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Review

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Synthesis and biological activity of potential antiviral compounds through 1,3-dipolar cycloadditions; Part 1: general aspects and reactions of azides

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Dedicated to Prof. Giovanni Desimoni, Master of Chemistry of GF and PQ, on the occasion of his 85th birthday

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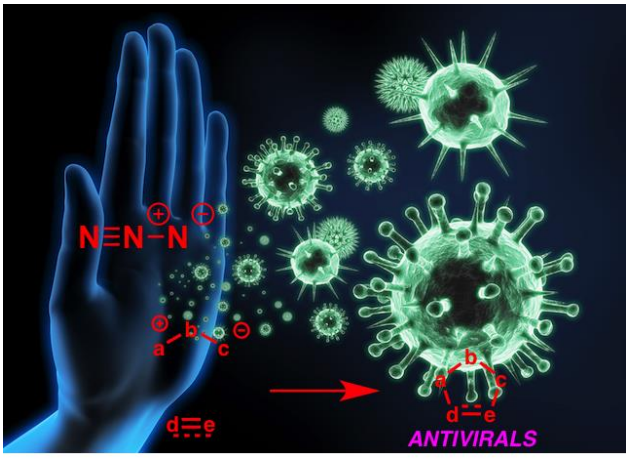
Abstract

Prominent in the current stage of drug development, antiviral compounds can be efficiently prepared through cycloaddition reactions. This review reports the use of 1,3-dipolar cycloaddition reactions of selected 1,3-dipoles, in particular azides, in the light of their application for the preparation of key intermediates in the design and synthesis of compounds that were tested for their antiviral activities against a variety of viruses. The products obtained from these pericyclic reaction approaches were tested for their activities in terms of blocking the virus replication and the relevant biological data are highlighted.



Keywords: Antivirals, 1,3-Dipolar cycloadditions, Azides, Synthesis, Biological evaluation, Nucleosides

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1. Introduction

Many research groups along the last fifty years have exhaustively investigated cycloaddition reactions, classified within the group of pericyclic reactions. These studies have been pursued both on the basis of experimental research and upon theoretical methodologies based on *ab initio* calculations.[1] The complex matter regarding mechanisms and potential synthetic applications of cycloaddition reactions is constantly being discussed in literature as the level of complexity of theoretical investigations and solution demands increases. The synthesis of molecules with high level of complexity and increasing importance, mainly in the medicinal chemistry field, places the topic "cycloaddition" in front of the dichotomy: to be useful and participate in the pivotal step of a synthesis or to be relegated to a simple chemical curiosity, not applicable to solve tremendous needs in modern organic chemistry. Cycloaddition reactions are a mature topic of organic chemistry, but abundantly used in different areas where chemistry represents the pivotal step for reaching the desired target. Books and reviews dedicated to cycloaddition reactions deal typically with either the synthesis of heterocycles, or general synthetic applications, or specific uses in natural product synthesis, or describe the use of a class of organic compounds as partners in the cycloaddition reactions.[2] However, other topics are still demanding for a general review since in recent years pericyclic reactions were extensively applied to different areas of chemistry, such as chemical biology, biological processes, catalyzed cycloaddition reactions, delivery of antiviral or anticancer drugs, targeting of biological active molecules, materials chemistry with applications in medicine, photovoltaic processes, photochemistry, energy harvesting, etc. Moreover, the chronological structure of the references regarding a general topic allows the reader to recognize the relevance of specific subjects during the past decades and the evolution of the synthesis with respect specific active compounds. Filling the gap between traditional pericyclic reaction uses and new synthetic strategies for specific target compounds will shed a new light over pericyclic reactions and demonstrating how these valuable tools may elegantly solve synthetic and mechanistic problems in modern organic chemistry.

The monumental work of Prof. Rolf Huisgen and co-workers led in the 1960s to the general concept of 1,3-dipolar cycloaddition reactions.[3] These valuable synthetic tools permit the preparation of molecules of higher complexity than the reactants. Few reactions rival these processes in synthetic utility, specifically in the field of heterocyclic chemistry. Five-membered heterocycles are the synthetic target of these reactions and their ability to produce heterocycles extends the importance of the 1,3-dipolar cycloaddition to other areas of organic chemistry, not strictly related to the ring formation processes. In addition, the use of chiral catalysts allowed for the control of newly formed stereocenters in the cycloaddition reactions with the valuable

stereochemical outcome borne by the newly synthetized molecules. If these latters are drugs, this is a key point for determining the success of a synthetic approach.

Here, we wish to propose a timely updated overview on the use of 1,3-dipoles for the synthesis of antiviral compounds, starting from the azides. There is no need to emphasize the great occurrence of information regarding the synthesis of antiviral compounds in the present days characterized by a worldwide pandemic emergency. Many drug developers dropped everything else to turn their attention to tackling the virus. The public suddenly became aware of the complexity of drug manufacturing, the fallibility of clinical trial design, and the tricky nature of immunity. Organic synthesis is constantly in search of methods for preparing new biological active molecules never imagined before and designed by modelling studies. This subject (the 1,3-dipolar cycloaddition) somewhat represents a valuable example of "ancient methodology" modernly used in chemistry and in modern organic synthesis. There is always need for a comprehensive view in a specific topic, especially when the urgency of novel applications forces the researcher in a rush to reach their synthetic goals.

This subject is still demanding for an updated review since in recent years pericyclic reactions were extensively applied to different chemistry areas, *e.g.* chemical biology, biological processes, catalyzed cycloaddition reactions, delivery of antiviral or anticancer drugs, targeting of biological active molecules, materials chemistry with applications in medicine, photovoltaic processes, photochemistry, energy harvesting, etc. None of these topics, where cycloaddition reactions spread out, has received a unifying overview in a single review with the specific background of the 1,3-dipolar cycloadditions, offering a solid base for future development in these and other subjects.

Literature covers the years from the 1990s, the period corresponding to the beginning of the use of 1,3-dipolar cycloadditions for the synthesis of biologically active molecules up to 2021. At a first glance, literature is increasing a lot and in the last ten years we count 131 papers over a total estimated of 188 manuscripts published on international journals (nearly 70%), testifying the increasing interest in 1,3-dipolar cycloadditions as synthetic tools to antiviral compounds. For the azides we count 119 manuscripts (source CAS SciFinder).
Due to this, the scope is to give a clear, comprehensive, and, as much as possible, exhaustive picture of the methodologies the chemists can apply for future developments in this pivotal strategic route. The main limitation relies into the choice of 1,3-dipoles that can be used in the context. Azides are for sure the largest used accompanied by nitrones and nitrile oxides. Few other 1,3-dipoles have been proposed with variable results. But, we believe that in the next years new 1,3-dipoles are expected to find applications in the field of interest.

This review aims to be a comprehensive text of general interest to the chemistry community because focuses the synthesis of antiviral compounds marking the key role of unconventional catalyzed and uncatalyzed cycloaddition reactions as valuable tools to prepare new derivatives in a unique reaction pathway, even scalable in industrial processes. It contains the most updated review regarding the use of catalyzed and uncatalyzed 1,3-dipolar cycloaddition reactions in the synthesis of antiviral compounds.

1.1 The viruses

The Viruses, the smallest and simplest biological structures lacking any type of cellular organization, are forced to live within cells, as intracellular parasites and the viral infections they produce deeply marked human history. The development in recent decades of new and powerful diagnostic methodologies allowed for an increasing capacity to identify new viruses. From the 1960s on, new frightening hemorrhagic african fevers were discovered and attributed to the filoviruses, while the AIDS appeared in 1981, although HIV was already present in human population in the 1930s.

Another emergency is the re-appearance of viral infections due to mutations and resistance to antiviral therapies. This is the case of the influenza viruses; the virus surface proteins have changed and are not recognizable by the human immune system any more. Sometimes the virus can be exchanged from humans to animals and back to the humans determining new pandemic situations and social alarms. Viral infections are detrimental since, contrary to bacteria, viruses cannot be defeated with antibiotics. Until very recently, only a relatively small number of antiviral compounds could be used for the pharmacological treatment of these diseases. Luckily, not only viruses are in progress but also the chemical research and the synthetic approaches to new and powerful method for the contrast to viral infections. The demand of antiviral compounds is constantly increasing and chemistry is constantly under exam to furnish novel strategies for a large scale preparation of active molecules.[4]

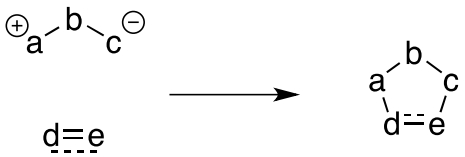
The term "emerging viruses" refers to both viruses that appear for the first time and those viruses that, after apparently disappearing, reemerge after many years. Marburg, Ebola and HIV are some of these but SARS, due to a new coronavirus, was identified in Hong Kong in early 2003. These emerging viral diseases all have a natural animal reservoir that do not develop the disease but produce a huge number of viral particles. Man can be infected by contact with these species.

Antiviral compounds, besides vaccines, are a chemical answer to these infections. Unfortunately, antivirals are subjected to resistance since RNA-dependent viruses are rapidly oriented to mutation. An antiviral compound, when administered, tends to select the viral strain that, as a mutation consequence, acquired the resistance to the drug itself. This is a huge problem of great relevance, in particular when the number of effective drug is limited. That is why the search of new and potent antiviral compounds is a constant need and the search of new synthetic methods is of great benefit for the contrast to viral infections.

2. 1,3-Dipolar Cycloaddition Reactions

2.1 General aspects

Cycloadditions can be classified according either to the number of new σ-bonds formed or considering to the size of the ring being formed. Usually, two reactants unite to form the cyclic compound, creating two new σ-bonds at the expense of two π-bonds. The thermal or photochemical formation of cyclobutanes from alkenes and the Diels-Alder (DA) synthesis are two important cycloadditions in which 4- and 6-membered rings are obtained, respectively. A cycloaddition of the type [3+2] affording an uncharged 5-membered ring cannot possibly occur with octet-stabilized reactants, which have no formal charges. A 1,3-dipole, *a-b-c*, must be defined, such that atom *a* possesses an electron sextet with a positive formal charge and that atom *c* is negatively charged. The combination of such a *1,3-dipole* with a multiple bond system *d-e* (alkene or alkyne), termed the *dipolarophile*, is referred to as a *1,3-dipolar cycloaddition* (Scheme 1) affording a 5-membered ring.[3]



Scheme 1. Schematic representation of a 1,3-dipolar cycloaddition reaction.

The term "1,3-dipolar cycloaddition", beyond any possible misunderstanding, is derived from the fact that compounds, which can only be represented by zwitterionic all-octet resonance structures, are ambivalent in the 1- and 3-positions, thus displaying electrophilic as well as nucleophilic reactivity.

2.2 Experimental and theoretical concerns

Compounds containing an electron sextet at *a* carbon, nitrogen, or oxygen atom are known to be not stable. Stabilization is possible if an unshared pair of electrons at atom *b* can relieve the electron deficiency at center *a* by formation of an additional bond. In the new mesomeric formula, in which *b* now has the positive charge, all the centers have completely filled valence shells. Such systems will be designated as 1,3-dipoles with internal octet stabilization (Figure 1).

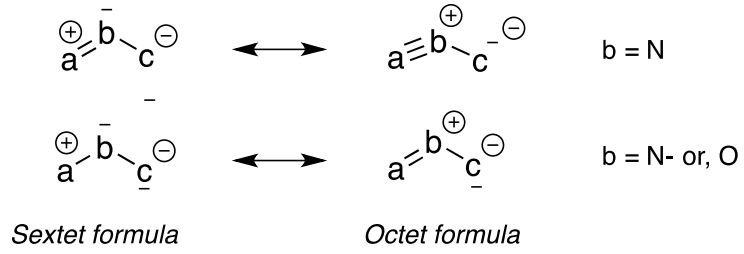


Figure 1. Sextet and octet structures of 1,3-dipoles.

The sextet structure of the 1,3-dipoles may contain either a double or a single bond between *a* and *b*. When a double bond is present and we confine ourselves to the lowest group of eight elements in the periodic table, then the middle atom, *b*, the free electron pair of which is borrowed by the sextet center, *a*, must be nitrogen. No other element has an extra pair of electrons available while remaining in the triply bonded neutral state.

In 1,3-dipoles where no double bond is present in the "unmasked" (sextet) formula, the role of the center *b* can be assumed by either a nitrogen function or an oxygen atom. A simple classification for 1,3-dipoles is obtained when C, N, and O atoms, in that order, are successively made to carry the positive and the negative charges of the sextet formula. With the centers *a*-*b*-*c* limited to these three elements, six 1,3-dipoles with double bonds can be visualized. However, a large number of 1,3-dipoles containing various combinations of carbon and hetero-atoms is theoretically possible and many have been made and studied. Figure 2 shows the historical classification made by Rolf Huisgen of propargyl and allyl type dipoles that is reference and canon for all those who investigate this topic.[3,5]

Normally, 1,3-dipolar cycloaddition reactions are concerted processes, like the DA reactions.[5] This determines the high regio- and stereo-selectivities, among other factors.[6] Some 1,3-dipolar cycloadditions are controlled mainly by the $HOMO_{(dipole)}$-$LUMO_{(dipolarophile)}$ interactions while others by the reverse interactions, *i.e.* $LUMO_{(dipole)}$-$HOMO_{(dipolarophile)}$; sometimes both the FMO interactions are important in determining reactivity and selectivity. In Figure 3, Huisgen depicted the behaviour of dipoles and dipolarophiles in the various types of cycloaddition reactions.

The smaller the energy gap between the controlling orbitals the faster the reaction. The former are accelerated by electron-donating substituents in the 1,3-dipoles and electron-withdrawing substituents in the dipolarophiles (Type I) while to opposite applies to the latter (Type III). In Type II cycloadditions, the controlling interaction depends on the nature of the dipolarophile and on the electronic nature of the substituents on the 1,3-dipole. This change in orbital control from $HOMO_{(dipole)}$-$LUMO_{(dipolarophile)}$ to *vice versa*

may have consequences in the observed regioselectivities. All these aspects must be taken into consideration and be carefully evaluated when planning a synthetic route.[7]

The 1,3-dipolar cycloaddition reactions are indeed extremely versatile. The range of available synthetic possibilities is so large to be comparable to that of the DA synthesis. Even today, 1,3-dipolar cycloadditions allow the preparation of a large number of 5-membered heterocycles.

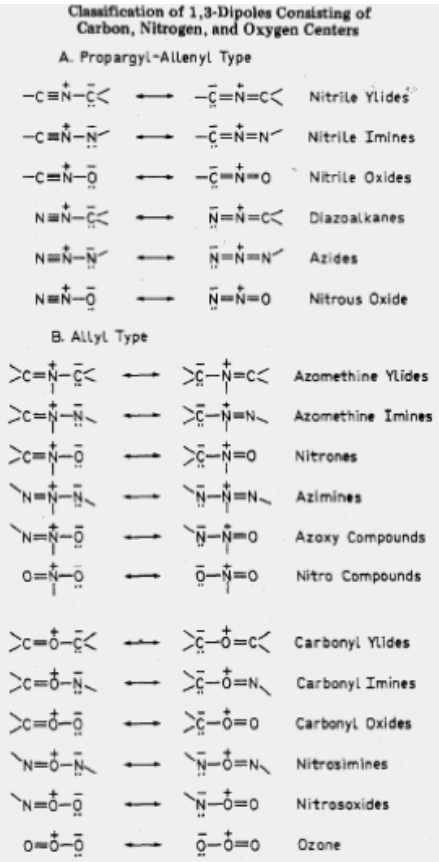


Figure 2. Classification of 1,3-dipoles (Reproduced with permission.[5] Copyright 1976, ACS).

The fact that the numerous individual reactions studied are patterned after a single principle, not only in a formal but also in a mechanistic sense, is particularly satisfying.

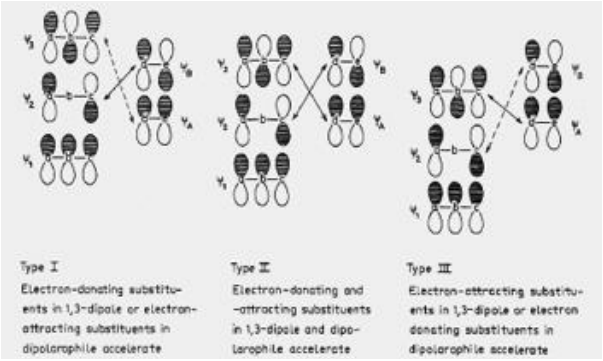


Figure 3. The HOMO-LUMO interactions between 1,3-dipoles and dipolarophiles (Reproduced with permission.[6] Copyright 1984, Wiley).

Many of the 1,3-dipoles are represented thus far by more or less exceptional structural types, if not indeed chemical curiosities and became rapidly representatives of the new classes of dipoles; their ability to undergo cycloadditions have been tested. The development of general synthetic routes leading to 1,3-dipoles has received closer attention in many studies. Moreover, the use of 1,3-dipoles in modern organic synthesis continues to fascinate many research groups and here finds a useful picture in the remarkable application towards the synthesis of antiviral compounds.

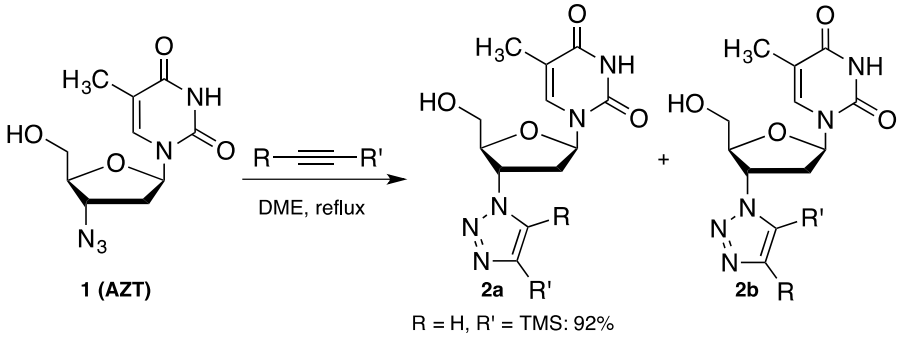
3. Synthesis of Antiviral Compounds

The synthesis of antiviral compounds by the use of 1,3-dipoles is here represented with some examples of application of these valuable highly reactive intermediates *in situ* generated or even in their stable forms, when possible.[8] The 1,3-dipole role is both in the introduction of suitable residues, necessary to display the antiviral activity, and as starting material for the generation of other reacting species, such as the case of nitrosocarbonyl intermediates,[9] which often represent the key intermediates for the synthesis of the target compounds.

In the search of the most frequently used 1,3-dipoles in the synthesis of antiviral compounds, not all the possible 1,3-dipoles have found application so far. Moreover, just those successfully employed for the preparation of biologically active molecules have been selected for this review and we report chronologically those cases where the *in vitro* biological data are also available.

3.1 Synthesis of antiviral compounds through azides

The most frequently used 1,3-dipoles in antiviral compound synthesis are azides. The versatility of this 1,3-dipole is well-known because of its use in "click chemistry" as well as for the preparation of triazole-based biologically active compounds.[10] Organic azides belong to the propargyl-allenyl category of dipoles and since the discovery of triazole formation from phenyl azide and dimethyl acetylenedicarboxylate (DMAD) in 1893,[3,6] they found a large success in the construction of heterocyclic frames and core structures of natural and bioactive compounds.[11]

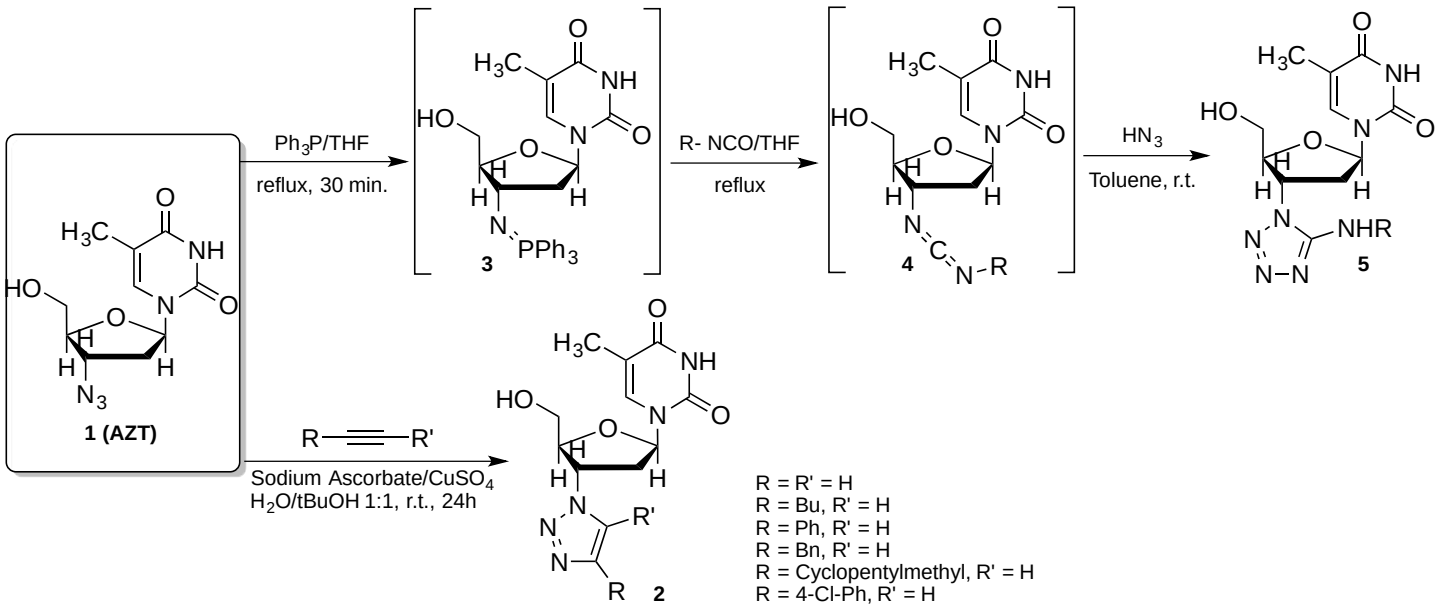


Scheme 2. Synthesis of 1-[(2*R*,4*S*,5*S*)-5-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]-tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione derivatives (**2**).

Unnatural 2',3'-dideoxynucleosides such as 3'-azido-3'-deoxythymidine (Scheme 2, **AZT**, **1**) are potent agents against the human immunodeficiency virus (HIV), giving rise to the acquired immunodeficiency syndrome (AIDS). After conversion to the 5'-triphosphates by cellular kinases, these compounds inhibit the

HIV-reverse transcriptase (RT). They act either as competitive inhibitors or as chain terminators of viral DNA polymerization due to the lack of the 3'-hydroxy group. However, long-term treatment limitations arise from its inherent bone marrow toxicity and the appearance of AZT-resistant mutants, thus making it necessary to search for novel analogs.

For this reason, compound **1** was coupled with a variety of alkynes, variably substituted, to give the regioisomeric cycloadducts **2a,b** generally in very good yields (36-92%). The regioselectivity is reflected by a slight preference for the regioisomer **2a** when R is an hydrogen atom[12] The simple procedure (reflux in DME) allowed for obtaining a large variety of products, but unfortunately none of the synthesized compounds exhibited good activity in HIV-1 infected CEM-V and MT-2V cells, nor did they inhibit syncytium formation in infected human peripheral blood monocytes. The inactivity was attributed to either lack of phosphorylation by cellular kinases or low affinity of the 5'-triphosphate to the HIV-RT. AZT **1** was similarly used in a one-pot synthesis of tetrazole derivatives. Compound **1** reacts with triphenylphosphine to form the intermediate **3** (Staudinger reaction) that can be functionalized with a variety of isocyanates to give the intermediates of type **4**. Upon treatment with hydrazoic acid in toluene solution, the tetrazole derivatives **5** were obtained in fair to very good yields (Scheme 3).[13]



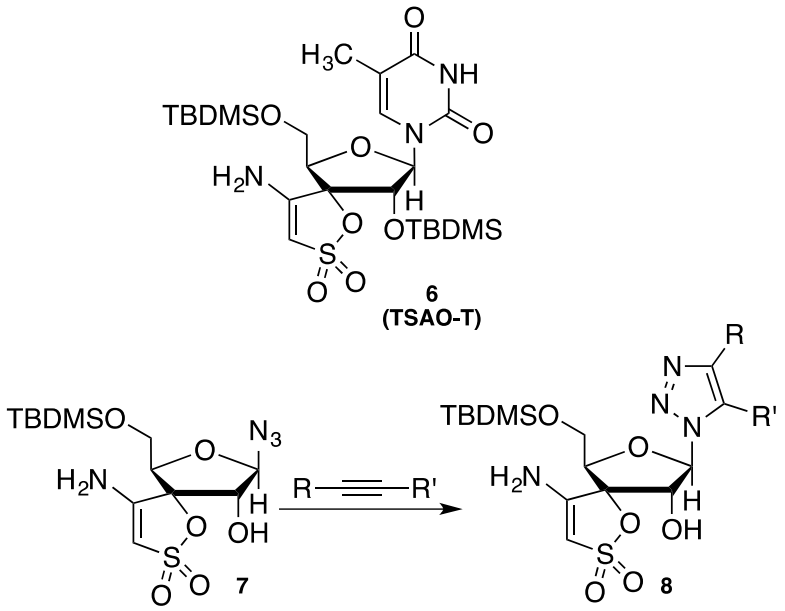
Scheme 3. Synthesis of tetrazolyl- and triazolyl-deoxythymidines.

