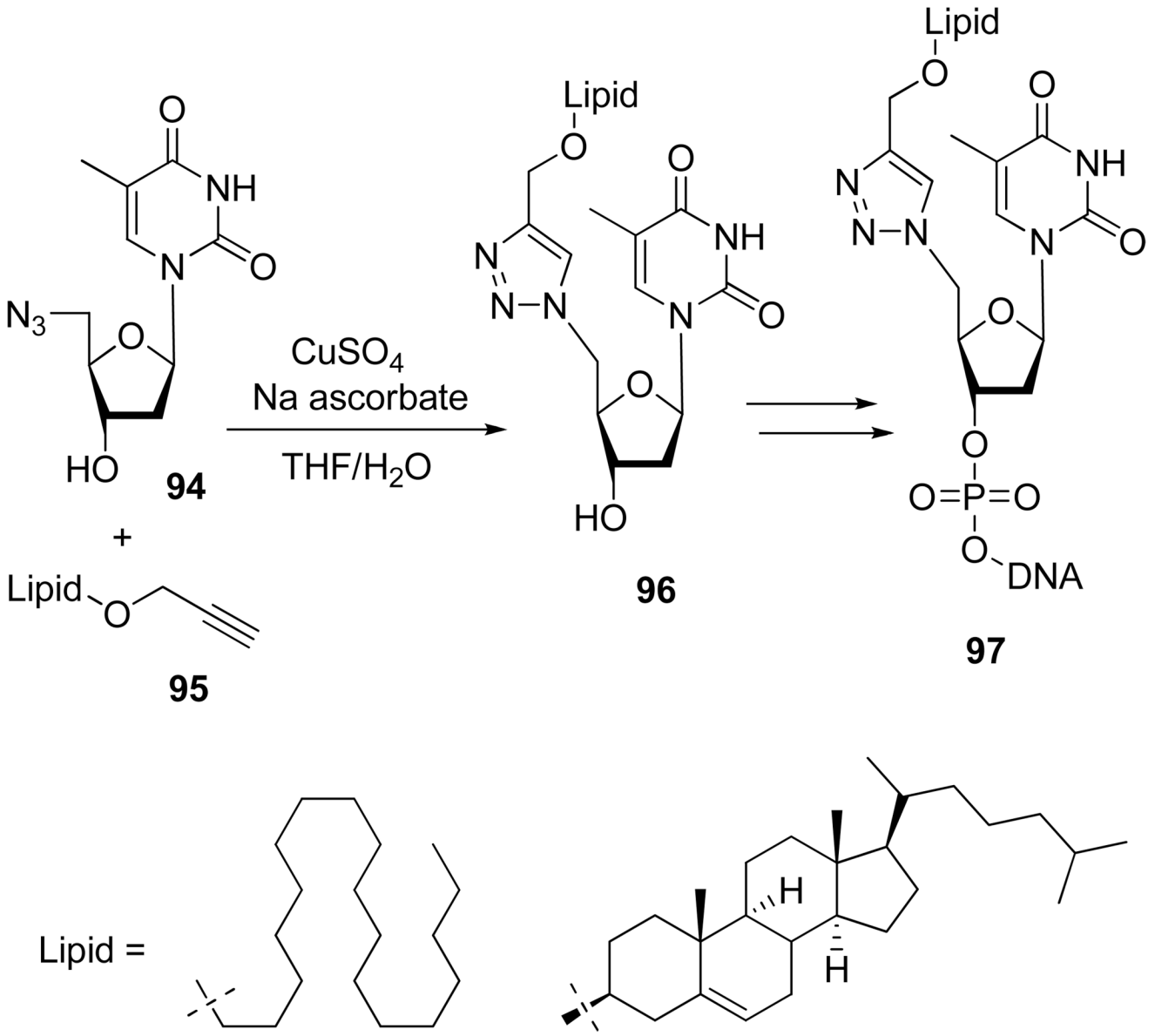

Scheme 26.
Synthesis of Lipid-Oligonucleotides Conjugates 97.