Unfortunately, the biological evaluation of these products did not give the expected results since none of them showed appreciable activity against HIV-1 or RNA- and DNA-viruses.

AZT (**1**) was conversely used to prepare a variety of 1,2,3-triazolyl derivatives of type **2** obtained in good yields through classical Cu-mediated azide 1,3-dipolar cycloaddition. By replacing the tetrazole ring by a 4,5-substituted triazole ring, a new generation of very potent thymidine kinase (TK-2) inhibitors was obtained. The modified sugar combined with a bromovinyl substituent at position 5 of the pyrimidine moiety further enhanced the selectivity towards TK-1.[14] A series of unknown 3'-α-[1,2,3]-substituted triazolo-2',3'-dideoxypyrimidine nucleoside analogues of the anti-HIV 3'-azido-3'-deoxythymidine (AZT) were synthesized through catalyzed alkyne-azide 1,3-dipolar cycloaddition. The obtained 3'-[1,2,3]-triazolo analogues were evaluated for their anti-HIV activity against HIV-1 in primary human lymphocytes as well as for their cytotoxicity in different cells. None of them inhibits HIV replication (EC_{50} > 20 μM).[15] Further developments

were achieved through the synthesis of triazolo-fused 3',5'-cyclic nucleoside analogues by an intramolecular 1,3-dipolar cycloaddition of nucleoside-derived azido-alkynes in a regio- and stereospecific manner. The thymine nucleoside base in the target compounds was transformed successfully into the corresponding 5-methylcytosine component. The synthesized compounds were tested for exploring the anti-HIV activity and in a H9 T lymphocytes assay for measuring the cell toxicity with modest results.[16]

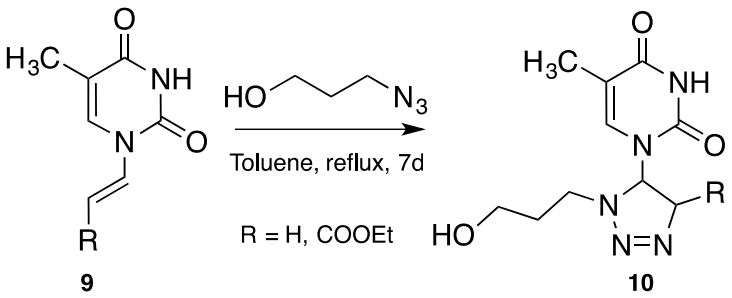
TSAO nucleoside analogues represent a class of HIV-1-specific agents, the prototype compound of which is the 1-[2',5'-bis-*O*-(tert-butyldimethylsilyl)-β-D-ribofuranosyl-thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide (**TSAO-T**, **6**). They are targeted at the HIV-1-encoded RT with which they interact at a non-substrate binding site; they are not antivirally effective against HIV-2 and other (retro)viruses. TSAO derivatives are the first HIV-1-specific RT inhibitors for which a well-defined part of the molecule has been identified as an essential pharmacophore interacting with a well-defined moiety of HIV-1 RT (Scheme 4).[17]



Scheme 4. Synthesis of triazole-TSAO derivatives **8**.

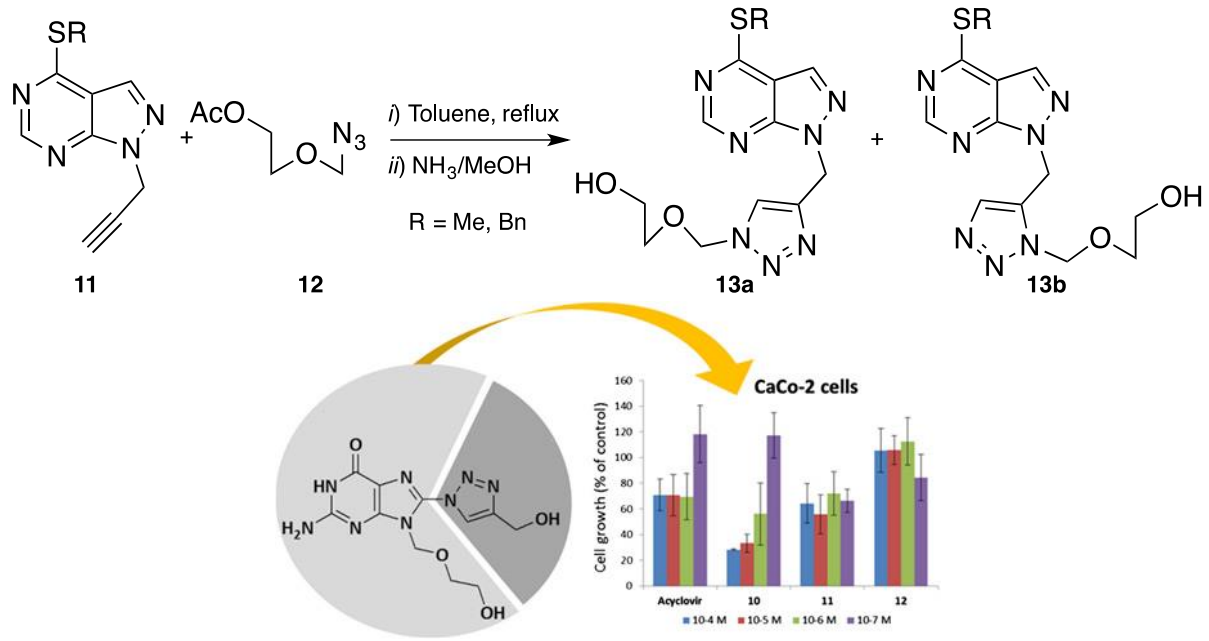
In order to determine the interaction points of TSAO compounds with the HIV-1 RT and to reveal the role that the nucleobase may play in this interaction, the synthesis and anti-HIV-1 activity of a series of TSAO analogues were developed. The thymine moiety of compound **6** was replaced by a series of 1,2,3-triazoles substituted with groups that may be involved in the interaction of the TSAO derivatives with the RT enzyme.[18] Scheme 4 reports the synthetic approach starting from the azido-derivative **7** that reacts with several substituted alkynes to afford the desired products **8**. These spironucleosides were tested for their inhibitory activity on HIV-1 and -2 induced cytopathicity in MT-4 and syncytium formation in CEM cells. None of them was active against HIV-2. Most of the 4- and 5-substituted 1,2,3-triazole TSAO derivatives proved to be more inhibitory to HIV-1, thus demonstrating that the nature of the substituent of the triazole plays a key role for the activity.

Vinyl heterobases, such as the thymine derivatives of type **9** (Scheme 5), were chosen to be reacted with 3-azidopropanol under harsh reaction conditions. These allowed for the obtaining of the triazoline compounds of type **10** in poor yields (11%) and, unfortunately, the products were found to be unstable under forcing conditions and none of the synthesized products was tested as antiviral.[19]



Scheme 5. Synthesis of the 1-[1-(3-hydroxypropyl)-4,5-dihydro-1*H*-1,2,3-triazol-5-yl)] -5-methylpyrimidine -2,4(1*H*,3*H*)-dione derivatives (**10**).

Similarly, suitably substituted heterobases, specifically pyrazolo-pyrimidines **11** bearing a triple bond were put to react with an azido molecule **12** resembling the acyclovir chain portion and the two regioisomeric products were obtained in very good yields (93-97%), which were separated and deprotected to give **13a,b** (Scheme 6).[20]



Scheme 6. Synthesis of regioisomeric 2-((4-(1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)methoxy)ethan-1-ol derivatives (**13**).

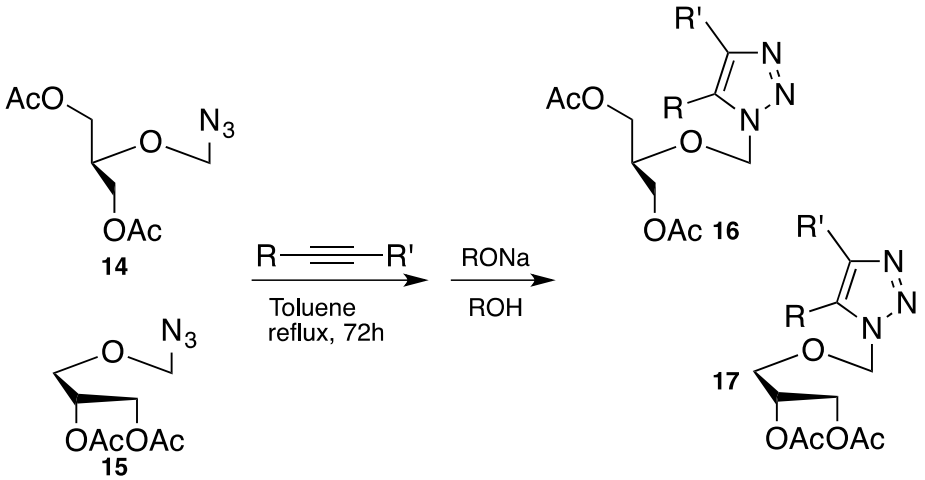
As a part of the research of another group dealing with the identification of new nucleobase derivatives with improved biological properties, a series of 8-substituted acyclovir derivatives was synthesized. The 8-azidoguanosine and novel 8-azidoacyclovir were synthesized from commercially available guanosine and acyclovir, and transformed into 8-bromopurine hydrazine derivatives. The 8-triazolylguanosine and 8-triazolylacyclovir analogs were successfully synthesized via the Cu(I) catalyzed reaction of azides with propargyl alcohol (4-pentyn-1-ol and 5-hexyn-1-ol). The new 1,4-disubstituted 1,2,3-triazolyl compounds were tested for antiviral activity against selected DNA and RNA viruses and cytostatic activity against normal Madine Darby canine kidney (MDCK I) cells, and seven tumor cell lines (HeLa, CaCo-2, NCI-H358, Jurkat, K562, Raji and HuT78). While the tested compounds exerted no antiviral activity at nontoxic concentrations, the 8-triazolyl

acyclovir derivative, with the shortest alkyl substituent at the C-4 of triazole ring, was found to be the most active against the CaCo-2 cell line (Scheme 6).[21]

The above-mentioned compounds (some of them shown in Scheme 6 and other derivatives) were also evaluated for the inhibitory effects against the replication of HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cells and for their anti-tumour activity. No important biological activity was found. Initial evaluation of these compounds showed that compound **11** (R = Bn) has marked activity against Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium (inhibition % > 90 %, MIC = 12.5 □g·mL^{-1}).[22] Other derivatives were also synthesized by replacing the SR group on the heterobase moiety with some NRR' and OR groups. The products obtained were evaluated for the inhibitory effects against the replication of HIV-1 (III_B), HIV-2 (ROD), various DNA viruses, a variety of tumor-cell lines and tuberculosis. Unfortunately, no significant biological activity was found.[23]

The same methodology was applied to the pyrimidine heterobases that were functionalized with a triple bond to become the dipolaropihile of choice for the azido cycloaddition with compound **12** (see Scheme 6). The regioisomeric compounds obtained in very good yields were tested for their inhibitory effect against the cytopathic effect of HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cells. No activity was observed against the replication of these viruses at compound concentrations up to 100 μg/mL.[24]

A valuable advancement is represented by the short and efficient synthesis of 1,2,3-triazole- and bis-1,2,3-triazole-acyclonucleosides, analogues of Acyclovir. A series of new 1,2,3-triazole acyclonucleosides linked to nucleobases were prepared *via* Cu(I)-catalyzed 1,3-dipolar cycloaddition of N-9 propargylpurine, N-1-propargylpyrimidines or N-1-propargylindazoles with the azido-pseudo-sugar under microwave-assisted synthesis followed by treatment with K_2CO_3/MeOH. The results were excellent in terms of yields and reaction time but no biological data were given.[25] Upon pursuing their investigations, Morocco's group proposed the preparation of 1,4-disustituted-1,2,3-triazolo-quinazoline ribonucleosides or acyclonucleosides by means of 1,3-dipolar cycloaddition between various *O*- or *N*-alkylated propargyl-quinazoline and 1'-azido-2',3',5'-tri-*O*-benzoyl ribose or activated alkylating agents under microwave conditions. Unfortunately, none of the compounds selected showed significant anti-HCV activity in vitro.[26]



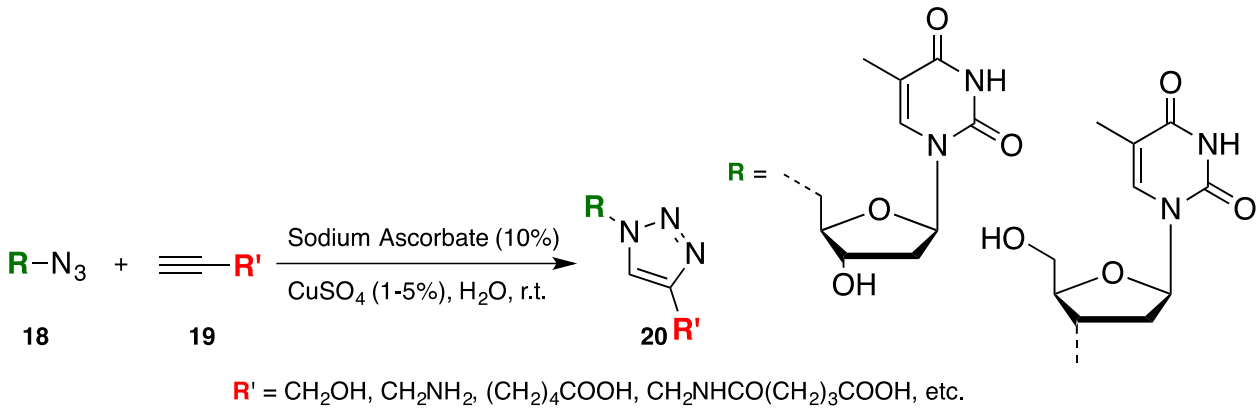
Scheme 7. Synthesis of 1,2,3-triazole acyclonucleosides.

Upon extending the investigations on acyclic nucleosides, new azido derivatives **14** and **15** were suitably prepared through a convenient method that uses glycerol as starting material. These 1,3-dipoles were then coupled with a variety of alkynes to afford the corresponding 1,3-dipolar cycloadducts of type **16** and **17**

(Scheme 7) in good yields (*ca.* 70%). The isolated and deprotected products were tested against the viruses Herpes simplex (HSV-1, HSV-2), vesicularstomatitis (VSV), vaccina (VV), cytomegalovirus (CMV), parainfluenza 3 (PIV) but they were found inactive.[27]

In a similar approach a series of mono-1,2,3-triazole and bis-1,2,3-triazole acyclonucleoside analogues was prepared via Cu(I)-catalyzed 1,3-dipolar cycloaddition of N-9 propargylpurines, N-1-propargylpyrimidines/triazine with the azido-pseudo-sugar 4-azidobutylacetate under solvent-free microwave conditions, followed by treatment with K_2CO_3/MeOH, or NH_3/MeOH. All studied compounds were screened for the antiviral activities [against human rhinovirus (HRV) and hepatitis C virus (HCV)] and antibacterial activities against a series of Gram positive and -negative bacteria. None of the products as antivirals exhibited specific activity, which means that they did not inhibit the replication of any of the tested viruses.[28]

An efficient regioselective synthesis of nucleoside conjugates was achieved by cycloaddition reaction of a variety of azides of type **18** with alkynes **19** using the sodium ascorbate/$CuSO_4$ system as a catalyst. These 16 novel thymidine analogues **20** were obtained in excellent yields (75–100%), employing mild reaction conditions with a broad scope of structural modification (Scheme 8). The synthesized compounds of type **20** were evaluated, but found inactive, against the following viruses: parainfluenza type 3, reo type1, Sindbis, Coxsackie B4, Punta Toro, vesicular stomatitis, respiratory syncytial, herpes simplex, vaccinia, cowpox, and human immunodeficiency virus (HIV). They were also screened for their antiviral activity against vaccinia and cowpox viruses using a cytopathic effect inhibition assay in HFF cells and none of them showed any activity (cidofovir as a positive control was active at about 8 µM).[29]

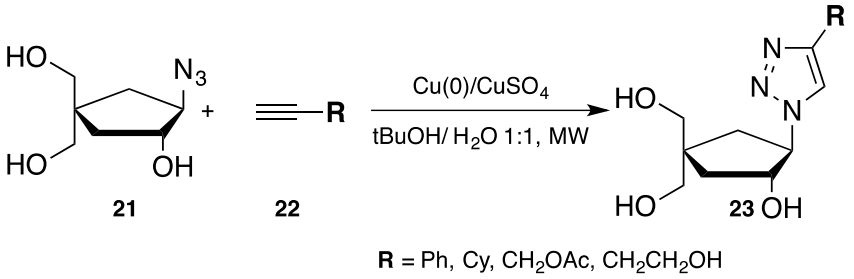


Scheme 8. Synthesis of thymidine analogues of type **20**.

The same method was applied for the preparation of carbanucleosides. Using a microwave assisted 1,3-dipolar cycloaddition, various alkynes of type **22** were reacted with the azido-carbocycle **21** (Scheme 9). The mixture copper/copper sulfate Cu(0)/Cu(II) *in situ* comproportionated was chosen as catalyst in water and *tert*-butanol as a co-solvent. All the cycloadditions reached a rapid complete conversion of the azidocarbocycle into the desired carbanucleoside **23**, obtained in excellent yields (95-98%).

Their antiviral activities and cellular toxicities were tested on the vaccinia virus. None of the synthesized compounds exhibited a significant antiviral activity.[30] The evolution of this work led to the synthesis of 1,2,3-triazolo-3'-deoxy-4'-hydroxymethyl carbanucleosides using different reaction conditions and diverse modulations on the heterocycle residues. Heterocyclic moieties were efficiently introduced on the pseudo-sugar either via nucleophilic substitution or via 1,3-dipolar Huisgen cycloaddition. With this latter approach, 1,4-disubstituted and 1,4,5-trisubstituted-[1,2,3]-triazolo-3'-deoxy-4'-hydroxymethyl carbanucleosides were

prepared from the corresponding azidocarbocycle and various terminal or internal alkynes. Antiviral activities and cellular toxicities of the final compounds were evaluated as smallpox inhibitors. Unfortunately, at concentrations up to 100 ?M, none of them inhibited production of vaccinia virus (Lister strain) or cowpox virus (Brighton strain) in Vero cells.[31]



Scheme 9. Synthesis of carbanucleosides **23**.

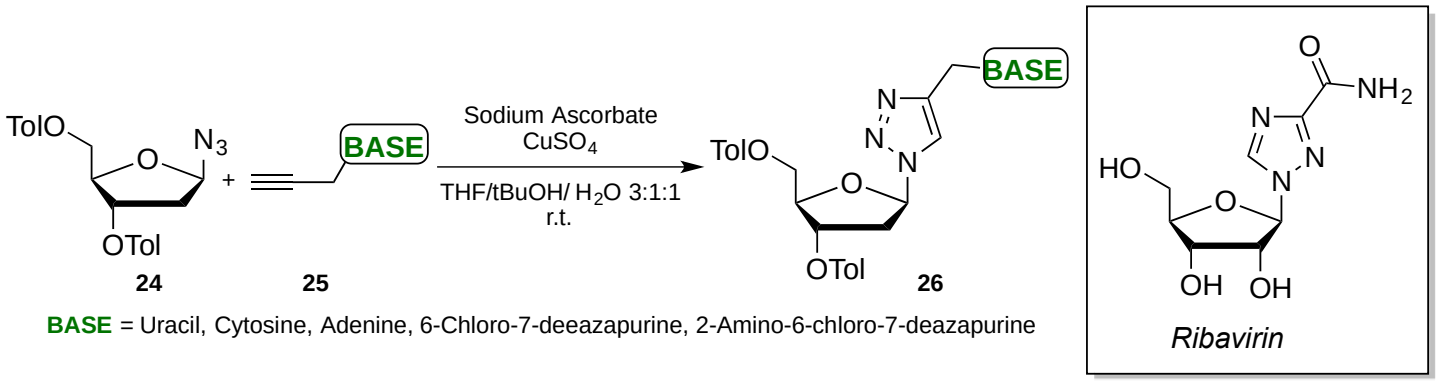
A simple structural modification of compounds **23** have been proposed by Spanish authors that prepared diastereoisomeric 2-azido-4-(hydroxymethyl)cyclopentan-1-ols that were used to synthesize a series of racemic 4-aryl-1,2,3-triazolyl carbanucleosides through a "click chemistry" strategy.[32] The target compounds were tested against a variety of viruses but unfortunately none of them displayed a significant inhibitory activity. A series of trisubstituted 1,2,3-triazole purine nucleosides were efficiently synthesized via Huisgen 1,3-dipolar cycloaddition in good yields from but-2-yne-1,4-diol and ethyl 2-azidoacetate. Bioactivity against cytomegalovirus (CMV) and varicella-zoster virus (VZV) in human embryonic lung cell cultures was evaluated but none of the cycloadducts show significant activity towards the selected viruses.[33] The same authors proposed a series of ribonucleosides of 1,2,3-triazolylbenzyl-aminophosphonates through the Kabachnik-Fields reaction using I_2 as catalyst followed by copper-catalyzed cycloaddition of the azide-alkyne reaction (CuAAC). The compounds were tested against various strains of DNA and RNA viruses with a modest inhibitory activity against respiratory syncytial virus (RSV) and modest inhibitory activity against Coxsackie virus B4.[34]

Later on, a reverse approach was proposed for the synthesis of ribavirin analogues under micellar catalysis. A *C*-alkynyl riboside engaged in a Huisgen 1,3-dipolar cycloaddition reaction with a variety of azides to afford a library of 1,2,3-triazole derivatives in good yields.[35]

In close similarity with the above reported synthetic scheme, a variety of 1,2,3-triazole nucleosides of type **26** linked to DNA nucleobases were prepared via Cu(I)-catalyzed 1,3-dipolar cycloaddition of *N*-9 propargyl-purines or *N*-1 propargyl-pyrimidines with the tolyl protected 1-azido-2-deoxyribofuranose **24** followed by treatment with MeONa/MeOH or aq. NH_3 (Scheme 10). In general, the yields were very good and the products were tested for their antiviral activity against selected RNA viruses.

Triazole nucleosides showed pronounced biological activities. The five-membered 1,2,4-triazole nucleoside ribavarin (virazole, see structure in the inset of Scheme 10) was the first synthetic nucleoside showing a broad spectrum of antiviral activities against many RNA and DNA viruses. These results prompted the authors to evaluate the antiviral activity of the related triazole compounds. Among the tested viruses, representatives of flavivirus, which belong to the class of positive-sense single-stranded RNA ($ssRNA^+$) viruses, were selected. These are Hepacivirus (hepatitis C; HCV), yellow fever virus (YFV), dengue virus type 2 (DENV-2), and West Nile virus (WNV). Unfortunately, none of the compounds show selective antiviral activity against the tested $ssRNA^+$ viruses. Only the tolyl protected 7-deazaadenine and 7-deazaguanine nucleosides developed less cytotoxicity than other triazole compounds in the HCV replicon system.[36] The synthesis of 1,4-

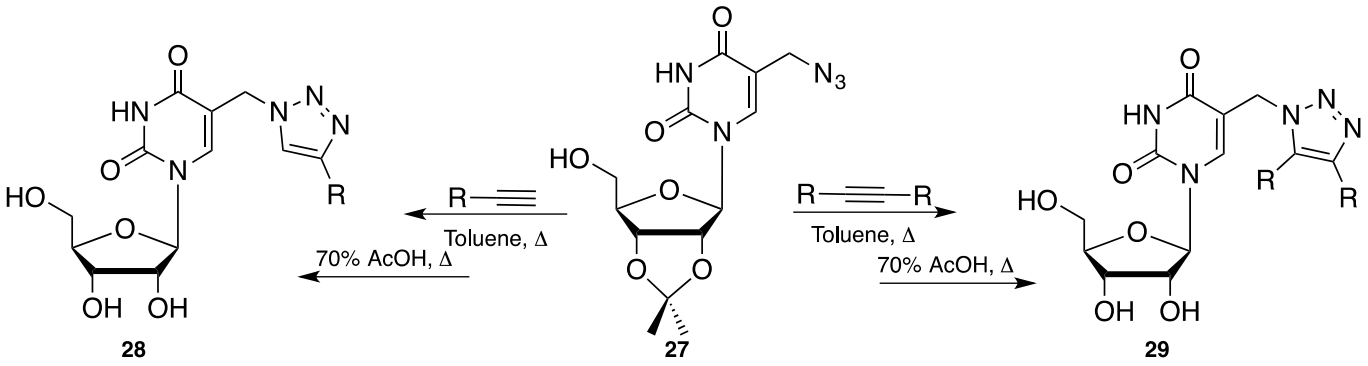
disustituted-1,2,3-triazolo-ribonucleosides was also performed by means of 1,3-dipolar cycloaddition between various *N*-1 propargyl-pyrimidines and 1'-azido-2',3',5'-tri-*O*-benzoylribose catalyzed by $Na_2CuP_2O_7$/sodium ascorbate. These derivatives were tested *in vitro* with the aim to evaluate their anti-HCV activity. None of the new compounds were found to inhibit HCV replication.[37]



Scheme 10. Synthesis of 1,2,3-triazole nucleosides **26**.

The same conceptual approach led to the synthesis of 1,4- and 1,5-disubstituted-1,2,3-triazolo nucleosides from several alkynes with 1'-azido-2',3',5'-tri-*O*-acetyl ribose using either copper-catalyzed azide-alkyne cycloaddition (CuAAC) or ruthenium-catalyzed azide-alkyne cycloaddition (RuAAC), respectively. Optimized RuAAC conditions were realized with the commercially available [Cp*RuCl(PPh_3)$_2$] under microwave heating, which allows a significant acceleration of the reaction times (from 6 h to 5 min). RuAAC and CuAAC were found to be useful tools for the synthesis of 1,2,3-triazolyl-nucleosides small libraries, but the investigated biological activity and toxicity of the synthesized triazoles against HCV in a replicon system in Huh-7 cells did not exhibit any significant activity or toxicity.[38]

Some 5-(1,2,3-triazol-1-ylmethyl)-uridine derivatives of type **28**,**29** were synthesized via the 1,3-dipolar cycloaddition of a 5-azidomethyl-uridine derivative **27** with substituted mono- and di-substituted acetylenes (Scheme 11).

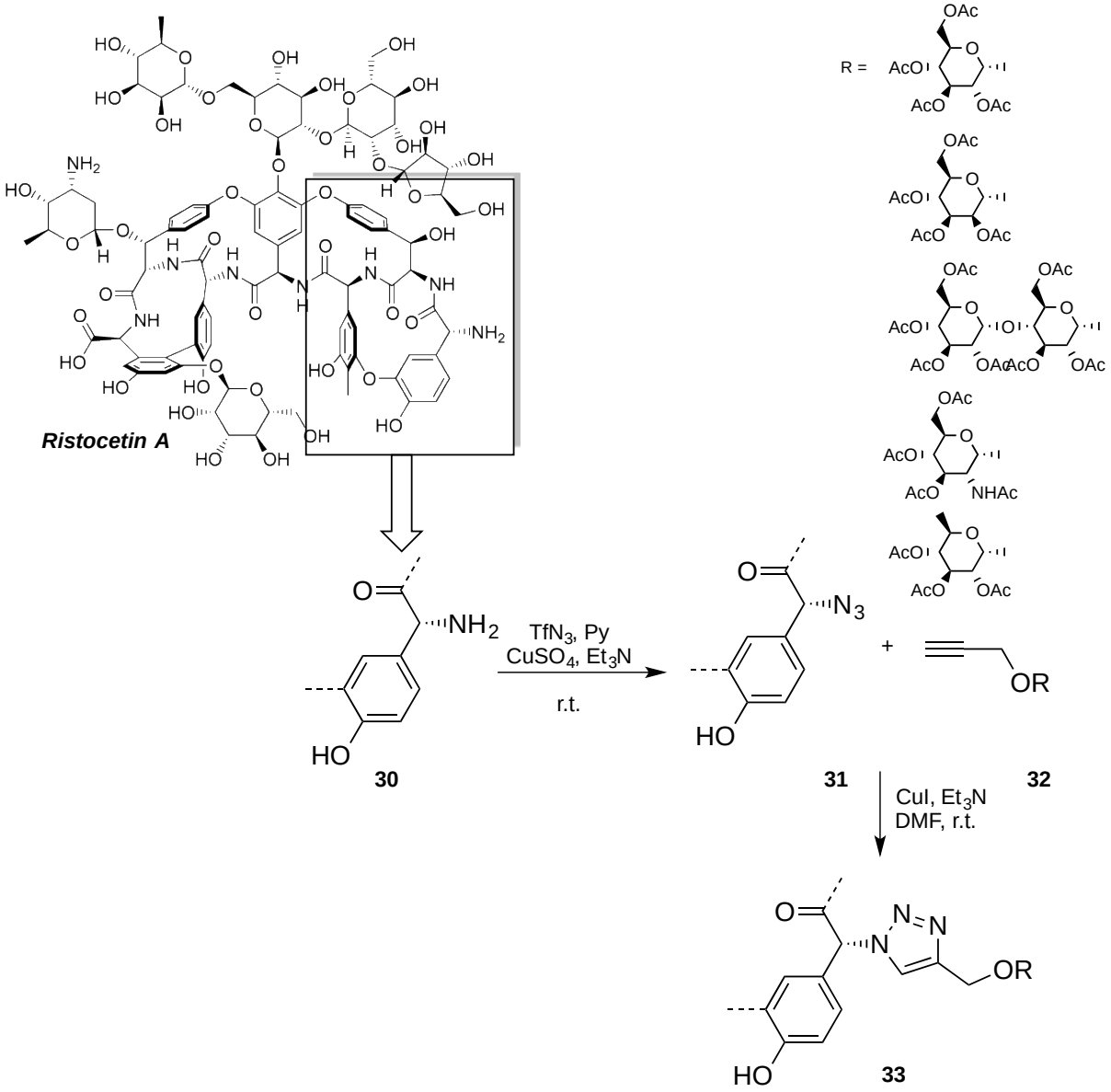


Scheme 11. Azidomethyl-uridine synthetic applications.

The antiviral activities of these compounds against hepatitis A virus (HAV, MBB cell culture-adapted strain) and Herpes simplex virus type-1 (HSV-1) were tested. For the antiviral activity against HAV-27, in both concentrations tested, 10 and 20 μg/10^5 cells, compounds of type **28** revealed the highest antiviral activity in

this series, and compounds of type **29** showed high activity at 10 μg/10^5 cells using amantadine as a control. For the antiviral activity against HSV-1, the results indicated that compounds of type **28** had the highest effect at 10 μg/10^5 cells, while compounds of type **29** showed moderate activity.[39] In close similarity, a series of novel 5-(1,2,3-triazolyl)-2′-deoxyuridines were synthesized using a simple and convenient one-step synthetic procedure via the Huisgen cycloaddition. The key step in these syntheses is a click reaction at the C-5 position under solvent free microwave irradiation and CuI as a catalyst. 5-Azidomethyl-2′-deoxyuridine was synthesized from thymidine and its CuAAC click reactions with several alkynes provided triazole derivatives of type **28** in good yields, dehydroxy at the position 2′. The synthesized compounds showed only marginal antiviral activities [against human rhinovirus (HRV), hepatitis C virus (HCV) and HIV].[40]

A series of flavonoid-triazolyl hybrids were synthesized through Cu(II)-promoted cycloaddition with azides and evaluated as novel inhibitors of HCV. The results of anti-HCV activity assays showed that most of the synthesized derivatives at a concentration of 100 μg/mL inhibited the generation of progeny virus.[41]

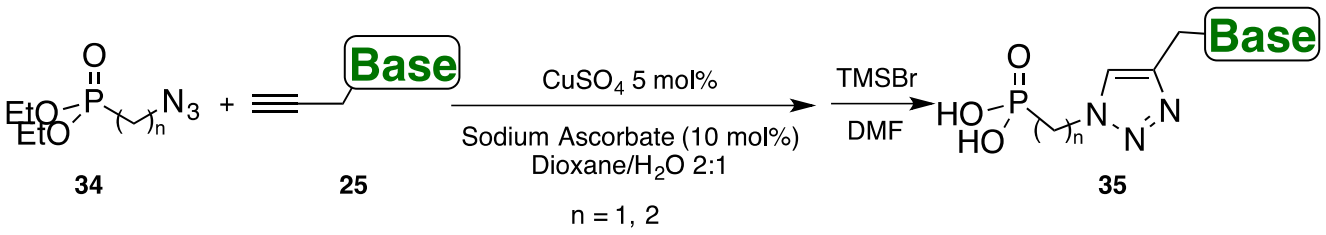


Scheme 12. Synthesis of triazole derivatives of ristocetin aglycon.

As in the examples reported in Scheme 11 where azido-methylene-uracil compounds were used for synthetic purposes, 6-azido-functionalized 1,3-dimethyluracil was also proposed as 1,3-dipole in the reaction with ethyl acetoacetate in the presence of sodium ethoxide to afford the 1,2,3-triazolyl derivate. The antiviral activity against HSV-1 revealed that some of the synthesized compounds showed the highest effect at concentration 10 μg/10^5 cells (unfortunately the authors used this notation to display the compound activity).[42]

Ristocetin A (Scheme 12) is a glycopeptide-type antibiotic produced that carries 6 sugar molecules (2 D-mannoses, D-glucose, D-arabinose, L-rhamnose and L-ristosamine) linked to the aglycon. It has a good antibacterial activity against Gram-positive strains. The aglycon **30** was modified into the corresponding azido derivative **31** and coupled with a variety of alkynes sugar-substituted **32** to give, in a typical "click chemistry" approach compounds **33**. The synthesized compounds **33** exhibited antiviral activity against influenza virus, with the highest activity being noted for the A/H1N1 subtype (antiviral EC_{50} values in the range of 6-16 μM). Some of these compounds produced minimal, if any, cytotoxicity at 70-100 μM.[43,44]

Triple bond functionalized heterobases of type **25** have been used with a different azides **34**, bearing a phosphonate group, in the synthesis of several triazoloacyclic nucleoside phosphonates **35** obtained in very good yields through 1,3-dipolar azide cycloaddition (Scheme 13).[45] The target compounds **35** were first evaluated for their antiviral effect on the replication of HCV in Huh 5.2 cells. Antiviral (IC_{50}), cytotoxicity (CC_{50}) and selectivity index (SI) properties of these compounds showed that some of these nucleoside mimics selectively operated as antivirals against HCV replication in the Huh 5-2 HCV replicon system. In particular the compound bearing the uracil moiety displayed anti-HCV activity with an IC_{50} value of 16 μM without any cytotoxicity at a concentration up to 100 μM. Interestingly, the length of the tether does not seem to have a detrimental effect on the activities and cytotoxicities of pyrimidines, whereas purines seem more affected.

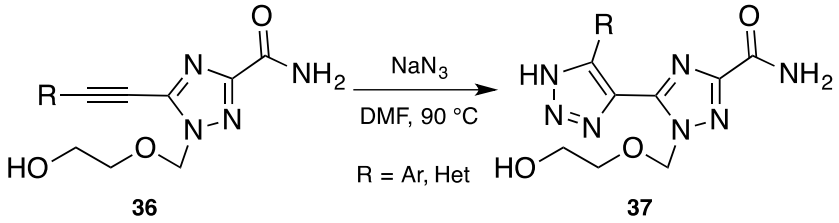


Scheme 13. Triazoloacyclic nucleoside phosphonates synthesized through the CuAAC reaction.

Similarly, the 1,3-dipolar cycloaddition of diethyl 2-azidoethyl-, 3-azidopropyl-, 2-azido-1-hydroxyethyl-, 3-azido-2-hydroxypropylphosphonates with selected *N*-propargyl nucleobases gave a series of the phosphonylated 1,2,3-triazole acyclo-nucleosides in which the phosphonate residue and nucleobases were linked by three- and four-carbon chains. The synthesized *O*,*O*-diethylphosphonates were transformed into the respective phosphonic acids. The compounds were evaluated *in vitro* for activity against a broad variety of DNA and RNA viruses. Unfortunately, no antiviral activity was observed at 100 μM.[46] A new series of acyclonucleotide analogues with a 1,2,3-triazole linker were prepared starting from diethyl azidomethyl-, 2-azidoethyl-, 3-azidopropyl-, 4-azidobutyl-, 2-azido-1-hydroxyethyl-, 3-azido-2-hydroxypropyl- and 3-azido-1-hydroxypropylphosphonates and selected alkynes under microwave irradiation. The *O,O*-diethylphosphonate acyclo-nucleotides were transformed into the respective phosphonic acids. The *in vitro* tests for the activity against a broad variety of DNA and RNA viruses showed that the acyclo-nucleotide bearing the N^3-benzoyl-quinazoline-2,4-dione moiety exhibited activity against both herpes simplex viruses (HSV-1, HSV-2) in HEL cell

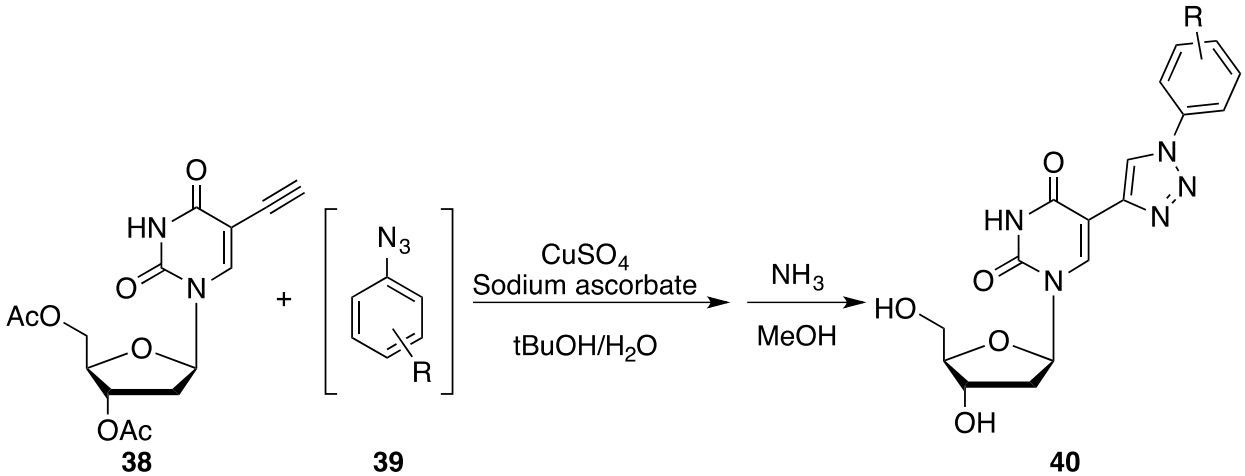
cultures (EC_{50} = 17 µM) and feline herpes virus (EC_{50} = 24 µM) in CRFK cell cultures.[47] The same authors proposed a general procedure for the preparation of 1,2,3-triazole analogues of nucleosides from diethyl 2-azidoethoxymethyl- and 2-azidoethoxyethylphosphonates. The application of microwave irradiation shortened the reaction time to 10 min in comparison to ca. 48 h when 1,3-dipolar cycloadditions were run under standard conditions. All compounds were evaluated *in vitro* for inhibitory activity against a broad variety of DNA and RNA viruses. None of the compounds were antivirally active at subtoxic concentrations.[48]

Alkynyl-derivatized heterobases were also coupled with either azidosugars or benzyl azide in the presence of Montmorillonite K10 impregnated with copper dichloride and potassium iodide ($CuCl_2$/KI/K10) as catalyst in the cycloaddition reactions, to provide the corresponding 1,4-disubstituted 1,2,3-triazoles that were obtained in good yields. All the compounds were evaluated for their antiviral activity *in vitro*. One of them showed moderate inhibition against influenza virus A (H3N2).[49]



Scheme 14. Synthesis of bis-triazolyl acyclo-nucleosides **37** via Huisgen reaction using NaN_3 and various 5-alkynyltriazole acyclonucleosides **36**.

A reversal approach where azido-pyrimidines and alkynyl-phopshonates reacted to successfully prepare triazolo analogues, was proposed but the cycloadducts did not give any inhibition against a variety of viruses.[50] A family of bis-triazolyl acyclo-nucleosides of type **37** were synthesized using a one-step synthetic procedure via the Huisgen reaction by addition of NaN_3 onto 1,2,4-triazole nucleosides of type **36** bearing internal alkynyl groups introduced at the 5-position of the triazole ring (Scheme 14). The yields were from fair to good and some of the compounds exhibited interesting antiviral activity against tobacco mosaic virus, demonstrating the importance of the bis-triazolyl motif to observe antiviral activity.



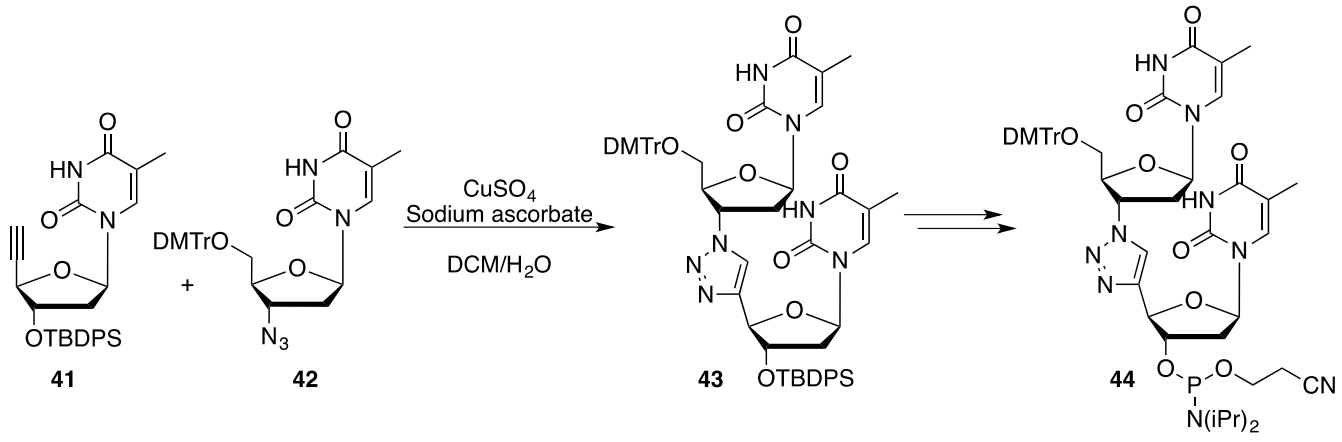
Scheme 15. Synthesis of C5-(1,4- and 1,5-disubstituted-1,2,3-triazolo)-nucleoside derivatives of type **40**.

The synthesized bis-triazolyl acyclo-nucleosides **37** were tested for their antiviral activity against TMV using the conventional half-leaf juice rubbing method with ribavirin as the positive control and water as the negative control. The levels of anti-TMV activity for some of the synthesized compounds were found to be either similar to or better than ribavirin, the standard reference for anti-TMV assay.[51]

The synthesis and antiviral evaluation of a series of C5-(1,4- and 1,5-disubstituted-1,2,3-triazolo)-nucleoside derivatives of type **40** was described by Agrofoglio and co-workers.[52] The key steps of these syntheses are the regioselective Huisgen's 1,3-dipolar cycloaddition reactions, using either copper-catalyzed azide-alkyne cycloaddition (CuAAC) or ruthenium-catalyzed azide-alkyne cycloaddition (RuAAC) under microwave activation starting from a triple bond functionalized and protected nucleoside structure **38** and a series of substituted aromatic azides **39** (Scheme 15).

Some compounds among the synthesized series possess activity against herpes simplex viruses 1 and 2, varicella-zoster virus, human cytomegalovirus and vaccinia virus. Compounds **40** were also evaluated on a wide panel of RNA viruses, including Vesicular stomatitis virus, influenza viruses type A (H1N1 and H3N2) and B in MDCK cell cultures, parainfluenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus in Vero cell cultures and Vesicular stomatitis, Coxsackie B4 and respiratory syncytial virus. However, no specific antiviral effect was demonstrated. Some of the synthesized compounds showed activities against several DNA viruses in the micromolar range and their cytostatic activities were determined against murine leukemia cells, human T-lymphocyte cells and cervix carcinoma cells.[52]

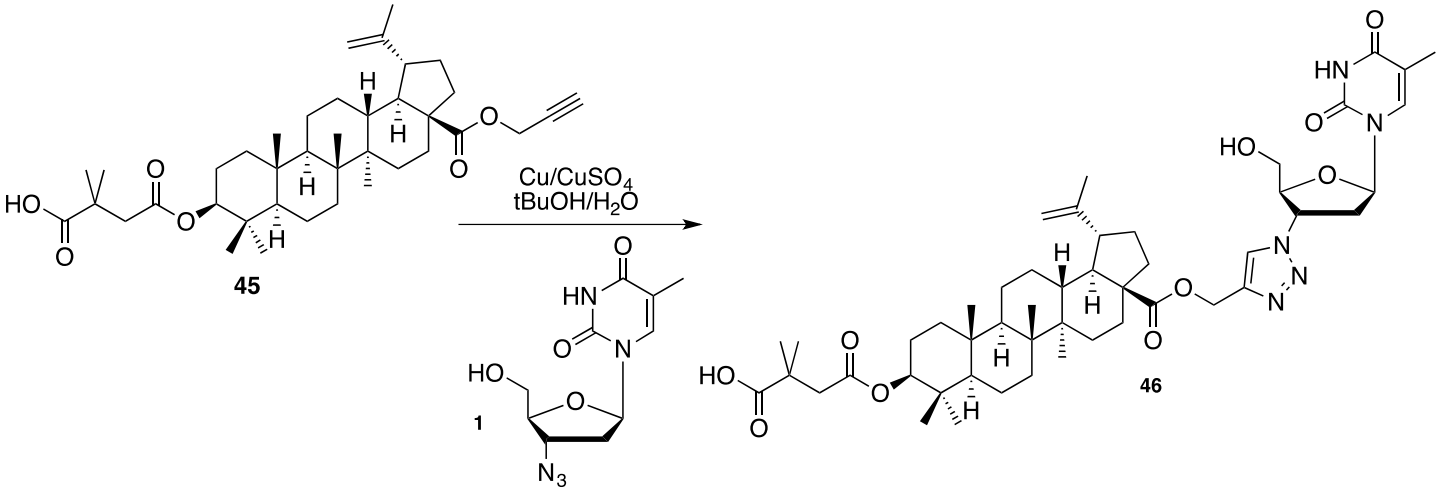
Contrary to the structure of compound **38**, the triple bond has been positioned on the sugar-like spacer to generate the dipolarophile **41** to be coupled with the structurally similar azide **42** to afford, after deprotection and introduction of the phosphorous group in **43**, the dinucleoside block **44** that was utilized directly for solid-phase synthesis of modified oligonucleotides (ON) using standard phosphoramidite protocols. The coupling time was increased to 15 min for the modified phosphoramidite (Scheme 16). No activity tests were however conducted on the synthesized ONs.[53]



Scheme 16. Synthesis of the dinucleoside block **43**.

A new strategy to link AZT **1** with betulinic acid **45** by click chemistry was designed and achieved through a classical azide cycloaddition (Scheme 17). This conjugation via the triazole ring offers a new direction for the modification of anti-HIV triterpenes. The cycloaddition chemistry provides an easy and productive way for linking two molecules. Among the newly synthesized derivatives, compound **46** showed potent anti-HIV activity with EC_{50} value of 0.10 μM, comparable to that of AZT (EC_{50}: 0.10 μM).[54]

AZT **1** was the first approved antiviral for the treatment of HIV. Reported efforts in clicking the 3'-azido group of AZT have not yielded 1,2,3-triazoles active against HIV or any other viruses. The first AZT-derived 1,2,3-triazoles with submicromolar potencies against HIV-1 was prepared by "clicking" **1** with 1-ethynylnaphtalene. The observed antiviral activities from the cytopathic effect-based assay were confirmed through a single replication cycle assay. SAR studies revealed two structural features key to antiviral activity: a bulky aromatic ring and the 1,5-substitution pattern on the triazole (the opposite of compound **46**). Biochemical analysis of the corresponding triphosphates showed lower ATP-mediated nucleotide excision efficiency compared to AZT, which along with molecular modeling suggests a mechanism of preferred translocation of triazoles into the P-site of HIV reverse transcriptase (RT). This mechanism is corroborated with the observed reduction of fold resistance of the triazole analogue to an AZT-resistant HIV variant (9-foldcompared to 56-fold with AZT).[55] This synthetic methodology has been extensively applied by various research groups for obtaining antiviral compounds.[56] Furthermore, Betulin derivatives containing a 1,2,3-triazole ring were found to possess a wide spectrum of biological activities, including antiviral, anticancer, and antibacterial activity. A series of triazoles were prepared by the 1,3-dipolar cycloaddition reactions between the alkyne derivatives of betulin and organic azides. The target triazoles were screened for their antiviral activity against DNA and RNA viruses. The cytotoxic activity of the obtained compounds was determined using five human cancer cell lines (T47D, MCF-7, SNB-19, Colo-829, and C-32) by a WST-1 assay. Compound **46** in particular gave promising results.[57]



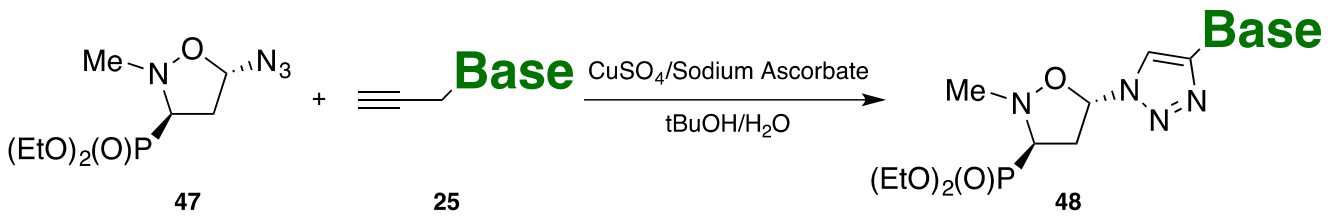
Scheme 17. Synthesis of AZT conjugates.

A newly designed protocol inserts the azido function on a 9:1 mixture of trans- and cis-5-acetoxy-2-methylisoxazolidin-3-yl-3-phosphonates at the anomeric carbon atom, leading to the formation of the equimolar mixture of cis- and trans-5-azido-2-methylisoxazolidin-3-yl-3-phosphonates **47**. The 1,3-dipolar cycloaddition of pure trans- and cis-5-azidoisoxazolidin-3-yl-3-phosphonates with selected alkynes-derivatized heterobases **25** gave the respective nucleoside mimetics containing a 1,2,3-triazole linker (Scheme 18).