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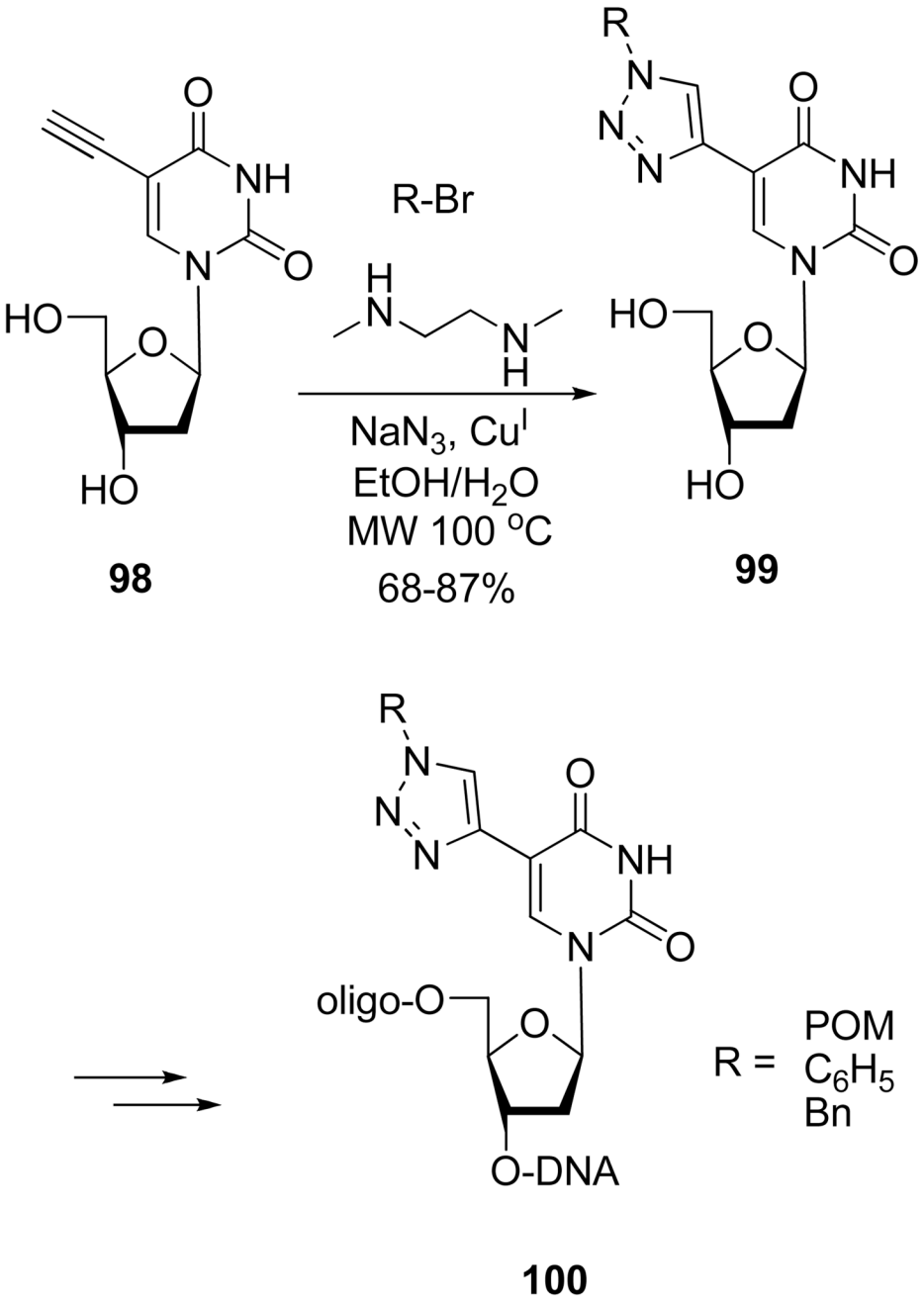

Scheme 27.
Synthesis of Modified Oligonucleotides 100.

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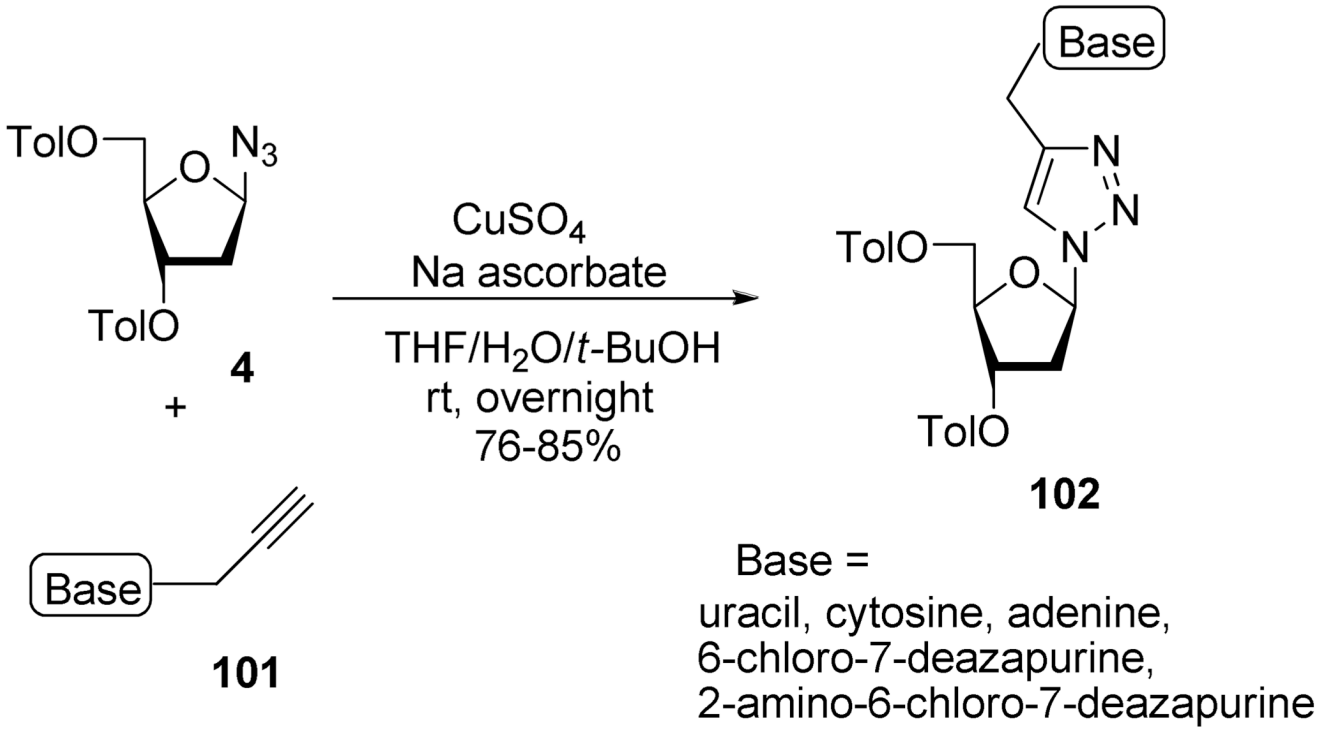

Scheme 28.
Synthesis of “Fleximers” 102.