The (1,2,3-triazolyl) isoxazolidine phosphonates of type **48** obtained herein were evaluated *in vitro* for activity against a variety of DNA and RNA viruses. However, none of the compounds were endowed with antiviral activity at subtoxic concentrations.[58] A novel series of 2'-oxa-3'-aza-4'a-carbanucleosides, equipped with a triazole linker at the 5'-position, has been developed by exploiting a click chemistry reaction of 5'-azido-2'-oxa-3'-aza-4'a-carbanucleosides with substituted alkynes. Biological tests indicate an antitumor activity for

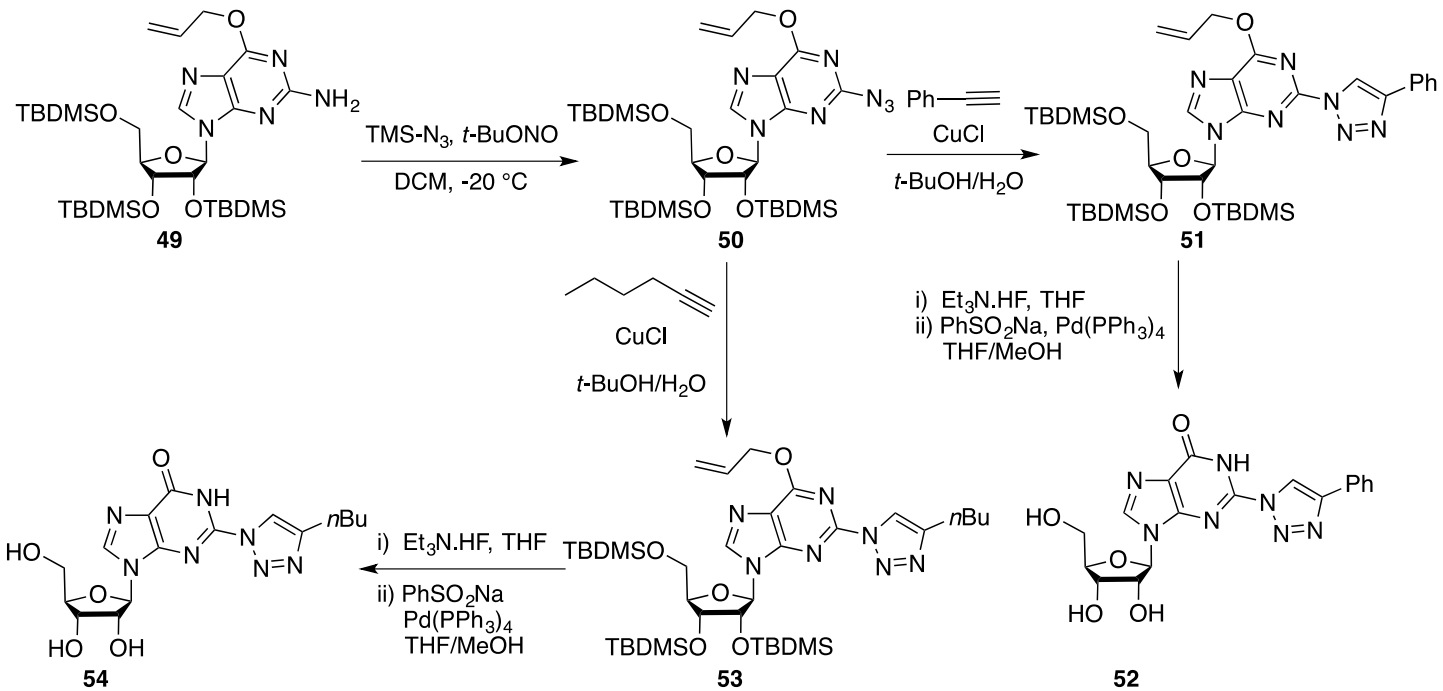
the synthesized compounds: most of them inhibit cell proliferation of Vero, BS-C-1, HEp-2, MDCK, and HFF cells with a CC_{50} in the range of 5.0-40 μM. The synthesized compounds do not show any antiviral activity.[59]

O^6-(Benzotriazol-1*H*-yl)guanosine and its 2'-deoxy analogue can be readily converted to the O^6-allyl derivatives of type **49** that upon diazotization with *t*-Bu-ONO and TMS-N_3 afford the C-2 azido derivatives of type **50** in 60 % yield (Scheme 19).



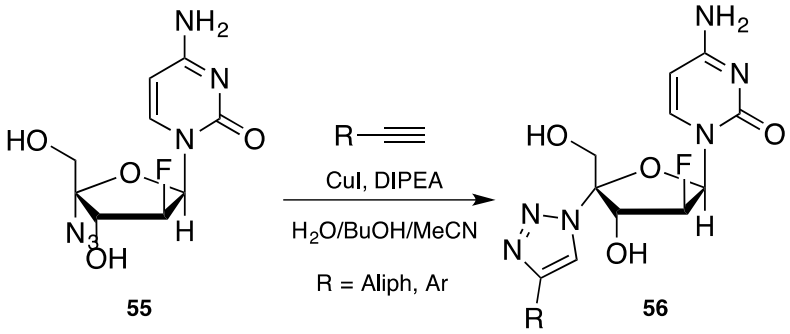
Scheme 18. 1,3-Dipolar cycloadditions of the 5-azidoisoxazolidines **47** with propargylated nucleobases **25**.

American and Belgian researchers preliminary studied the solvent dependent azide-tetrazole equilibrium of C-6 azidopurine nucleosides.[60] A screening of the conditions for the ligation of the azido nucleosides with alkynes showed that CuCl in *t*-BuOH/H_2O was found optimal, yielding C-2 1,2,3-triazolyl nucleosides **51,53** in 70-82% yields. Removal of the silyl groups with $Et_3N{\cdot}3HF$ followed by deallylation with $PhSO_2Na/Pd(PPh_3)_4$ gave the C-2 triazolylinosine nucleosides **52,54**. The two C-2 triazolyl adenosine analogues demonstrated pronounced antiproliferative activity in human ovarian and colorectal carcinoma cell cultures. When evaluated for antiviral activity against a broad spectrum of DNA and RNA viruses, some of the C-2 triazolylinosine derivatives showed modest inhibitory activity against cytomegalovirus with the exception of compound **54**, the anti-CMV activity of which was more pronounced (EC_{50} 73 μM).[61]



Scheme 19. Synthesis of nucleoside **54**.

A series of 4'-[1,2,3]triazole-2'-deoxy-2'-fluoro-β-D-arabinofuranosylcytosines of type **56** were prepared by CuAAC reactions of 1-(4'-azido-2'-deoxy-2'-fluoro-β-D-arabinofuranosyl) cytosine **55** with appropriate alkynes in good yields (Scheme 20). Most of these nucleoside analogs exhibited potent anti-HIV-1 activity with no cytotoxicity observed at the highest tested concentration up to 25 μM. Some of them exhibited extremely potent antiviral activity, with a great potential for further developments as novel nucleoside RT inhibitors (NRTIs) for the HIV-1 infection.

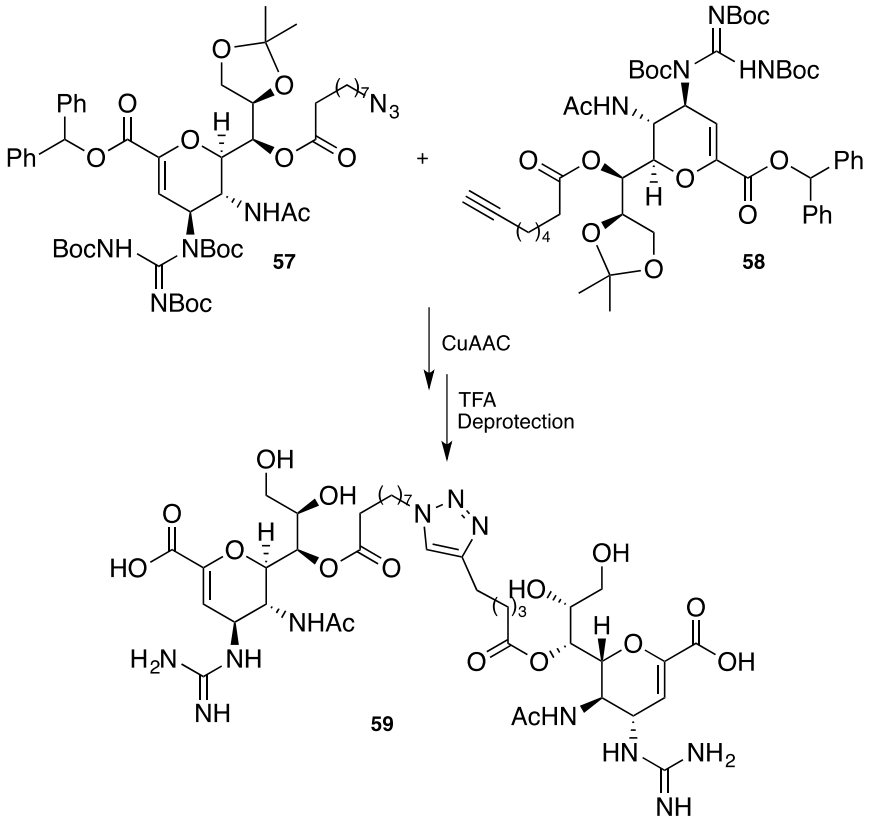


Scheme 20. Synthesis of 4'-[1,2,3]triazole-2'-deoxy-2'-fluoro-β-D-arabinofuranosylcytosines of type **56**.

In particular, the antiviral activity against HBV infection was tested in HepG2.2.15 cell line. The inhibitory activities of tested compounds against the production of HBsAg and HBeAg were examined by ELISA assay and their cytotoxicity was measured by MTT assay. Some of the compounds showed low toxicity in the HepG2.2.15 cell line and exhibited nanomolar inhibitory activity against HBsAg production (EC_{50} = 0.01 μM, SI = 49,800).[62] An intramolecular version of the above reported synthetic approach was proposed by Chinese research groups where the dipolarophile alkyne moiety was linked to the distal secondary OH group close to the azido moiety. Triazolo-fused 3′,4′-cyclic nucleoside 4′-spiro nucleoside analogues were synthesized via an intramolecular 1,3-dipolar cycloaddition in a regio- and stereo-specific manner. The thymine nucleoside base in these target compounds was transformed into the corresponding 5-methyl cytosine component. The synthesized compounds were examined in a multinuclear-activation galactosidase indicator (MAGI) assay for exploring the anti-HIV activity and in a human T lymphocytes H9 (H9 T) assay for measuring the cell toxicity, but with modest results.[63] The same authors proposed the synthesis of medium-sized cyclic nucleosides containing a fused triazole ring via intramolecular 1,3-dipolar cycloadditon reaction. 2',3'-seco-Uridine was employed as the key intermediate for the introduction of azido and alkynyl moieties in the defined positions of the reaction precursors. The cycloaddition reactions were achieved in high yields by heating the precursor in refluxing toluene. The uracil base in these target compounds was successfully transformed into the corresponding cytosine. The synthesized compounds were evaluated in a MAGI assay for their anti-HIV activities, and in a H9 T lymphocytes assay for their cell toxicities: even if the anti-HIV activities of these compounds were inferior to that of AZT, the unique structural features of these new molecules remain interesting to be tested for antiviral activities to other viruses.[64]

The CuAAC reaction was used to synthesize the dimers of the neuraminidase inhibitor zanamivir in high yields. The effect upon antiviral activity of varying the linker length and the number of triazole units was explored. All dimers were tested for anti-viral activity against influenza A/Sydney/5/97 and B/Harbin/7/94 in a cythopatic effect (CPE) assay. Dimer **59** is 100 times more potent than oseltamivir carboxylate and 1000-3000 fold more potent than zanamivir. Dimer **59** was prepared from click reaction form the azido derivative **57** and the dipolarophile **58** (Scheme 21).[65] Structurally similar, a series of 1,2,3-triazole oseltamivir derivatives, which

could simultaneously occupy the classical NA catalytic site and the newly reported 430-cavity, were designed, synthesized, and evaluated for their anti-influenza activities. The results demonstrated that four compounds showed robust anti-influenza potencies against H5N1, H5N2 and H5N6 strains in both enzymatic and cellular assay. One of them was proved to possess the most potent and broad-spectrum anti-influenza activity, with IC_{50} values of 0.12 μM, 0.049 μM and 0.16 μM and EC_{50} values of 2.45 μM, 0.43 μM and 2.8 μM against H5N1, H5N2 and H5N6 strains, respectively, which were slightly weaker than oseltamivir carboxylate. In the embryonated egg model, the same compound achieved the similar protective effect against H9N2 strain with oseltamivir carboxylate.[66]



Scheme 21. Synthesis of dimer **59**.

The exploitation of the 150-cavity in the active site of influenza A viral neuraminidases for the design of novel C-6 triazole-containing Tamiflu derivatives was reported by a Canadian research group. A synthetic route was developed by utilizing a highly substituted cyclic Baylis-Hillman acetate as an active precursor for azide substitution via suprafacial allylic azide [3,3]-sigmatropic rearrangement. From D-(−)-quinic acid, triazole derivatives were prepared (Figure 4).

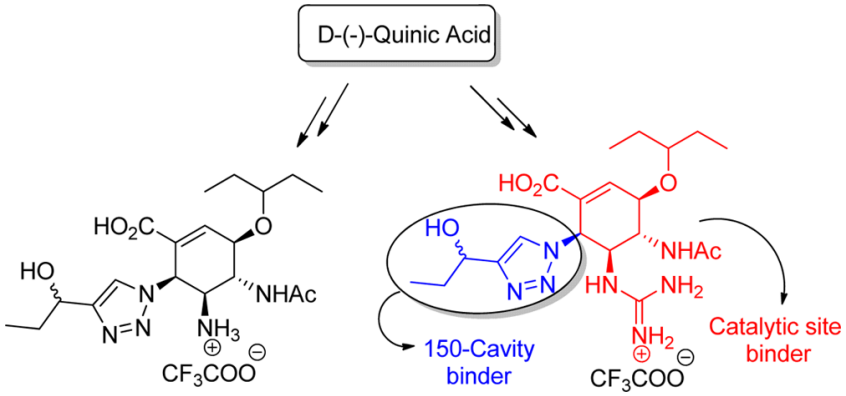
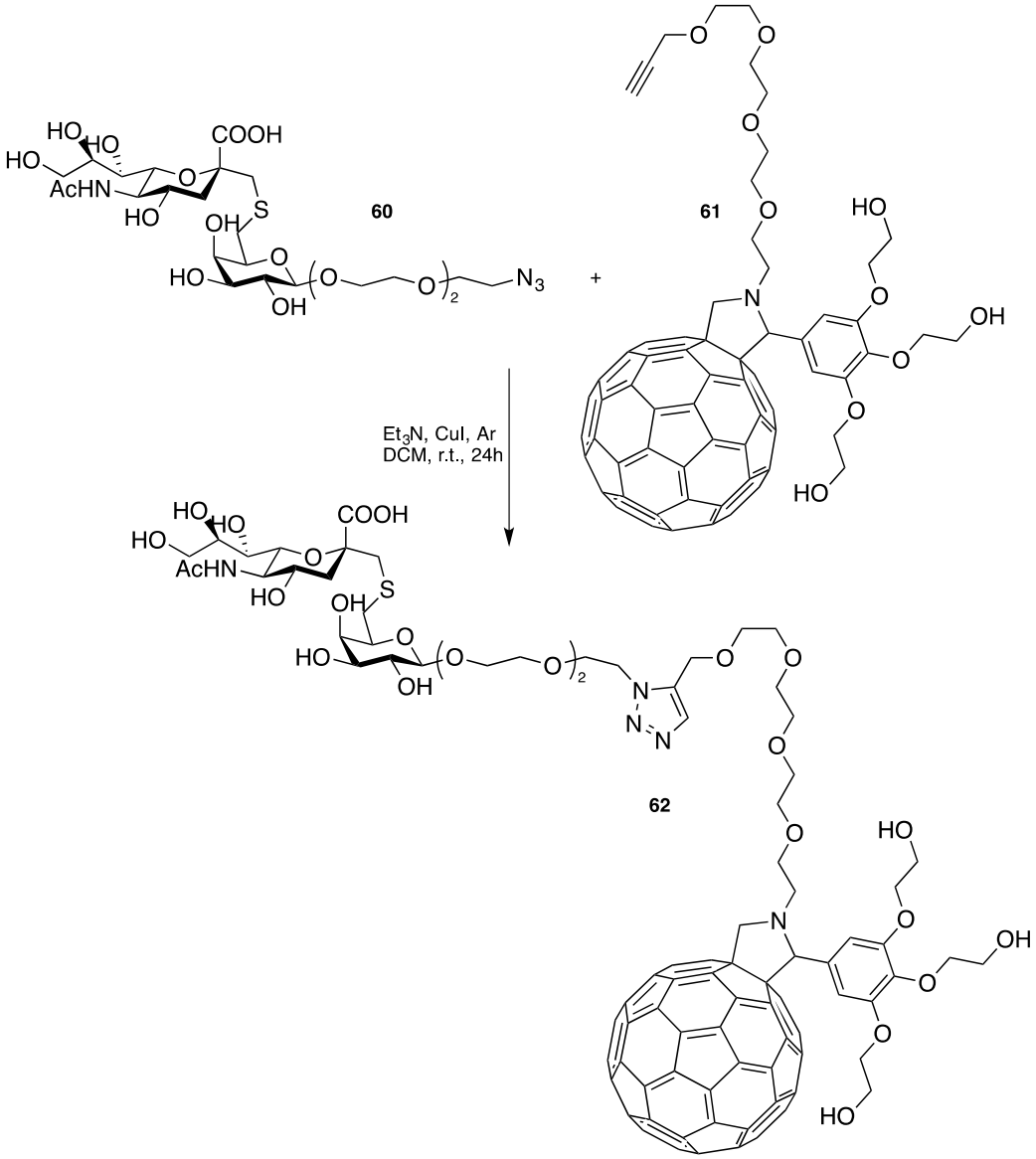


Figure 4. D-(−)-quinic acid strategy to triazole derivatives.

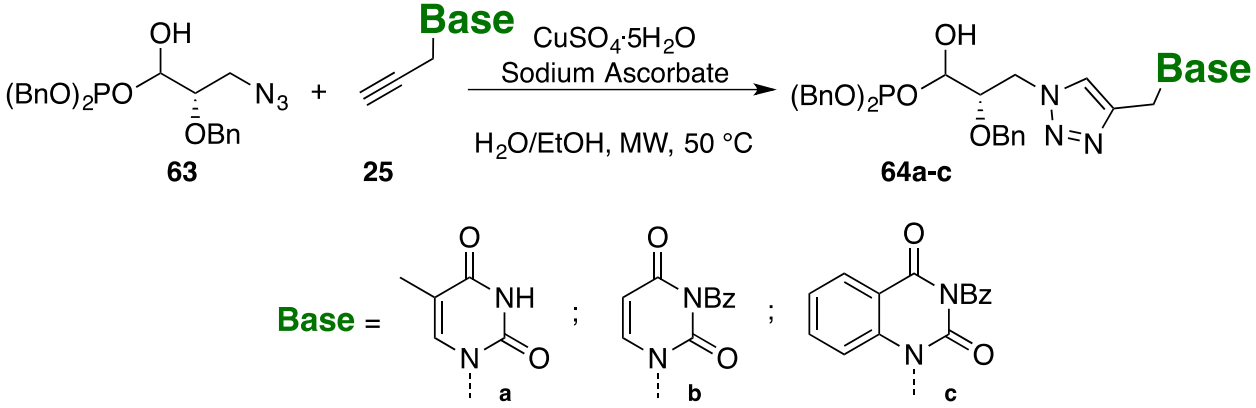
Virus replication inhibitory assays *in vitro* of these triazole derivatives containing either an amino or guanidino function indicated that the guanidinium compound showed the higher efficacy against a strain with N2 subtype at a concentration of 2 x 10^{-5} M but did not inhibit replication of a strain with N1 subtype even at a concentration of 10^{-4} M. To probe the nature of the enzyme-inhibitor interactions, molecular dynamics simulations were performed on complexes of these compounds with different neuraminidase enzymes. The results indicated that the candidate inhibitors occupy both the 150-cavity and catalytic site but with alternating occupancy.[67] More recently, a strategy to prepare chemically programmed antibodies that have the long half-life and effector function of an antibody and the therapeutic activity of a conjugated small-molecule drug, peptide, or oligonucleotide has been proposed. The agent to be conjugated to the antibody is first functionalized with a β-lactam and is then selectively treated with low pK_a lysine residues, which are key to the catalytic activity of aldolase monoclonal antibody (mAb) 38C2, to form an amide bond. A chemically programmed antibody was linked to a small-molecule enzyme inhibitor that targets neuraminidase, in order to create a novel potent neuraminidase inhibitor that maintains long-term systemic exposure, having the potential for enhanced activity through antibody-associated effector function and valency.[68]

In order to obtain self-assembly, multivalent ligand for influenza virus hemagglutinin α-*N*-acetylneuraminyl-α(2,6)-D-galactopyranose **60** was synthesized and bonded to a water-soluble fullerene derivative **61** using 1,3-dipolar cycloaddition reactions (Scheme 22).

The aggregating amphiphilic compound did not inhibit the influenza virus hemagglutinin, but it proved to be an inhibitor of its neuraminidase with a 50% inhibitory concentration of 81 μM. The numerous tetraethylene glycol chains applied in the molecule improved its water solubility, probably shielded the active disaccharide part, thus preventing its multimeric interaction with viral hemagglutinin. On the other hand, this shielding did not seem to disturb the interaction of **62** with the viral neuraminidase.[69] Among the pathogen-associated carbohydrate patterns, the Man α (1→2) Man α disaccharide motif is of particular interest because its multivalent derivatives are considered as potential antiviral or antibacterial agents through interaction with mannose-binding lectins. The authors of this work present a straightforward synthesis of amphiphilic compounds containing a hydrolytically stable *S*-linked 1,2-mannobioside residue. Various lipophilic carriers, such as a hexadecyl chain and two pyrrolidinofullerene derivatives, have been proposed by the same research group. According to a dynamic light scattering study, the obtained amphiphiles form nanoscale aggregates in water producing multivalent presentation of the thiomannobioside residue.[70]



Scheme 22. Synthesis of compound **62**.

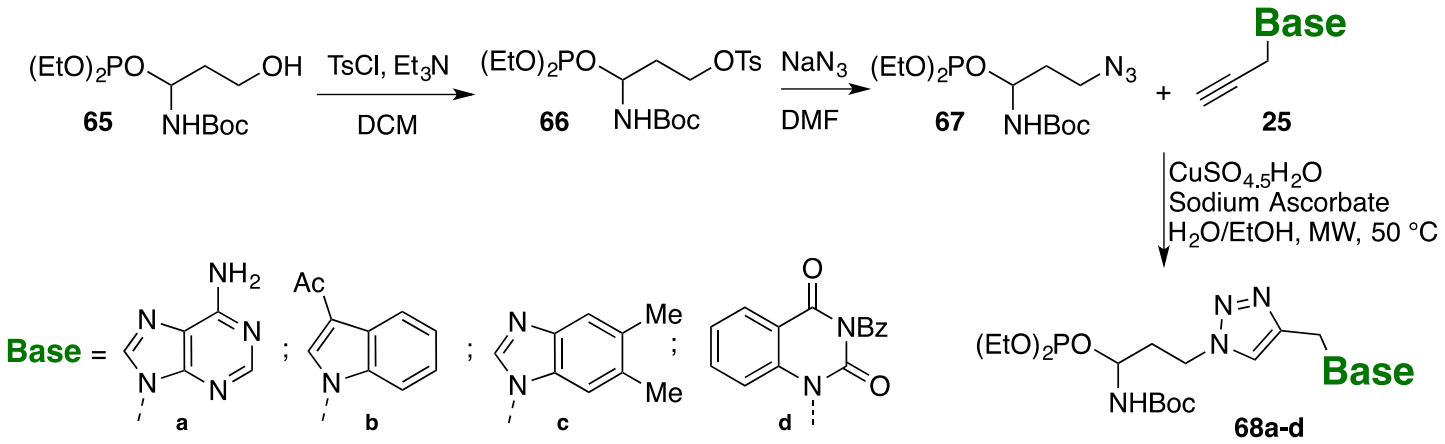


Scheme 23. Synthesis of analogues **64**.

The efficient synthesis of a series of polyhydroxylated dibenzyl ω-(1*H*-1,2,3-triazol-1-yl)alkylphosphonates as acyclic nucleotide analogues of type **64** was described by Belgian and Polish researchers starting from dibenzyl ω-azido-(polyhydroxy)alkyl-phosphonates **63** and selected alkynes **25** under microwave irradiation (Scheme 23).

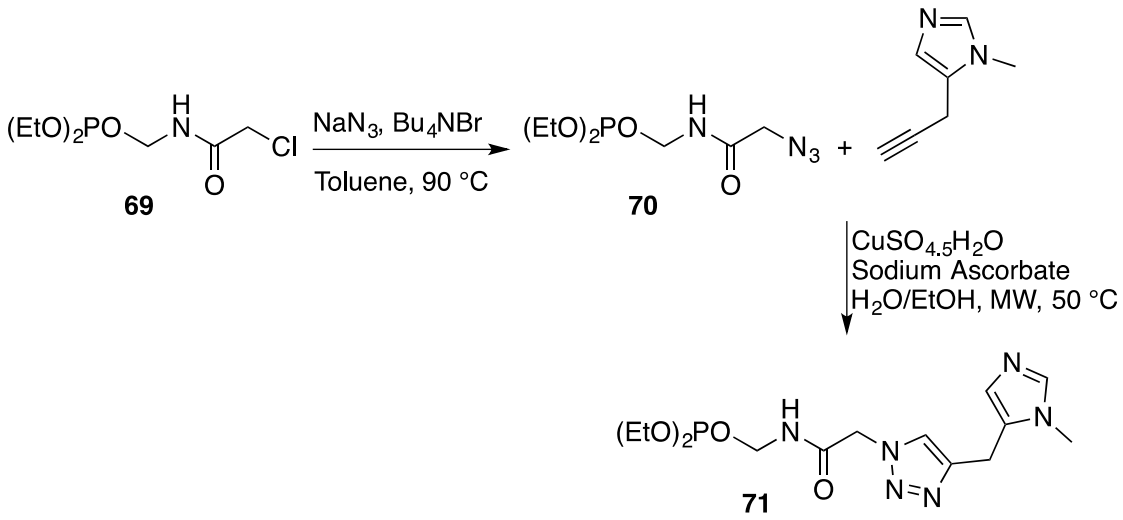
Selected dibenzylphosphonate acyclo-nucleotides were transformed into the corresponding phosphonic acids and were evaluated *in vitro* for activity against a broad variety of DNA and RNA viruses and for cytostatic activity against murine leukemia L1210, human T-lymphocyte CEM and human cervix carcinoma HeLa cells.

Compound **64a** (thymine) exhibited antiviral activity against the influenza A H3N2 subtype (EC_{50} = 20 μM-visual CPE score; EC_{50} = 18 μM-MTS method; MCC >100 μM, CC_{50} >100 μM) in Madin Darby canine kidney cell cultures (MDCK). Compound **64b** (uracil-Bz) was active against vesicular stomatitis virus and respiratory syncytial virus in HeLa cells (EC_{50} = 9 and 12 μM, respectively). Moreover, compound **64d** (benzouracil-Bz) showed activity against both HSV-1 and HSV-2 in HEL cell cultures (EC_{50} = 2.9 and 4 μM, respectively) and feline herpes virus in CRFK cells (EC_{50} = 4 μM), but at the same time it exhibited cytotoxicity toward uninfected cell. Several other compounds have been found to inhibit proliferation of L1210, CEM as well as HeLa cells with IC_{50} in the 4-50 μM range.[71]



Scheme 24. Synthesis of derivatives **68**.

A series of 1-amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates (*R*)- and (*S*)-**68a-d** were obtained from enantiomerically pure (*R*)- and (*S*)-1-*tert*-butoxycarbonyl (Boc)-amino-3-azidopropyl-phosphonates **67** and *N*-propargylated nucleobases in good yields (Scheme 24). The synthesis stands on the tosylation of the alcohol **65** and azido functionalization of **66**. The cycloaddition to the *N*-propargylated nucleobases afforded the protected compounds **68a-d**. All 1,2,3-triazolylphosphonates (*R*)- and (*S*)-**68** were evaluated for their activities against a broad range of DNA and RNA viruses. Compound (*R*)-**68b** (B = 3-acetylindole) was moderately active against vesicular stomatitis virus in HeLa cell cultures (EC_{50} = 45 μM). Compounds (*S*)-**68a** (B = adenine), (*R*)-**68d** (B = *N*3-Bz-benzuracil), (*R*)-**68b** (B = 3-acetylindole), and (*R*)-**68c** (B = 5,6-dimethylbenzimidazole) were cytotoxic toward Crandell-Rees feline kidney (CRFK) cells (CC_{50} = 2.9, 45, 72, and 96 μM, respectively).[72] The phosphonylated 1,2,3-triazolo[4,5-*d*]pyrimidines (8-azahypoxantines) were synthesized employing the 1,3-dipolar cycloaddition of corresponding ω-azidoalkylphosphonates and 2-cyanoacetamide followed by the pyrimidine ring closure with triethyl orthoformate. All the compounds were evaluated *in vitro* for their inhibitory activities against a variety of DNA and RNA viruses but none was found active below 100 μM.[73]

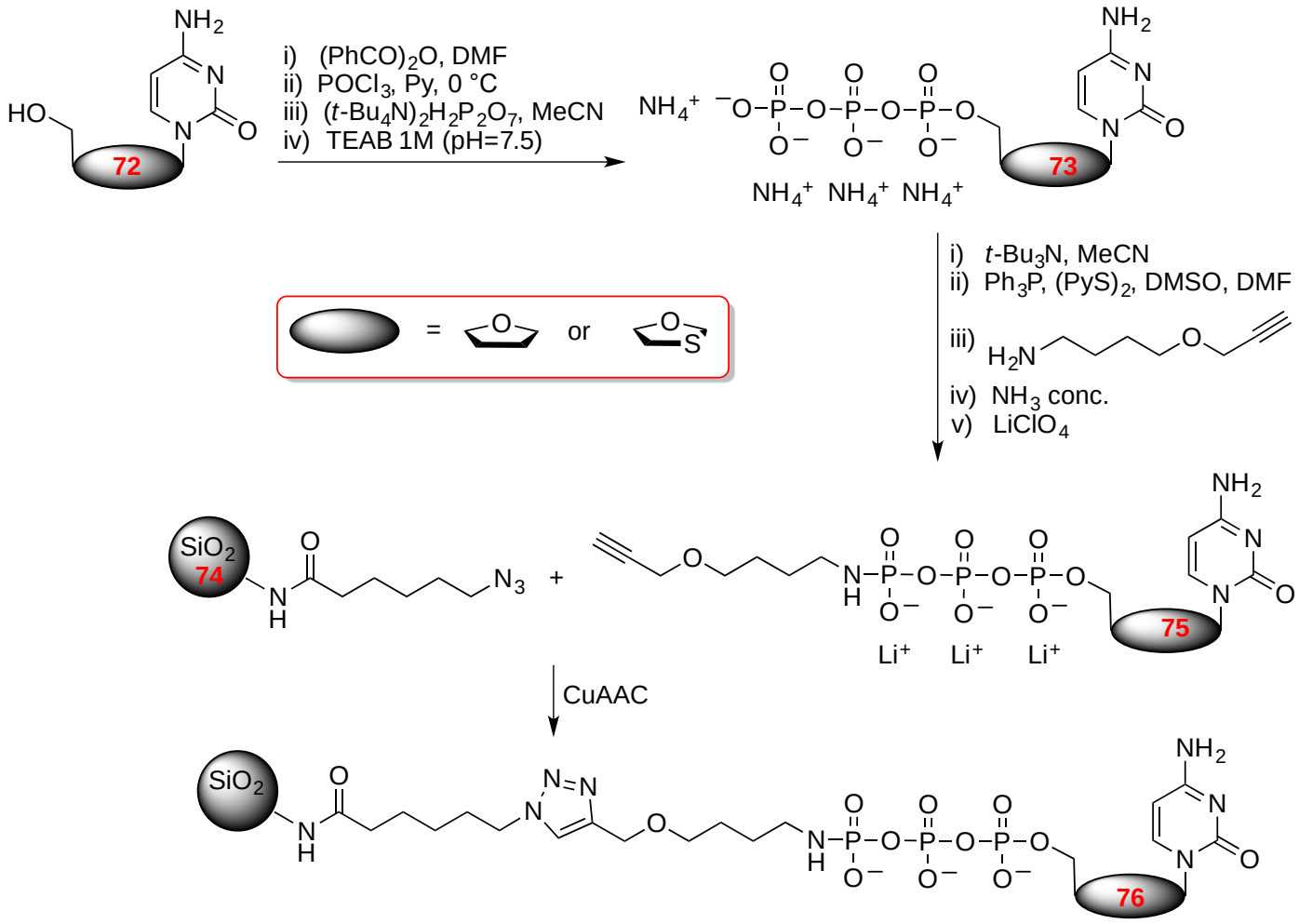


Scheme 25. Synthesis of [(1,2,3-triazol-1-yl)acetamido]-methylphosphonate **71**.

The same authors (of the previous work) prepared a series of 4-substituted [(1,2,3-triazol-1-yl)acetamido] methyl phosphonates of type **71** as acyclic nucleotide analogs, synthesized from diethyl (2-chloroacetamido) methylphosphonate **69** via azidation followed by 1,3-dipolar cycloaddition with selected alkynes derived from either natural nucleobases or their mimetics (Scheme 25). All compounds were tested for their antiviral activities against DNA and RNA viruses as well as for cytostatic activity or cytotoxicity. Among the tested compounds, [(1,2,3-triazol-1-yl)acetamido]-methylphosphonate **71** bearing a methyl-imidazole substituent showed activity against the vesicular stomatitis virus (EC_{50} = 45 μM) in HeLa cell cultures.[74]

Nucleoside analogues are commonly used in HIV/AIDS therapy and, after entering through the membrane, are phosphorylated by intracellular nucleoside kinases into active 5'-mono-, di-, and triphosphates. The drug resistance to nucleoside analogues refers to nucleoside transporter deficiency, reduced nucleoside kinase activity, over-expression of multi-drug resistance proteins or modifications in apoptotic pathways.[75] A conceivable strategy to avoid resistance is to use a pre-phosphorylated nucleoside directly as a drug that can thus bypass intracellular phosphorylation. However, nucleoside triphosphates are very poorly internalized by cells. Attempts to get around this problem by masking the phosphate groups by turning them into phosphate esters have not been successful until recently.[76] The fully substituted di- and triphosphate esters become unstable. Difficulties in the development of lipophilic nucleoside triphosphate (NTP) pro-drugs arise due to hydrolytic instability of the pyrophosphate bond, whilst in the natural NTPs this bond is kinetically stable due to negative charges that slow down cleavage by nucleophiles.

An alternative to the lipophilic pro-drug approach could be in using drug delivery systems such as liposomes, nanogels, nanoparticles etc., which could potentially solve the problem of poor cell absorption of NTPs. SiO_2 nanoparticles represent a simple and versatile method for the preparation of SiO_2-dNTP conjugates. There are several examples when nucleoside analogues were originally approved as antiviral agents and then it has been shown that they display promising anticancer properties. Conjugates of antiviral phosphorylated nucleoside analogues may also possess potential antitumor activity. Russian researchers aimed to prepare triphosphates of anti-HIV nucleoside drugs lamivudine and zalcitabine with γ-alkynyl phosphoramidate group, obtain their conjugates with SiO_2 nanoparicles (SiO_2-dNTP) via Cu(I)-catalyzed azide-alkyne cycloaddition and assess their antiviral and cytotoxic properties. Scheme 26 shows the synthetic route.



Scheme 26. SiO_2 nanoparticles synthesis.

The pivotal step is represented by the insertion of a triple bond on the phosphorylated derivative **73** to give **75** that undergoes 1,3-dipolar cycloaddition with the azide **74** to afford the final compound **76**. The conjugates of phosphorylated lamivudine and dideoxycytidine (zalcitabine) showed higher potency than the parent nucleosides. The conjugate of phosphorylated azidothymidine was less active against HIV-1 than the parent nucleoside probably because of the replacement of its 3'-azido group by 1,2,3-triazole ring. These results show an opportunity for using SiO_2 nanoparticles as a transport for delivering phosphorylated nucleosides to cells in order to increase their efficiency as antiviral and anticancer drugs.[77] Non-obligate chain terminating nucleosides with a linear substituent (azido or ethynyl group) at the 4' position represent an important class of compounds in antiviral discovery, particularly against hepatitis C virus (HCV) and human immunodeficiency virus (HIV). 3'-Azidothymidine (AZT)-derived 1,2,3-triazoles can be potent inhibitors of HIV-1. To gauge the medicinal chemistry impact of functionalizing the 4'-linear substituent and possibly generate novel antiviral nucleoside scaffolds, azide-alkyne cycloaddition reactions with 4'-AZT were investigated. Figure 5 shows the structures of families of thymidine and triazole derivatives.

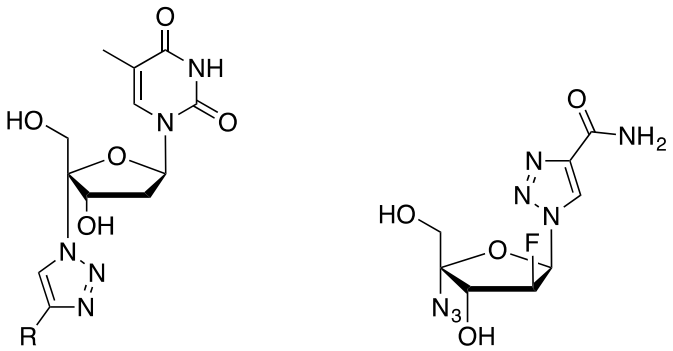
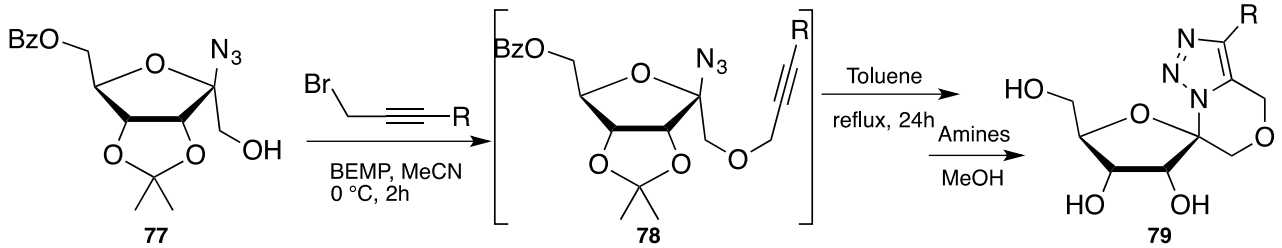


Figure 5. Structures of Triazole-thymidine and Azido-triazole derivatives.

Antiviral screening identified a few triazole analogues with moderate activity against HIV-1 (18-62% inhibition at 10 μM) and/or influenza A virus (15-50% inhibition at 10 μM). None of them was found active against West Nile virus (WNV) or HCV. These results suggested that the linear 4' azido group of azido-triazole is essential for target binding and that its chemical manipulation could largely compromise antiviral potency.[78] Azido-triazole derivatives were found potent anti-HBV agents.[79] Novel 4-monosubstituted 2'-deoxy-2'-β-fluoro-4'-azido-β-D-arabino furanosyl-1,2,3-triazole nucleoside analogues were prepared and screened for in vitro anti-HBV activity. At 5.0 μM in the cellular model, all the synthesized compounds displayed activities comparable to that of the positive control, lamivudine (20 μM). Among the compounds tested, the amide-substituted analogue showed the most promising anti-HBV activity and low cytotoxicity in the cell model. It retained excellent activity against lamivudine-resistant HBV mutants. In duck HBV (DHBV)-infected duck models, both the serum and liver DHBV DNA levels (67% and 53%, respectively) were remarkably reduced.
An efficient synthesis of spirocyclic triazoloxazine nucleosides was achieved by the conversion of β-D-psicofuranose to the corresponding azido-derivative **77**, followed by alkylation of the primary alcohol with a range of propargyl bromides, obtained by Sonogashira chemistry. The products of these reactions **78** underwent 1,3-dipolar addition to generate the protected spirocyclic adducts (Scheme 27). These were easily deprotected to give the corresponding ribose nucleosides **79**. The library of compounds obtained with different amines was investigated for its antiviral activity using mouse hepatitis virus (MHV) as a model where the 4-chloroaniline derivative showed the most promising activity and tolerability.[80]



Scheme 27. Synthesis of spiro-compound **79**.

The same research group prepared a number of spirocyclic ribonucleosides containing either a triazolic or azetidinic system, along with two analogous phosphonate derivatives of the former. These systems were prepared from the same β-D-psicofuranose as the starting material. The triazole spirocyclic nucleosides were obtained using the 1-azido-1-hydroxymethyl derived sugars, where the primary alcohol was alkylated with a

range of propargyl bromides, whereas the azetidine systems originated from the corresponding 1-cyano-1-hydroxymethyl sugars. Due to their close similarity with ribavirin, the library of compounds was investigated for the antiviral properties using murine hepatitis virus (MHV) as a model and with promising results.[81]

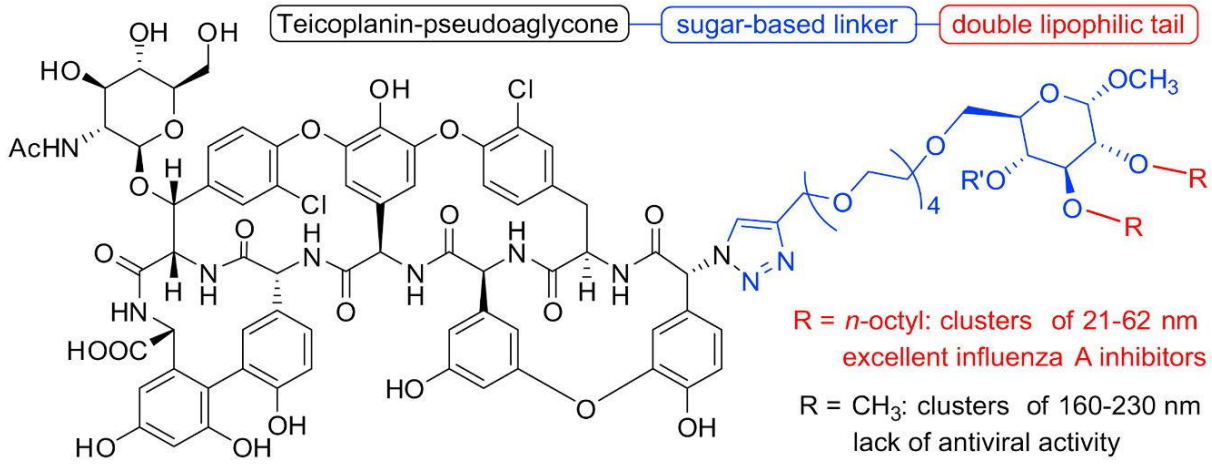
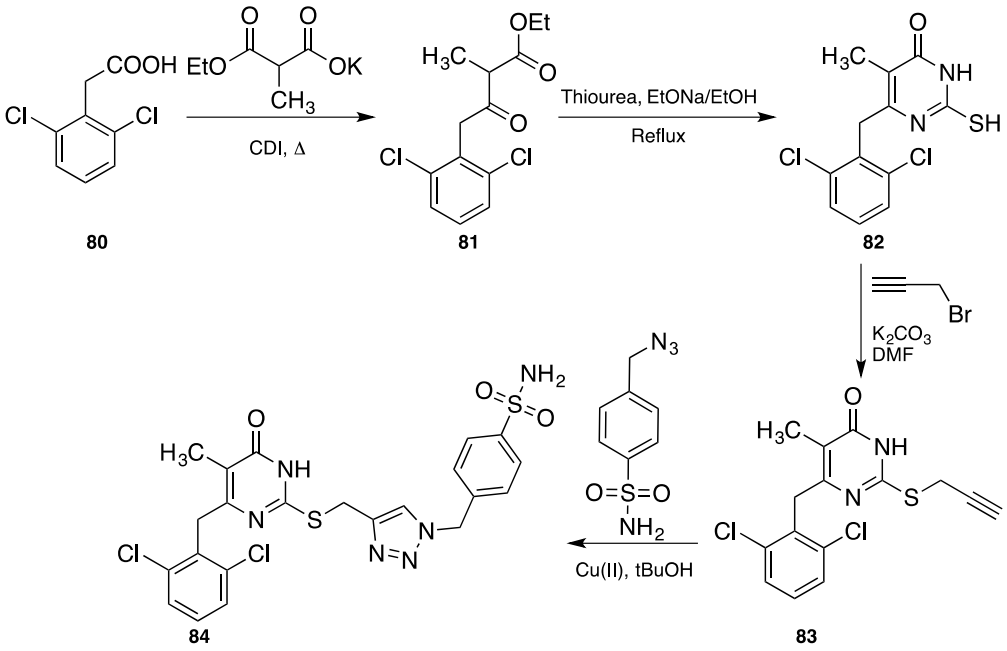


Figure 6. Teicoplanin pseudoaglycone derivatives (Reproduced with permission.[82] Copyright 2014, Elsevier).

To obtain cluster-forming antibiotic compounds, teicoplanin pseudoaglycone derivatives containing two lipophilic *n*-octyl chains were synthesized through azide methodology (Figure 6). The compounds proved to be poor antibacterials, but, surprisingly, they exhibited potent anti-influenza virus activity against influenza A strains. Their antiviral EC_{50} values range from 1-2 μM to 4- to 20-fold lower than the compound concentrations causing cytotoxic effects. This antiviral action was related to inhibition of the binding interaction between the virus and the host cell. Related analogs bearing methyl substituents in lieu of the octyl chains, displayed no anti-influenza virus activity. Hence, an interaction between the active, dually *n*-octylated compounds and the lipid bilayer of the host cell can be postulated, to explain the observed inhibition of influenza virus attachment.[82]

A series of *S*-DABO (Dihydro-Alkoxy-Benzyl-Oxopyrimidine) derivatives with the substituted 1,2,3-triazole moiety on the C-2 side chain were synthesized using the simple and efficient CuAAC reaction, and biologically evaluated as inhibitors of HIV-1. The most active HIV-1 inhibitor was compound **84** (Scheme 28), which exhibited similar HIV-1 inhibitory potency (EC_{50} =3.22 μM) compared with 3TC (EC_{50} = 2.24 μM). However, none of the synthesized compounds demonstrated any kind of inhibition against HIV-2 replication. It is worth noting that, in many types of non-nucleoside RT inhibitors (NNRTIs), the optimum activity was often found with substituents containing the hydrophilic SO_2NH_2 group, indicating its nature can better accommodate the chemical environment in this region of RT and provide potential interactions with amino acids.[83] By combining the structural features of dihydropyrimidinone and 1,2,3-triazole heterocycles, novel hybrid compounds were synthesized via CuAAC under microwave irradiation. The newly synthesized compounds were evaluated for their antiviral activity against varicella-zoster virus (VZV). Some of them showed valuable antiviral activities, with EC_{50} values ranging from 3.6 to 11.3 μM against TK+ and TK- VZV and without measurable cell-growth inhibition.[84] Similarly, a new series of 1,4-disubstituted-1,2,3-triazolethymine derivatives were synthesized and tested for their *in vitro* cytotoxic activities against human cancer cell line (MDA-MB 231). These derivatives were useful as starting points for further study of new anticancer drugs and to confirm the potential of triazole-sulfonamide analogues as lead compounds in anticancer drug discovery. In addition, 1,4-disubstituted-1,2,3-triazolethymine derivatives were evaluated *in vitro* for antiviral activity against the replication of HIV-1

and HIV-2 in MT-4 cells. The results showed that 1,4-disubstituted-1,2,3-triazolethymine derivatives possess a potent activity against HIV-1.[85]



Scheme 28. Synthetic pathway to compound **84**.

The use of multivalent carbohydrate compounds to block cell-surface lectin receptors is a promising strategy to inhibit the entry of pathogens into cells and could lead to the discovery of novel antiviral agents. One of the main problems with this approach, however, is that it is difficult to make compounds of an adequate size and multivalency to mimic natural systems such as viruses. Hexakis-adducts of [60]fullerene are useful building blocks in this regard because they maintain a globular shape at the same time as allowing control over the size and multivalency. Water-soluble tridecafullerenes, decorated with 120 peripheral carbohydrate subunits, the so-called 'superballs', were prepared through the collaboration of several research European groups and could be efficiently synthesized from hexakis-adducts of [60]fullerene in one step by using CuAAC (Figure 7). Infection assays show that these superballs are potent inhibitors of cell infection by an artificial Ebola virus with half-maximum inhibitory concentrations in the subnanomolar range.[86]

The C-type lectin expressed on immature dendritic cells (dendritic cell-specific intercellular adhesion, DC-SIGN) is also a promising target for antiviral drug development. Mono- and divalent C-glycosides based on D-manno and L-fuco configurated saccharide units are promising DC-SIGN ligands. The convergent synthesis of C-glycoside dendrimers decorated with 4, 6, 9, and 12 α-L-fucopyranosyl units and with 9 and 12 α-D-mannopyranosyl units (Figure 8A) allowed to demonstrate the affinity against DC-SIGN by surface plasmon resonance assays. For comparison, parent *O*-glycosidic dendrimers were synthesized and tested. A clear increase of both affinity and multivalency effect was observed for *C*-glycomimetics of both types (mannose and fucose). When dodecavalent *C*-glycosidic dendrimers were compared, there was no difference in affinity regarding the sugar unit (L-fuco, IC_{50} 17 μM; D-manno, IC_{50} 12 μM). For the rest of glycodendrimers with L-fucose or D-mannose attached by the *O*- or *C*-glycosidic linkage, *C*-glycosidic dendrimers were significantly more active. These results showed that the biological activity of *C*-glycoside mimetics is higher in comparison to the corresponding *O*-glycosides and therefore these glycomimetic multivalent systems represent potentially promising candidates for targeting DC-SIGN.[87]

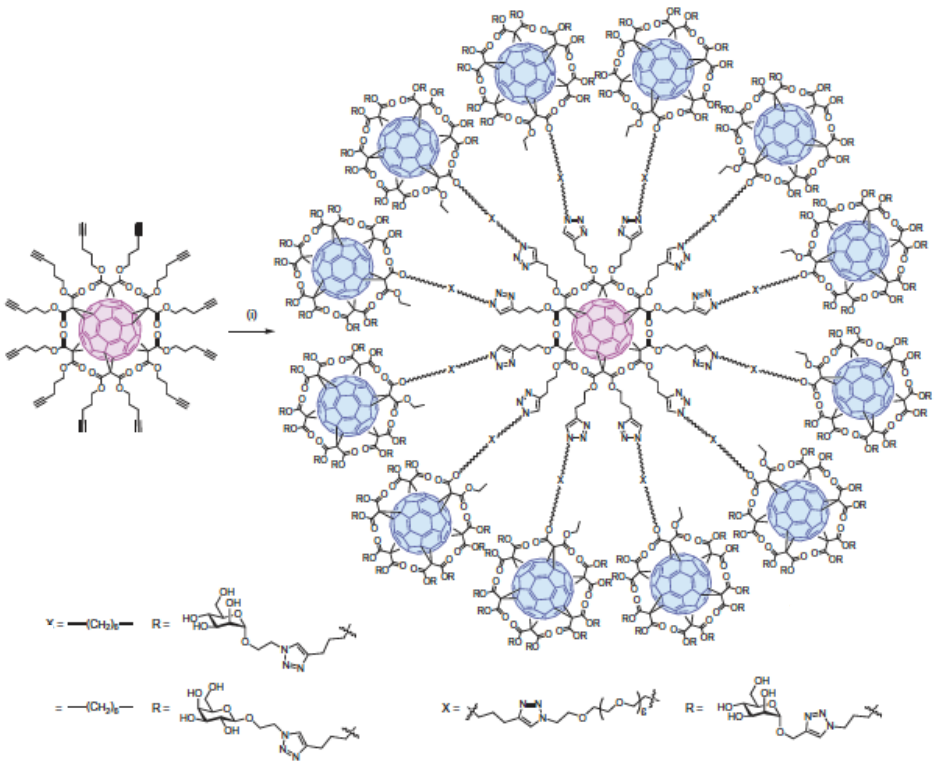


Figure 7. Syntheses of the tridecafullerenes using CuAAC click chemistry. The core fullerene (endowed with 12 alkyne groups) is joined to the peripheral fullerene-based compounds by click chemistry. Reagents and conditions: (i) $CuBr{\cdot}S(CH_3)_2$, sodium ascorbate, Cu(0), DMSO, 25 °C, 48 hours, 73-79%; (ii) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF/H_2O, 80 °C (MW), two hours, 76%.

Novel antiviral nanotherapeutics, which may inactivate the virus and block it from entering host cells, were prepared using a combination of bioorthogonal CuAAC and photoinitiated thiol-ene coupling. The conjugates were found to be biocompatible and demonstrate no toxicity to cells at biologically relevant concentrations. The resulting bioconjugates inhibit HSV-1 at both the early and the late stages of the infection process.[88]

Single wall carbon nanotubes (SWCNTs), multiwall carbon nanotubes (MWCNTs), and single wall carbon nanohorns (SWCNHs) have been employed as virus-mimicking nanocarbon platforms for the multivalent presentation of carbohydrates in an artificial Ebola virus infection model assay. These carbon nanoforms have been chemically modified by the covalent attachment of glycodendrons and glycofullerenes using the CuAAC approach. This modification increases the water solubility of these structurally different nanocarbons. Their efficiency in blocking DC-SIGN-mediated viral infection by an artificial Ebola virus was tested in a cellular experimental assay, finding that glycoconjugates based on MWCNTs functionalized with glycofullerenes are potent inhibitors of viral infection.[89]

An analogous strategy to that shown in Figure 8A has been applied as illustrated in Figure 8B where several ribavirin analogues were synthesized and incorporated into a multivalent arrangement. The antiviral activity was tested against Junìn virus, the etiological agent responsible for Argentine hemorrhagic fever. Some compounds inhibited Junìn virus in the range of 13.2-389.1 µM. Two modified ribavirin analogues showed an effective concentration comparable to ribavirin but with a higher selectivity index.[90]

The C-type lectin receptor DC-SIGN has been also identified as an entry receptor for epidemic and pandemic pathogens such as SARSCoV-2, Ebola virus, and HIV-1. A new class of potent glycomimetic DC-SIGN

antagonists has been prepared from a focused library of triazole-based mannose analogues. Structure-based optimization yielded a glycomimetic ligand with a more than 100-fold improved binding affinity compared to methyl α-D-mannopyranoside. The identified ligand was employed for the synthesis of multivalent glycopolymers that were able to inhibit SARS-CoV-2 spike glycoprotein binding to DC-SIGN-expressing cells, as well as DC-SIGN-mediated transfection of ACE2+ cells by SARS-CoV-2 spike protein-expressing viruses, in nanomolar concentrations.[91]

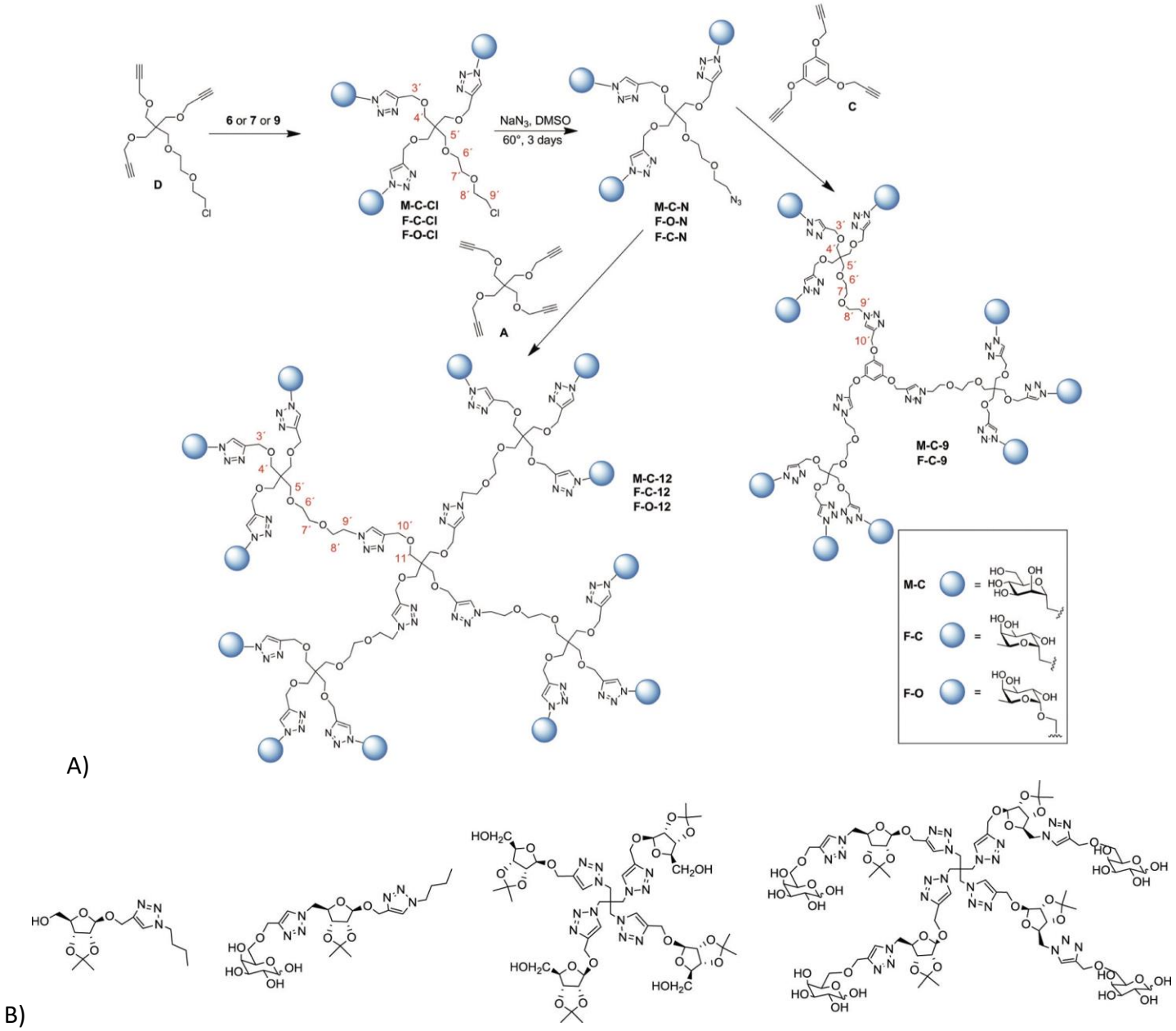


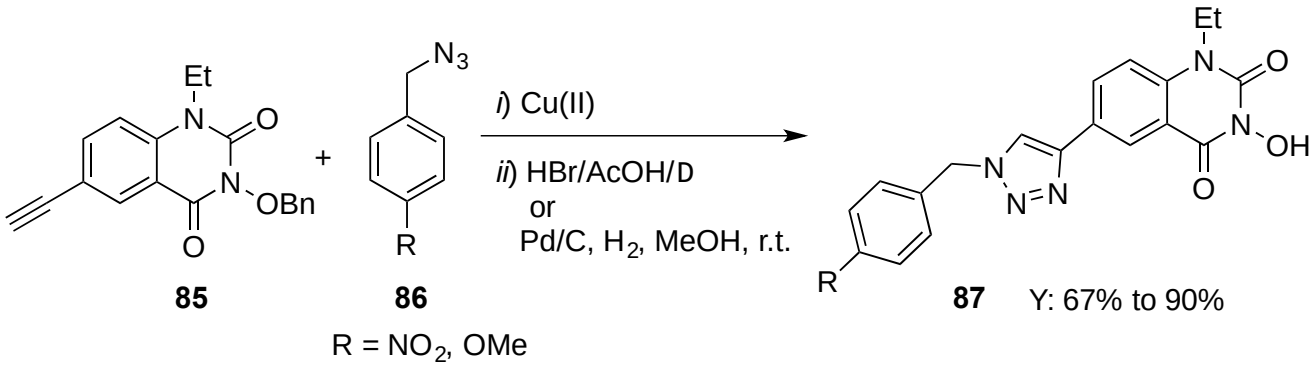
Figure 8. A) C-glycoside dendrimers decorated with 4, 6, 9, and 12 α-L-fucopyranosyl units and with 9 and 12 α-D-mannopyranosyl units. B) Ribavirin analogues incorporated into a multivalent arrangement.

Unprecedented 3D hexa-adducts of [60]fullerene peripherally decorated with twelve tryptophan (Trp) or tyrosine (Tyr) residues have been synthesized and tested on the antiviral activity against HIV. The potent activity against HIV demonstrates the relevance of the globular 3D presentation of the peripheral groups

(Trp/Tyr) as well as the length of the spacer connecting them to the central core to interact with the viral envelopes, particularly in the case of HIV, and support the hypothesis that [60]fullerene can be an alternative and attractive biocompatible carbon-based scaffold for this type of highly symmetrical dendrimers.[92]

New p-t-Bu-calixarene glycoclusters bearing tetrahydroxamic acid groups exhibit micromolar inhibition of soluble DC-SIGN binding and provide nanomolar IC_{50} inhibition of both DC-SIGN dependent Jurkat cis-cell infection by viral particle pseudotyped with Ebola virus glycoprotein and the HCMV-gB-recombinant glycoprotein interaction with monocyte-derived dendritic cells expressing DC-SIGN. A unique cooperative involvement of sugar, linker, and calixarene core is likely behind the strong avidity of DC-SIGN for these low-valent systems.[93]

A series of 1,2,3-triazolyl 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones **87** were constructed utilizing the CuAAC protocol. The biological significance of the synthesized quinazolines was highlighted by evaluating the *in vitro* antiviral activity. Several compounds exhibited excellent activities, specifically against vaccinia and adenovirus. In particular, the nitro derivative **87** (Scheme 29, R = NO_2) displayed the most potent inhibitory activity against vaccinia with an EC_{50} value of 1.7 µM, which was 15 fold lower than that of the reference drug cidofovir (EC_{50} = 25 µM). The methoxy derivative **87** (R = OMe) was the most potent compound against adenovirus-2 with an EC_{50} value of 6.2 µM, lower than all the reference drugs. No data are present in the literature on antiviral activity of 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones against DNA-viruses.[94]



Scheme 29. Synthetic route to 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones.

Quinazoline derivatives were synthetized and found active toward herpes simplex virus-1 wildtype and thymidine kinase deficient (HSV-1 TK^-) strains (EC_{50} values in the range of 4.6-13.8 µM). Some of these compounds exhibited activity toward varicella-zoster virus (VZV) TK+and TK^- strains (EC_{50} = 2.1-9.5 µM).[95]

Figure 9. Multivalent scaffolds strategy (Reproduced with permission.[96] Copyright 2016, Elsevier).

Mono-, di-, tetra-, and octa-valent difluorinated zanamivir analogs were prepared to become potent inhibitors of influenza virus.[96] The mono difluorinated zanamivir with an azide linker attached at the C-7 position was synthesized in good yield from sialic acid using Selectfluor® and DAST (diethylaminosulfur trifluoride) as the fluorination reagent. This intermediate was attached on various alkynlyated scaffolds via ‘‘click Chemistry” to afford multivalent glycoclusters (Figure 9). A NA inhibition assay was used to evaluate the compounds for their inhibitory activity using H7N9 virus like particle. These multivalent sialosides show enhanced inhibition with IC_{50} values ~100-fold better than the corresponding monomer, indicative of the strength of using a multivalent approach. The increased intrinsic affinities of these analogues were achieved by ‘‘cluster” effect. Among them the increase of IC_{50} values for the octavalent analogue is maximal 145-fold to the monomer. With these promising results, polyvalent DFSAs and their high inhibitory activities on cell level are now under investigation. These results will provide a basis for investigating polymer-attached inhibitors as more efficacious therapeutics, especially against the drug-resistant influenza virus.[96]

C-Nucleosides have been defined as an underexplored and important class of nucleosides with antiviral and anticancer activity. In addition, triazole heterocycles are well employed as a strategy to modify nucleobase in nucleoside analogues, although rare examples were described for triazoyl *C*-nucleosides. N^2-Aryl-1,2,3-triazole *C*-nucleoside compounds that could be obtained by selective 1,2,3-triazole heterocycle N^2 arylation in 1-β-D-ribofuranosyl-2*H*-1,2,3-triazole substrate were designed in a study by French-Brazilian researchers.[97] The optimized condition used AdBrettPhos/$[PdCl(allyl)]_2$ as the catalyst system. This transformation was accomplished by aryl halides bearing either electron donor or withdrawing groups, as well as by heterocyclic halides in good to excellent yields (Figure 10). The transformation developed represents a significant contribution to the nucleoside field, since it allows for the synthesis of valuable scaffolds through selective functionalization of triazole nucleosides.

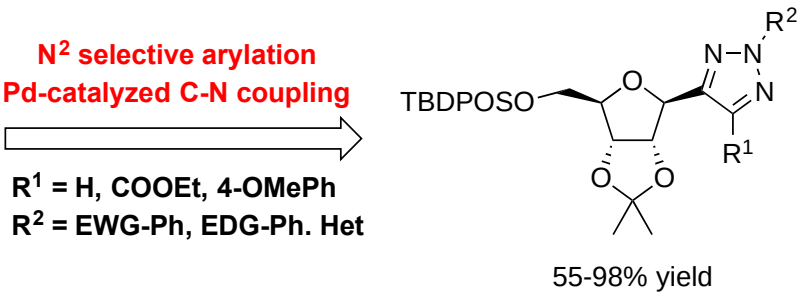
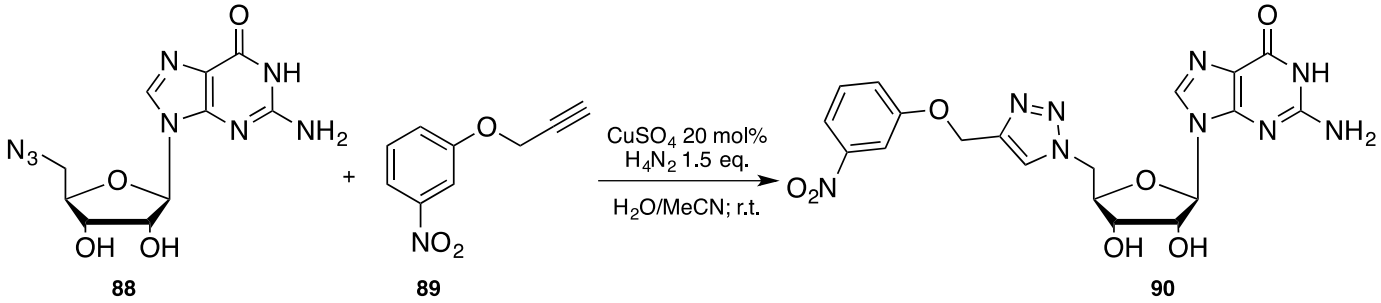


Figure 10. Functionalization strategy of the triazole ring (Reproduced with permission.[97] Copyright 2016, ACS).

Unfortunately, the authors did not test the synthesized compound properties against some viruses.[97] Similarly, no biological tests were done for the compounds obtained through this interesting synthetic strategy towards 8-azanebularine analogues displaying interesting antiviral, antitumor and biochemical activities. The typical glycosylation of 8-azapurines always resulted in the desired products in low yields due to the lack of stereo- and regioselectivity of the glycosylation reaction. The key steps involve a Cu-catalyzed 1,3-dipolar cycloaddition of a 1-β-azido sugar moiety with ethyl 3-bromopropiolate and a Pd-catalyzed cascade amidine arylation-intramolecular ester amidation reaction to build the hypoxanthine structural motif. Unfortunately, no biological data are available.[98]

A series of non-hydrolysable 5'-aryl substituted GDP analogs has been synthesized by reacting 5'-azido-5'-deoxyguanosine **88** with different aryl- and benzyloxy-alkynes. Cu(I) nanoparticles in water were found to be the most efficient catalyst, producing the desired 5'-arylguanosines in good yields (Scheme 30). The

synthesized compounds were screened for *in vitro* activity against *Leishmania donovani* axenic amastigotes and intramacrophage amastigotes stages.



88 **89** **90**

Scheme 30. Synthetic pathway to compound **90**.

The 4-(3-nitrobenzyl)-1,2,3-triazole 5'-substituted guanosine analog **90** was found to be the most active in the series with an IC_{50} of 8.6 µM on axenic amastigotes. Despite a rather low in vitro antileishmanial activity on the intramacrophage amastigotes, the absence of cytotoxicity on RAW 264.7 macrophages justifies further pharmacomodulations making this antileishmanial series promising. *In silico* docking experiments highlighted that the most active compound **90** present a binding conformation similar to the GDP-mannose. This observation and the good qualitative accordance between the results of biological essays and the prediction of the molecular docking calculations seem to correlate the antileishmanial activity with the interaction of the GDP-MP active site.[99]

Longer peptides corresponding to the *C*-terminal heptad repeat (*C*-peptides) of HIV-1 gp41 were found to be potent inhibitors against virus-cell fusion. Designing short *C*-peptide-based HIV-1 fusion inhibitors could potentially redress the physicochemical and technical liabilities of a long-peptide therapeutic. However, designing such inhibitors with high potency has been challenging. A conjugated architecture by incorporating small-molecule inhibitors of gp41 into the *N*-terminus of a panel of truncated *C*-peptides was developed by a Chinese research group (Figure 11). Among these small molecule-capped short peptides, the 26-residue peptide Indole-T26 inhibited HIV-1 Env-mediated cell-cell fusion and viral replication at low nanomolar levels, reaching the potency of the only clinically used 36-residue peptide T20 (Enfuvirtide).

The methodology relies upon the azide 1,3-dipolar cycloaddition reaction between the azido-functionalized protected peptide and the dipolarophile of choice. Thus, the small molecule-peptide conjugation provides a viable strategy for designing short peptide based HIV-1 fusion inhibitors to expand the repertoire of antiretroviral therapy and perhaps to develop pharmaceutical interventions targeting helical PPIs in other biological processes.[100]

It was found that multivalent ligands that exhibit high binding affinity to influenza hemagglutinin (HA) trimer, can block the interaction of HA with its sialic acid receptor. Multivalent pentacyclic triterpene-functionalized per-*O*-methylated cyclodextrin (CD) derivatives were thus designed and synthesized using click reactions. A cell-based assay showed that some compounds (Figure 12) exhibited strong inhibitory activity against influenza A/WSN/33 (H1N1) virus. The compound in Figure 12 showed the most potent anti-influenza activity with IC_{50} of 4.7 µM. The time-of-addition assay indicated that the compound at hand inhibited the entry of influenza virus into host cell. Furthermore, hemagglutination inhibition and surface plasmon resonance (SPR) assays indicated that the compound shown in Figure 12 tightly bound to influenza HA protein with a dissociation constant (K_d) of 4.0 µM.

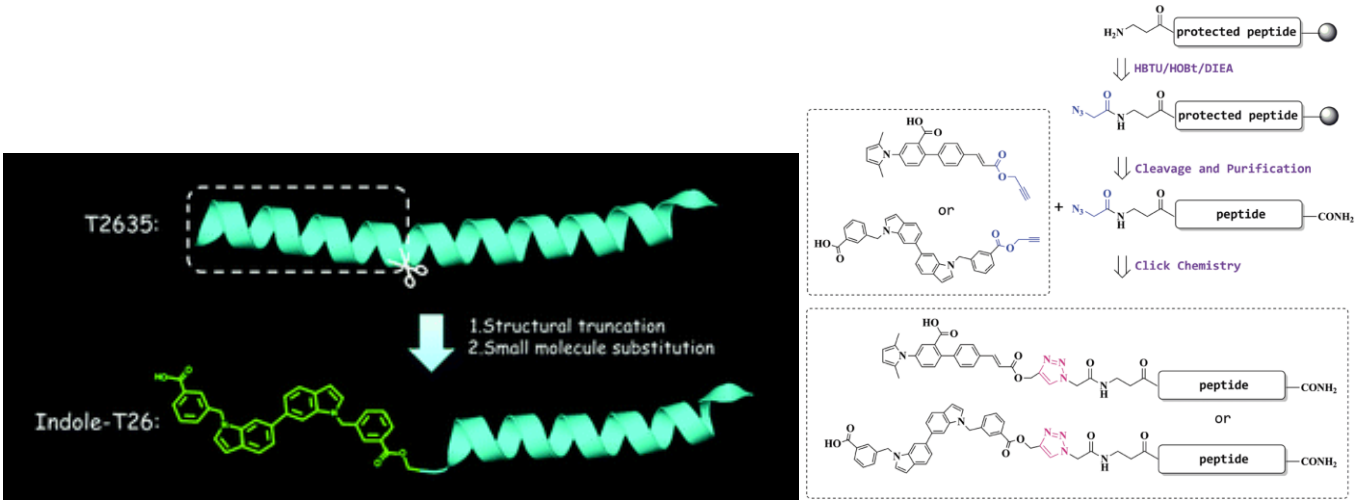


Figure 11. Truncation of peptide chain and strategy for the synthesis of small molecule–peptide conjugates (Reproduced with permission.[100] Copyright 2016, RSC).

The results demonstrated a strategy of using per-*O*-methylated β-CD as a scaffold for designing multivalent compounds to disrupt influenza HA protein-host receptor protein interaction and thus block influenza virus entry into host cells.[101]

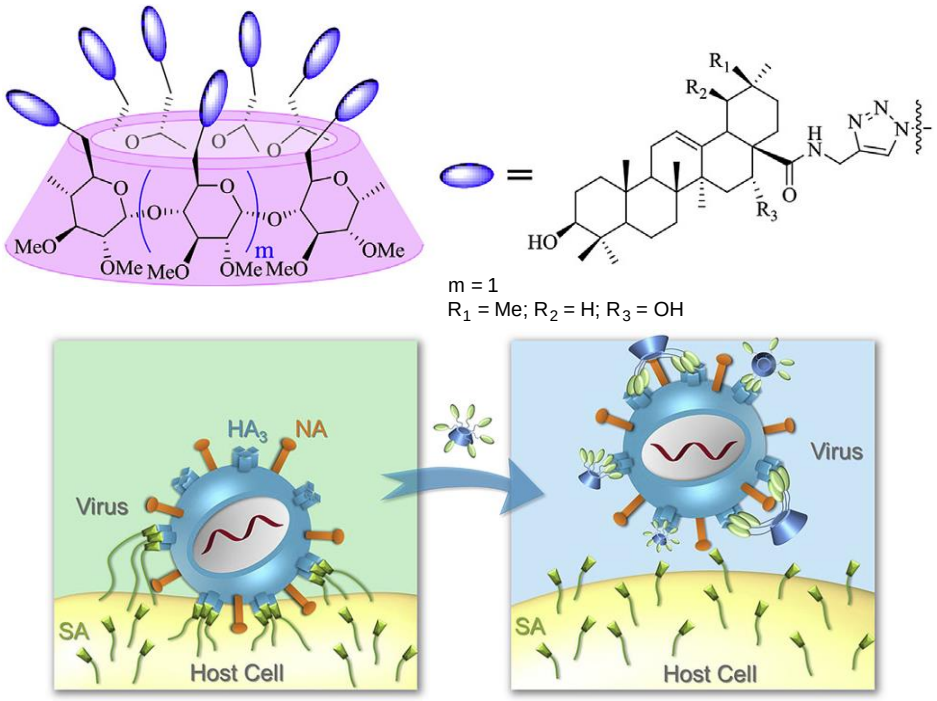
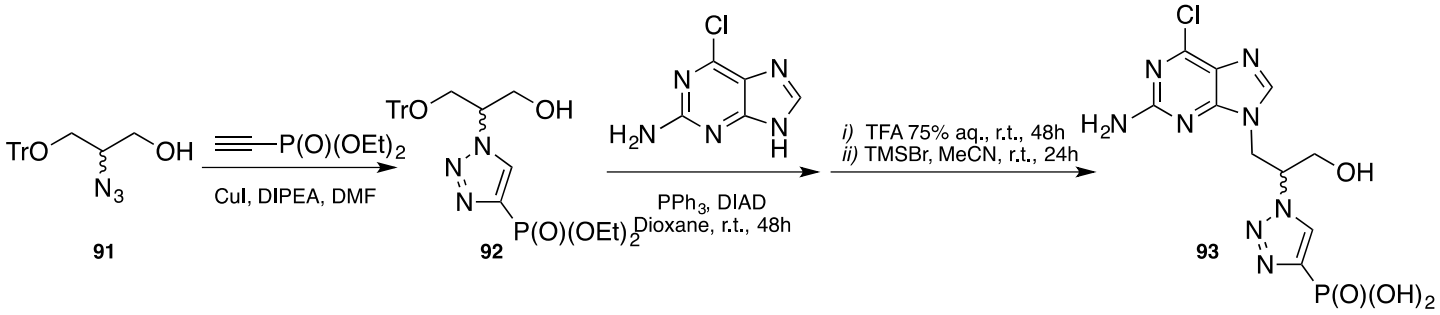


Figure 12. Cyclodestrin derivatives structures and anti-H1N1 inhibition scheme (Reproduced with permission.[101] Copyright 2017, Elsevier).

The emergence of drug resistant variants of the influenza virus leads to a great need to identify new and effective antiviral agents. For this reason, the synthesis and anti-influenza activity of novel sialic acid (C-5 and C-9)-pentacyclic triterpene conjugates was carried on to prepare two conjugates that showed strong cytotoxicity to MDCK cells in the Cell Titer-Glo assay at a concentration of 100 μM. They showed no significant

cytotoxicity to HL-60, Hela, and A549 cell lines in MTT assay under the concentration of 10 μM. These compounds displayed weak potency towards influenza A/WSN/33 (H1N1) virus (100 μM, ~20–30%), and no significant anti-influenza activity was found for the other conjugates. The data suggested that both the C-5 acetamide and C-9 hydroxy of sialic acid were important for its binding with hemagglutinin during viral entry into host cells, while C-4 and C-2 hydroxy were not critical for the binding process and could be replaced with hydrophobic moieties.[102]

A family of acyclic nucleoside phosphonates (ANPs) bearing a (1*H*-1,2,3-triazol-4-yl)phosphonic acid group of type **93** (Scheme 31) in the acyclic side chain were prepared in order to study the influence of the hetaryl rigidizing element on the biological properties of such compounds. The key synthetic step consisted of a CuAAC between diethyl ethynylphosphonate and the corresponding azidoalkyl precursor to afford the intermediate **92**. ANPs of type **93** in this family, bearing a guanine base, exhibited the highest potency for the human 6-oxopurine phosphoribosyl transferase irrespective of the stereochemistry at C-20. Four compounds inhibited *Plasmodium falciparum* 6-oxopurine phosphoribosyl transferase with little differences in their K_i values irrespective of whether the base was guanine, hypoxanthine or xanthine but only two, with guanine as base, inhibited PvHGP RT.[103]



Scheme 31. Synthetic pathway to compound **93**.

In previous examples we have seen the application of a CuAAC methodology for the synthesis of antiviral compounds. Since the breakthrough of copper-assisted azide-alkyne cycloadditions (CuAAC), there have been several reports describing the synthesis and properties of novel triazole-modified nucleic acid derivatives for potential downstream DNA- and RNA-based applications.

A recent review focuses on highlighting representative novel nucleic acid molecular structures that have been synthesized via the “click” azide-alkyne cycloaddition. Many of these derivatives show compatibility for various applications that involve enzymatic transformation, nucleic acid hybridization, molecular tagging and purification and gene silencing. The details of these applications can be found in these manuscripts where the chemistry of triazoles seems to be really promising for future developments of antiviral and anticancer compounds.[104,105]

A variety of triterpene dimers bearing different scaffolds were designed and synthesized via the CuAAC reaction and their anti-HCV entry activities were evaluated by HCVpp and VSVpp entry assays. It was found that echinocystic acid and its dimer were still necessary for maintaining anti-HCV entry activity, and replacement of echinocystic acid by other triterpenes might significantly decrease its anti-viral activities. Using a linker bearing a piperazine group (pink ring in Figure 13), the dimer dramatically increased its potency with IC_{50} at 2.87 nmol/L. In addition, the undesired hemolytic effect of all these type of compounds was removed.[106]

New conjugates of substituted pyridine and carbohydrate moieties linked by 1,2,3-triazoles were synthesized by preparing the dipolarophile via insertion of a propargyl group by *O*-propargylation of pyridone derivatives. Attachment of carbohydrate molecules of type **95** to the substituted pyridine core of type **94** was performed by Cu-catalyzed cycloaddition of propargyl sugars with azidoethoxypyridine derivative or azido-sugars with substituted (propargyl)oxypyridines, which afforded the corresponding 1,2,3-triazoles **96** in high yields (Scheme 32). Antiviral activity of synthesized compounds was studied against H5N1 influenza virus and triazolyl glycoside **96** demonstrated high activity in addition to low toxicity.[107]

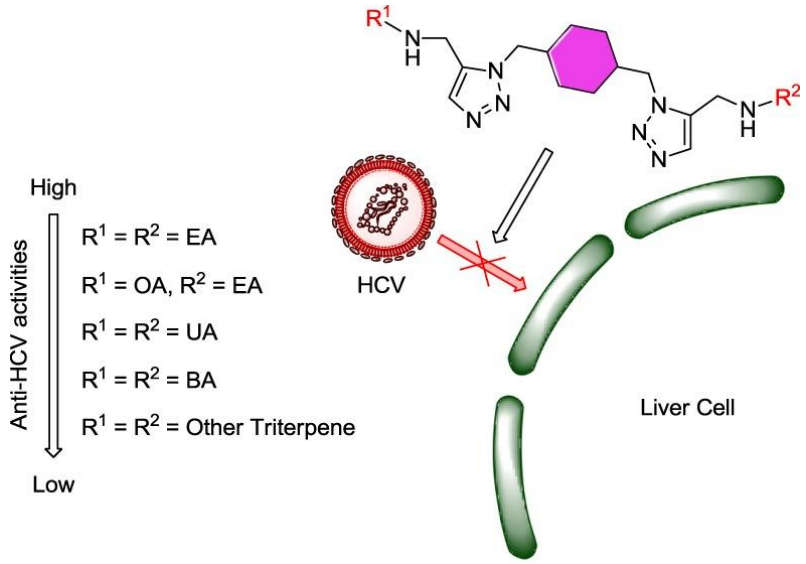
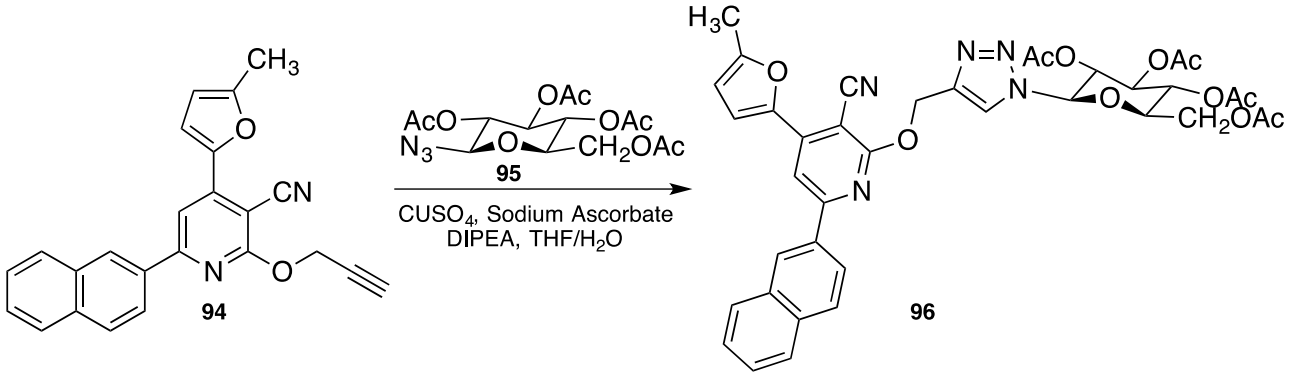


Figure 13. Inhibition scheme in HCV (Reproduced with permission.[106] Copyright 2017, Wiley).

The synthesis of a biotinylated bivalent zanamivir analog as a probe for influenza viruses was achieved by using a glycan-based sandwich assay where glycans were immobilized on glass slides to capture strains of influenza A H1N1, A/Brisbane/59/2007 virus. The biotinylated bivalent zanamivir analog-labeled streptavidin complex was used as exemplified in Figure 14. The coupling was ensured by azide chemistry where triazole rings are the linkers between the two moieties. This research strongly suggests that glycans can be used for capturing and reporting influenza viruses and the biotinylated compounds can be used as probes for capturing and isolating influenza viruses from complex mixtures.[108]



Scheme 32. Cu-catalyzed cycloaddition of azido-sugar.

It is known that the HIV-1 capsid (CA) protein plays essential roles in both early and late stages of HIV-1 replication and it is considered an important, clinically unexploited therapeutic target.

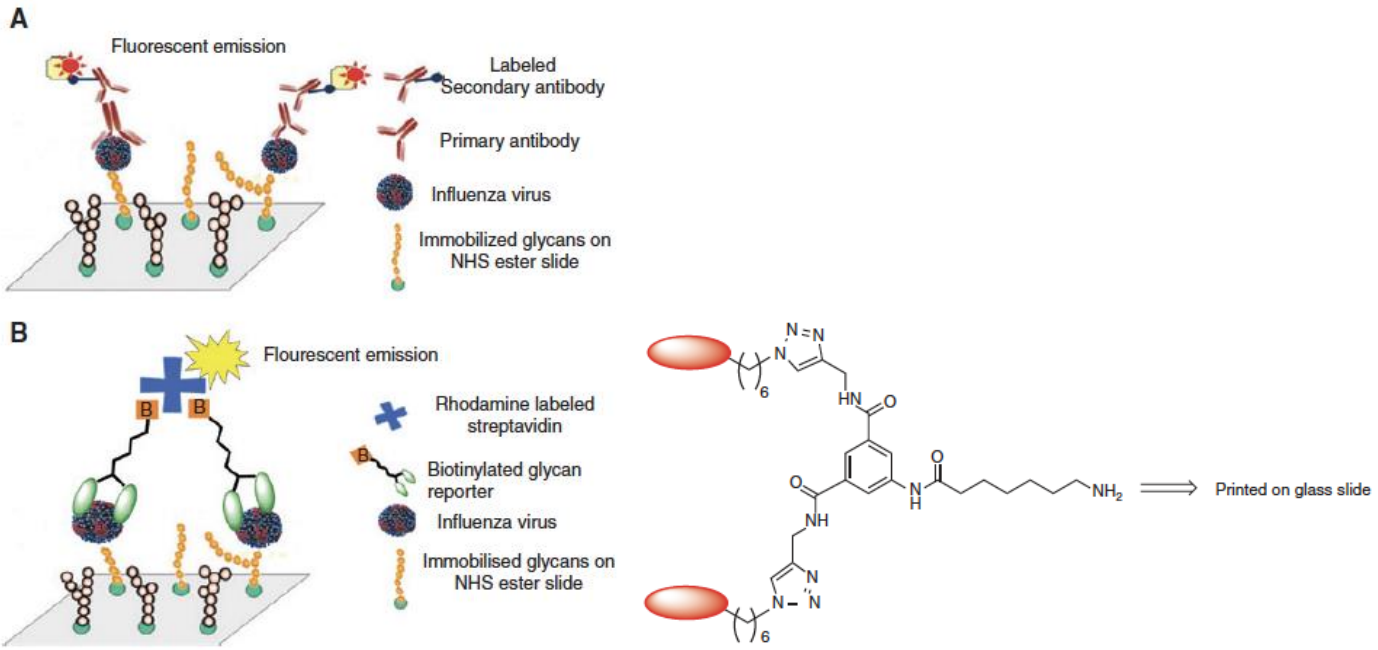


Figure 14. (A) Representation of the sandwich assay using glycans as capture and labeled antibody as reporters. (B) Representation of the sandwich assay using glycans as capture and biotinylated glycan-labeled streptavidin as reporters. Azide chemistry produces the triazole rings as the linkers between the two moieties.

For this reasons, small drug-like molecules that inhibit this critical HIV-1 protein have become a priority in research projects. The synthesis of small molecule targeting of the CA protein, and in particular a very attractive inter-protomer pocket, was reported and specifically the design, the parallel synthesis, and the anti-HIV-1 activity evaluation of a series of novel phenylalanine derivatives as HIV-1 CA protein inhibitors. These molecules were synthesized via CuAAC-reaction. Figure 15 shows the structure of a compound that exhibited the greatest potency and lowest toxicity within this new series with an EC_{50} value of 4.33 µM and CC_{50} value of >57.74 µM (SI > 13.33). These values are very similar to the lead compound PF-74 (EC_{50} = 5.95 µM, CC_{50} > 70.50 µM, SI > 11.85). Furthermore, it was demonstrated via surface plasmon resonance (SPR) binding assays that the compound at hand interacts robustly with recombinant HIV-1 CA and exhibits antiviral activity in both the early and late stages of HIV-1 replication. It is worth noting that the novel parallel synthesis and SARs set the foundation for further rational optimization and discovery of CA-targeting compounds with improved potency.[109]

An interesting approach was developed to offer distinct and well-defined glycopolymers for deciphering the biological roles of natural bioactive polysaccharides. Fucose monomers were chemically synthesized and decorated with specific sulfation patterns including unsulfate (with no SO_3Na groups), monosulfate, disulfate, and trisulfate groups (Figure 16).

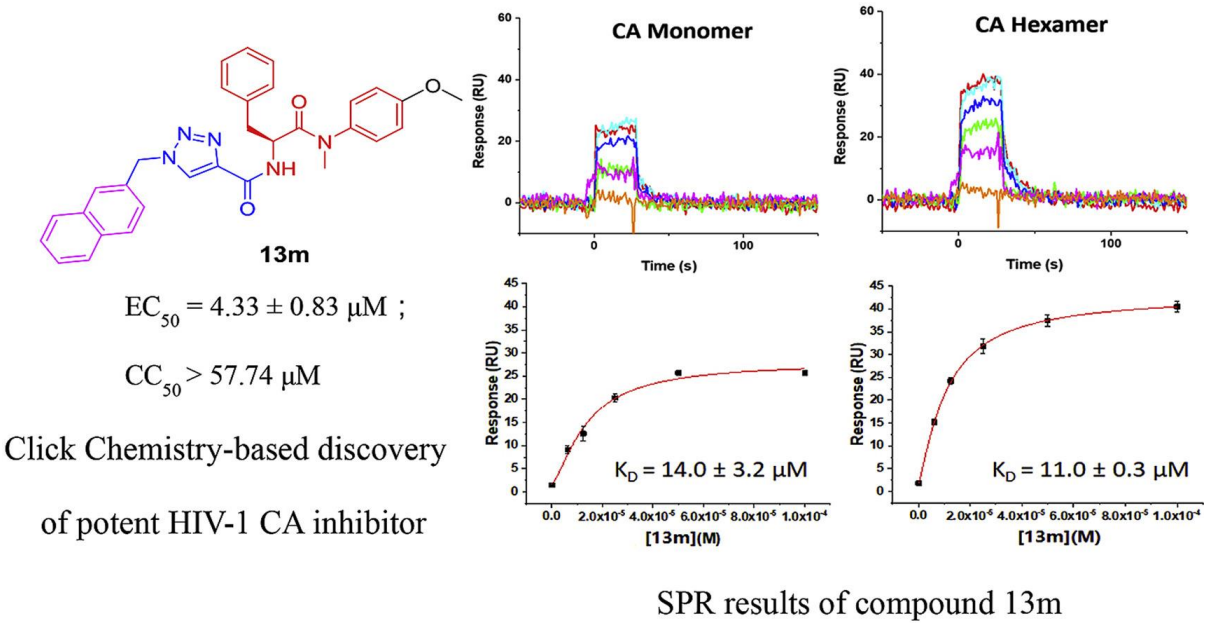


Figure 15. Structure of active compound and activity data (Reproduced with permission.[109] Copyright 2018, Elsevier).

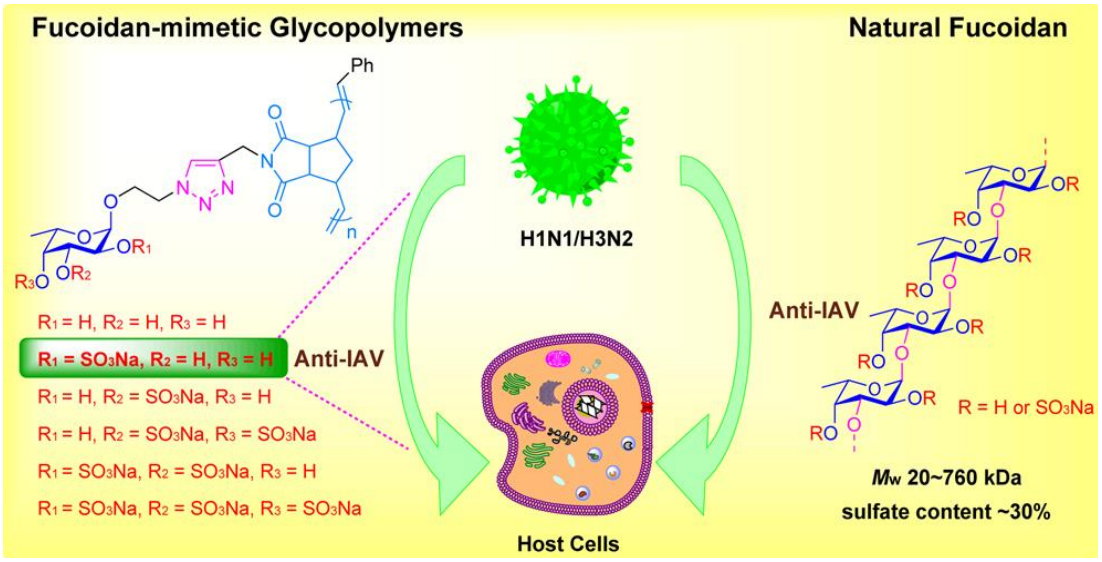


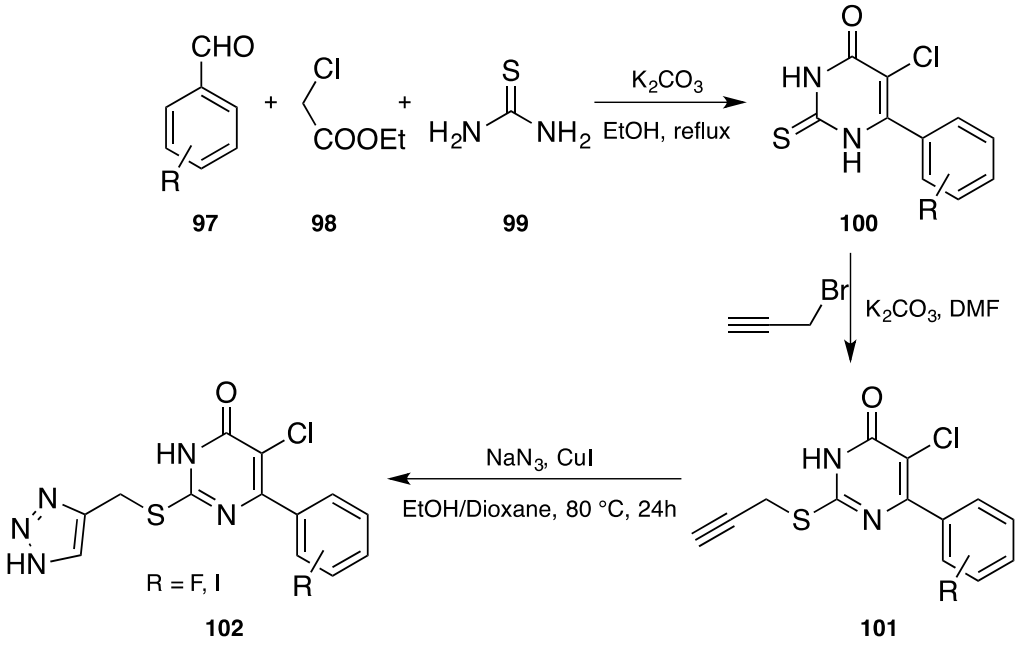
Figure 16. Fucoidan-mimetic glycopolymers structures and action mechanism (Reproduced with permission.[110] Copyright 2018, ACS).

Six fucoidan-mimetic glycopolymers were successfully prepared through microwave assisted ring-opening metathesis polymerization (ROMP) in an emulsion system. Three glycopolymers associated with 2-*O*-sulfation exhibited better inhibitory effects on the H1N1 virus, while other glycopolymers with monosulfate groups were more effective against the H3N2 virus. These findings would promote the development of novel anti-influenza A virus drugs based on natural fucoidans.[110]

A novel series of 2-[(1*H*-1,2,3-triazol-4-yl)methylthio]-5-chloro-6-phenylpyrimidin-4-(3*H*)-one derivatives has been synthesized and their molecular docking as well as *in vitro* assay as anti-HIV-1 non-nucleoside reverse

transcriptase inhibitors were studied. The synthesis started from the reaction of aldehydes **97**, ethyl chloroacetate **98**, and thiourea **99** to give the 6-aryl-5-chloro-2-thiouracils derivatives **100**. Then, reaction of the latter compounds with propargyl bromide **101** followed by azide click reaction led to the formation of compounds **102** in good yields (Scheme 33). The obtained derivatives were evaluated as anti-HIV-1 non-nucleoside RT inhibitors.

The enzyme assay results demonstrated that 4-fluorophenyl- and 4-iodophenyl-substituted compounds were more potent than the remaining derivatives, compared against RT enzyme. The inhibitory activity of HIV-1 viral replication was also assessed by cell-based assay. The compounds above mentioned were the most potent inhibitors of HIV-1 replication against HIV-1 IIIB (EC_{50} = 0.69 and 0.9 μg/mL, respectively for fluoro and iodo derivatives; the selectivity index = 58.69 and 43.66, respectively; CC_{50} with HIV-1IIIB = 40.5 and 39.3 μg/mL, respectively) and HIV-1ADA5 (EC_{50} = 1.05 and 0.29 μg/mL, respectively; the selectivity index = 42.33 and 4.14, respectively; CC_{50} = 40.36 and 154.48 μg/mL, respectively).[111]



Scheme 33. Synthesis of 2-[(1*H*-1,2,3-triazol-4-yl)methylthio]-5-chloro-6-phenylpyrimidin-4-(3*H*)-one derivatives **102**.

Divalent oseltamivir and guanidino oseltamivir analogues with esterification on the carboxyl acid group as potent inhibitors of influenza virus neuraminidase were prepared via click reaction (Figure 17). The primary structure activity relationship study demonstrated that appropriate distance between two oseltamivir monomers around 30 Å can crosslink two adjacent neuraminidase tetramers on the virion surface and result in highly effective NA inhibitors against three strains of influenza virus and H7N9 virus like particle. This strategy also provides a basis for the multivalent modification on oseltamivir.

The most potent NA inhibitor, whose structure is shown in Figure 17, exhibits approximately 68-fold increase in inhibition against H1N1 strain compared with its monomer, which further stresses the importance of the linker for the dimer attachment.[112]

The synthesis and biological evaluation of 5-chloro-2-thiophenyl-1,2,3-triazolylmethyl dihydroquinolines (Figure 18) as dual inhibitors of *Mycobacterium tuberculosis* and influenza virus have been conducted taking advantage of the Huisgen's [3+2] cycloaddition of 6-(azidomethyl)-5-chloro-2-(thiophen-2-yl)-7,8-

dihydroquinoline with various alkynes using sodium ascorbate and copper sulphate. The products were obtained from good to excellent yields. The new compounds were evaluated for *in vitro* antimycobacterial against *M. tuberculosis* H37Rv (Mtb) and antiviral activity against influenza virus A/Puerto Rico/8/34 (H1N1). Among the fifteen new analogues prepared, the compounds bearing the substituents shown in Figure 18 were identified as potent antitubercular agents. The virus-inhibiting activity of all the fifteen compounds was found to be moderate, and among them the compound bearing thiophene moiety appeared the most active with good selectivity index (IC_{50}=19.5 µg/mL; SI=15). These results help in developing newer dual inhibitors of tuberculosis and influenza virus.[113]

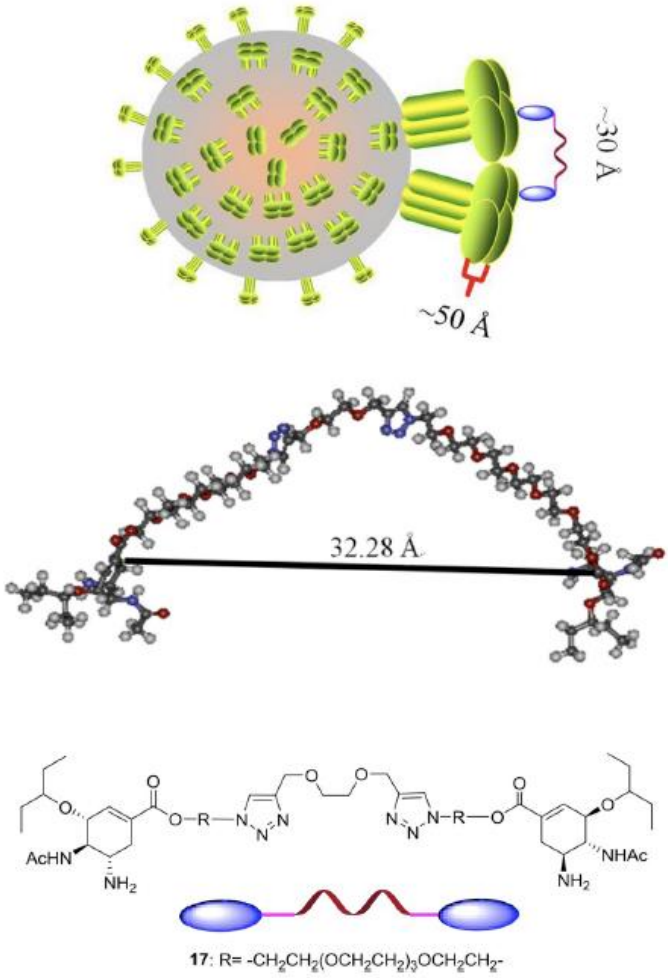


Figure 17. Schematic representation of distance between two NA monomer active sites on a same virion and the low-energy extended conformation of dimeric compound (Reproduced with permission.[112] Copyright 2019, Elsevier).

Two new pharmacophoric models based on quinoline derivatives and incorporating 1,2,3-triazole glycosides have been synthesized in good yields via 1,3-dipolar cycloaddition reaction based on the click strategy. Copper catalyzed click dipolar cycloaddition of the acetylenic centers with azido quinolines leads to the targeted compounds. Docking of the designed structures into Influenza virus neuraminidase (NA) active site was studied. Three molecular structures exhibit a good fit within the binding site of the protein. Antiviral activity against H5N1 avian influenza virus strainA/Egypt/M7217B/2013 was tested, and a number of compounds demonstrate high inhibition activity.[114]

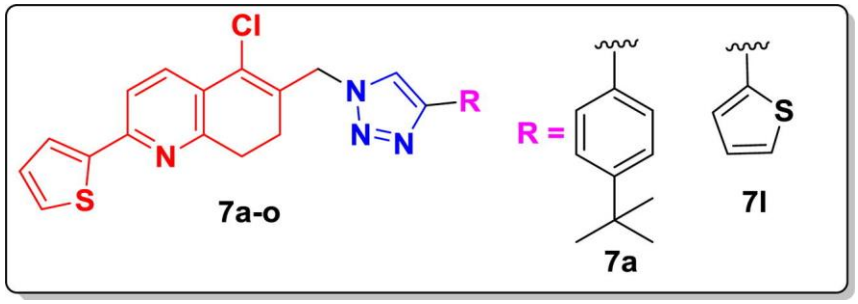


Figure 18. Structures of antitubercular agents (Reproduced with permission.[113] Copyright 2019, Elsevier).

New therapeutic possibilities were proposed for cardiac glycosides traditionally used to treat heart diseases, such as anticancer and antiviral activities. Readily accessible 3β-azido-3-deoxydigitoxigenin from digitoxigenin (Figure 19) were obtained: (i) *O*-glycosyl triazoles through click chemistry with propargyl glycosides; and (ii) compounds substituted in the alpha carbonyl position with different residues linked via an amino-group. All obtained derivatives have their chemical structures confirmed, and their anti-herpes (against HSV-types 1 and 2 replication) and cytotoxic (against PC3, A549, HCT-8and LNCaP cell lines) activities evaluated. Some of these compounds exhibited most promising results against HSV-1 (KOS and 29-R strains) and HSV-2 (333 strain) replication with SI values > 1000. They were also the most cytotoxic for the human cancer cell lines tested with IC_{50} values similar to those of paclitaxel. They also presented reduced toxicity toward non-cancerous cell lines (MRC-5 and HGF cells). Promising compounds were tested in regard to their ability to inhibit Na^+/K^+-ATPase. The inhibition rate correlates suitably with the bioactivity demonstrated by those compounds against the different human cancer cells tested as well as against HSV replication. It was possible to obtain novel digitoxigenin-derivatives with remarkable cytotoxic and anti-herpes activities as well as low toxicity and high selectivity.[115]

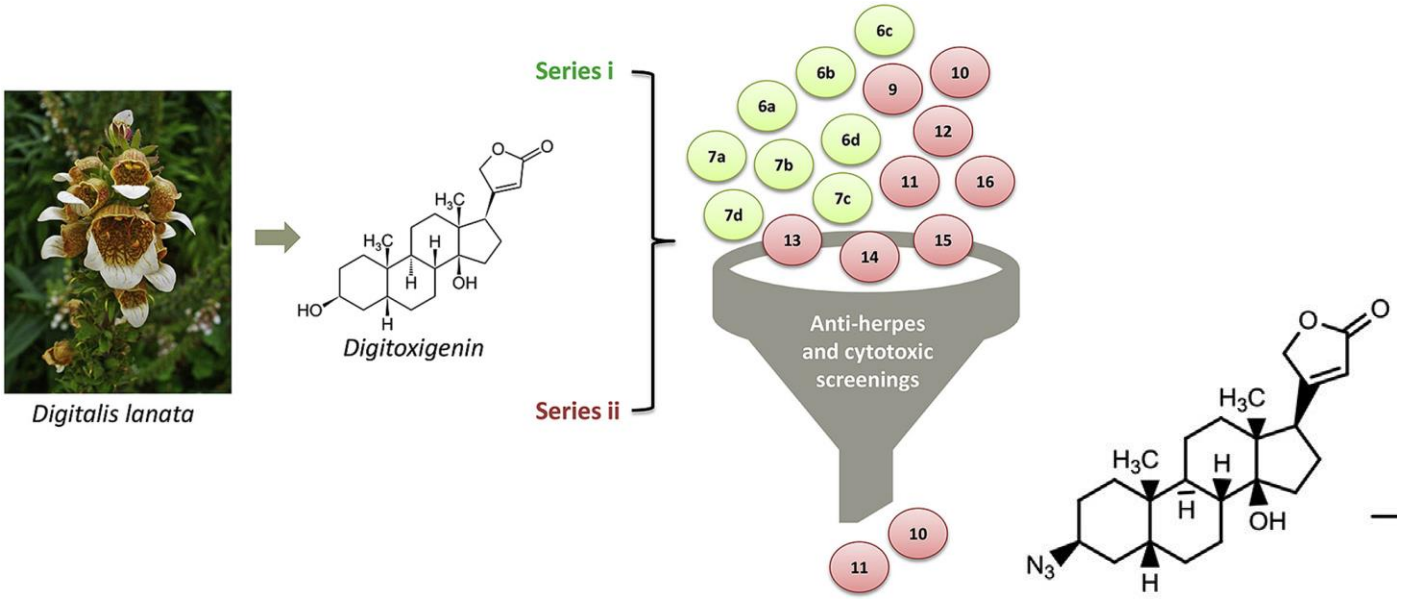


Figure 19. Source and structure of Digitoxigenin (Reproduced with permission.[115] Copyright 2019, Elsevier).

The development of entry inhibitors is an emerging approach to the inhibition of influenza virus. The oleanolic acid (Figure 20) was discovered as a mild influenza hemagglutinin (HA) inhibitor. The preparation of a

series of oleanolic acid-saccharide conjugates via the CuAAC reaction has been developed and the anti-influenza activity of these compounds was evaluated *in vitro*. Among them, compound shown in Figure 20, a glucose conjugate, showed a significantly increased anti-influenza activity with an IC_{50} of 5.47 μ, and no obvious cytotoxic effect on MDCK cells was observed at 100 μ. Hemagglutination inhibition assay and docking experiment indicated that this compound might interfere with influenza virus infection by acting on HA protein.[116]

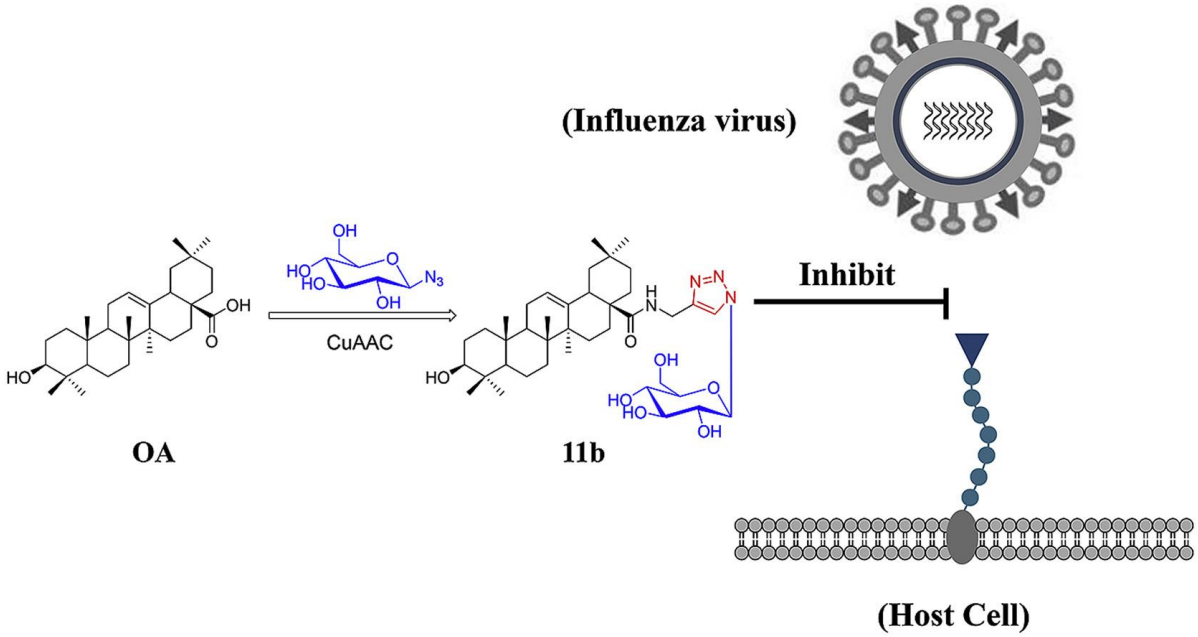
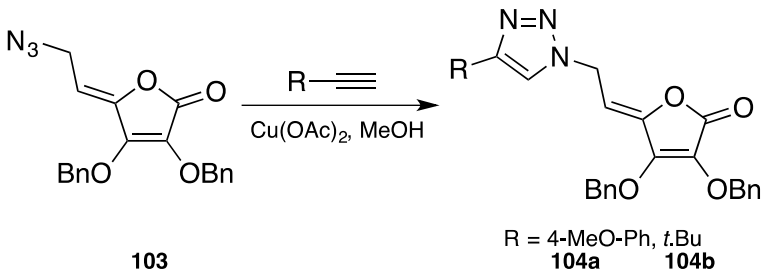


Figure 20. Synthetic strategy to oleanoic acid-saccharide conjugates (Reproduced with permission.[116] Copyright 2019, Elsevier).

Taking inspiration from the 3D structure of HA as a homotrimeric receptor, the same research group designed and synthesized 15 OA trimers with different linkers and central region via the CuAAC reaction. All of the OA trimers were evaluated for their antiviral activities in vitro, and some of them were observed to exhibit robust potency (IC_{50} in the submicromolar range) against influenza H1N1 virus that was stronger than that observed with oseltamivir. The results of hemagglutination inhibition assays and surface plasmon resonance binding assays suggest that these OA trimers may interrupt the interaction between the HA protein of influenza virus and the host cell sialic acid receptor, thus blocking viral entry. These findings highlight the utility of multivalent OA conjugates to enhance the ligand−target interactions in anti-influenza virus drug design and are also helpful for studying antiviral drugs derived from natural products.[117]

Two series of 6-(1,2,3-triazolyl)-2,3-dibenzyl-L-ascorbic acid derivatives with the hydroxyethylene and ethylidene linkers were synthesized and evaluated for their antiproliferative activity against seven malignant tumor cell lines and antiviral activity against a broad range of viruses. The conformationally unrestricted spacer between the lactone and 1,2,3-triazole units had a great effect on antitumor activity. The introduction of a long side chain at C-4 of 1,2,3-triazole that led to the synthesis of decyl-substituted 2,3-dibenzyl-L-ascorbic acid resulted in a selective and potent antiproliferative activity on breast cancer MCF-7 cells cells in the nM range. The p-methoxyphenyl-substituted derivative **104a** displayed specific anti-cytomegalovirus (CMV) potential, whereas the *tert*-butyl substituted **104b** had the most potent, yet relatively non-specific, anti-varicella zoster (VZV) activity (Scheme 34).



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Scheme34. Synthesis of compounds **104a,b**.

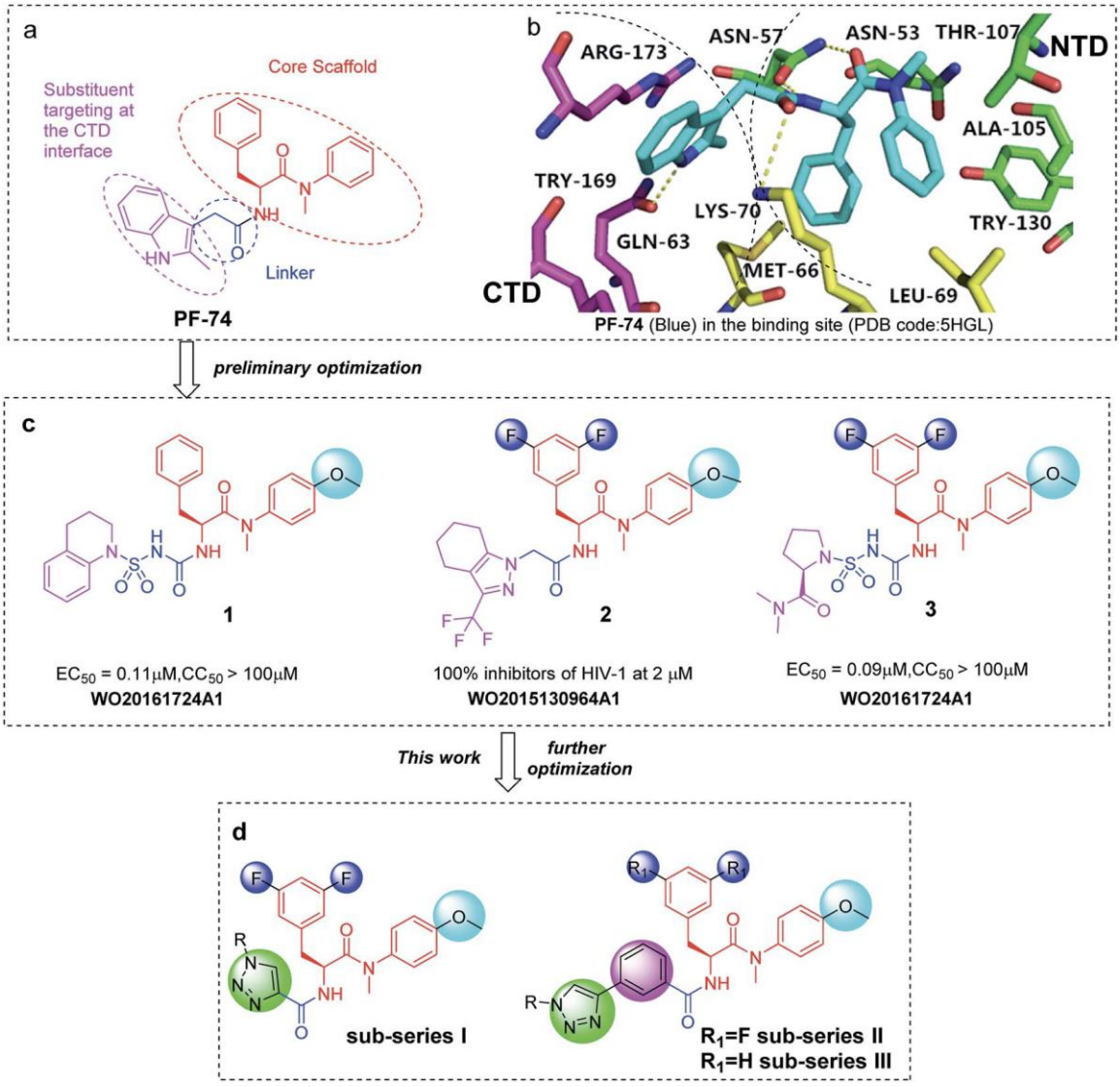

Figure 21. Design of novel phenylalanine derivatives as HIV-1 CA inhibitors: (a) Structure of PF-74; (b) the binding mode of PF-74 in the NTD-CTD interface of CA protein hexamer. Yellow dashed lines indicate H-bond interactions. (c) Phenylalanine derivatives as HIV-1 CA protein inhibitors. (d) Designed target compounds (Reproduced with permission.[119] Copyright 2019, RSC).

Compound **104a** expressed the highest activity (EC_{50} = 5.74 μ) with no cytotoxic effect. The analogue **104b** expressed anti-VZV activity (EC_{50} =11.70 μ), albeit not highly specific, within the same order of magnitude as that of brivudine (EC_{50} =18.44 μ).[118]

The HIV-1 capsid protein plays a crucial role in both early and late stages of the viral life cycle. This fact has intrigued researchers to target it to develop anti-HIV drugs. Accordingly, the design, synthesis and biological evaluation of a series of novel phenylalanine derivatives as HIV-1 capsid-protein inhibitors has been developed using the CuAAC reaction.

Among this series of inhibitors, compound II of Figure 21 displayed a remarkable anti-HIV activity (EC_{50} = 2.13 μ, CC_{50} > 35.49 μ). Furthermore, surface plasmon resonance binding assays showed that compounds II and PF-74 have similar affinities to HIV-1 capsid monomer.

New investigations showed that the weak permeability and water solubility of representative compounds were probably the important factors that restricted their cell-based activity. Preliminary structure-activity relationships were inferred based on the activities of these compounds, and their known structure. The most promising new compound was studied with molecular dynamics simulation to determine the preferred interactions with the drug target.[119] The same authors pursued the investigation on HIV-1 since their efforts demonstrated HIV-1 capsid protein is a prospective therapeutic target for the development of new antivirals. The most extensively studied capsid inhibitor, PF-3450074 (PF-74, discovered by Pfizer), targets an inter-protomer pocket within the capsid hexamer. In a subsequent work the design, synthesis, and biological evaluation of a series of 4-phenyl-1*H*-1,2,3-triazole phenylalanine derivatives as HIV-1 inhibitors based on PF-74 scaffold was proposed. Most of the analogues demonstrated potent antiviral activities, among them, the anti-HIV-1 activity (EC_{50} = 3.13 μ) is particularly prominent.[120]

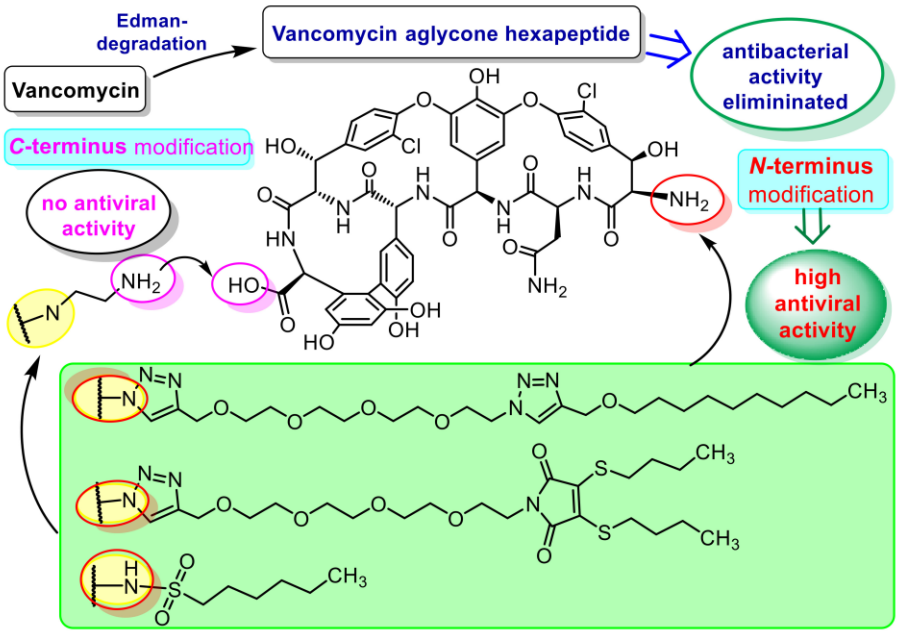

Figure 22. Synthetic strategy to the Vancomycin aglycone hexapeptide.

Since Influenza A and B viruses are a global threat to human health and increasing resistance to the existing antiviral drugs, new glycopeptide derivatives have emerged as a promising new class of antiviral agents. To avoid potential antibiotic resistance, these antiviral glycopeptides are preferably devoid of antibiotic activity. Six vancomycin aglycone hexapeptide derivatives were prepared (Figure 22) with the aim of

obtaining compounds having anti-influenza virus but no antibacterial activity. Two of them exerted strong and selective inhibition of influenza A and B virus replication, while antibacterial activity was success fully eliminated by removing the critical *N*-terminal moiety. In addition, these two molecules offered protection against several other viruses, such as herpes simplex virus, yellow fever virus, Zika virus, and human coronavirus, classifying these glycopeptides as broad antiviral molecules with a favorable therapeutic index.[121] Hybridization of two chemically diverse compounds into a new bioactive effector product is a successful concept to improve the properties of a hybrid drug relative to the parent compounds. (Iso)quinoline-artemisinin hybrids, obtained through copper-catalyzed azide-alkyne cycloaddition or metal-free click reactions (in organic solvents or in the presence of water), were analyzed *in vitro*, for the first time, to determine their inhibitory activity against human cytomegalovirus (HCMV), relative to their parent compounds and the reference drug ganciclovir (Figure 23).

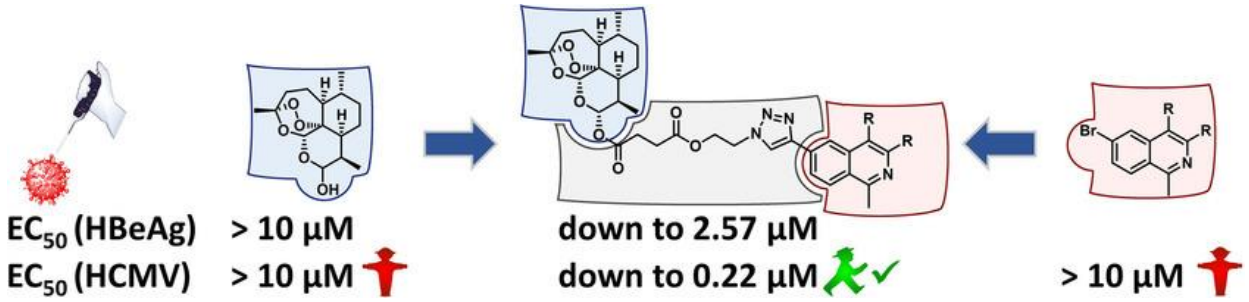

Figure 23. Isoquinoline derivatives structures and antiviral activities (Reproduced with permission.[122] Copyright 2020, Wiley).

EC_{50} (HCMV) values were obtained in a range 0.22-1.20 µ, which indicated highly potent antiviral properties in the absence of cytotoxic effects on normal cells (CC_{50} >100 µ). The most active hybrid, shown in Figure 23 (middle) (EC_{50} = 0.22 µ), is 25 times more potent than its parent compound artesunic acid (EC_{50} = 5.41 µ) and 12 times more efficient than the standard drug ganciclovir (EC_{50} = 2.6 µ). Interestingly, the hybrid at hand also shows inhibitory activity against hepatitis B virus in vitro (EC_{50} (HBeAg) = 2.57 µ).[122]

The highly conserved HIV-1 transactivation response element (TAR) binds to the trans-activator protein (Tat) and facilitates viral replication in its latent state. The inhibition of Tat-TAR interactions by selectively targeting TAR RNA has been used as a strategy to develop potent antiviral agents. Therefore, HIV-1 TAR RNA represents a paradigmatic system for therapeutic intervention. Biotin-tagged TAR RNA was used to assemble its own ligands from a pool of reactive azide and alkyne building blocks.

To identify the binding sites and selectivity of the ligands, the *in situ* cycloaddition has been further performed using control nucleotide (TAR DNA and TAR RNA without bulge) templates (Figure 24). The hit triazole-linked thiazole peptidomimetic products have been isolated from the biotin-tagged target templates using streptavidin beads. The major triazole lead generated by the TAR RNA presumably binds in the bulge region, shows specificity for TAR RNA over TAR DNA, and inhibits Tat-TAR interactions.[123]

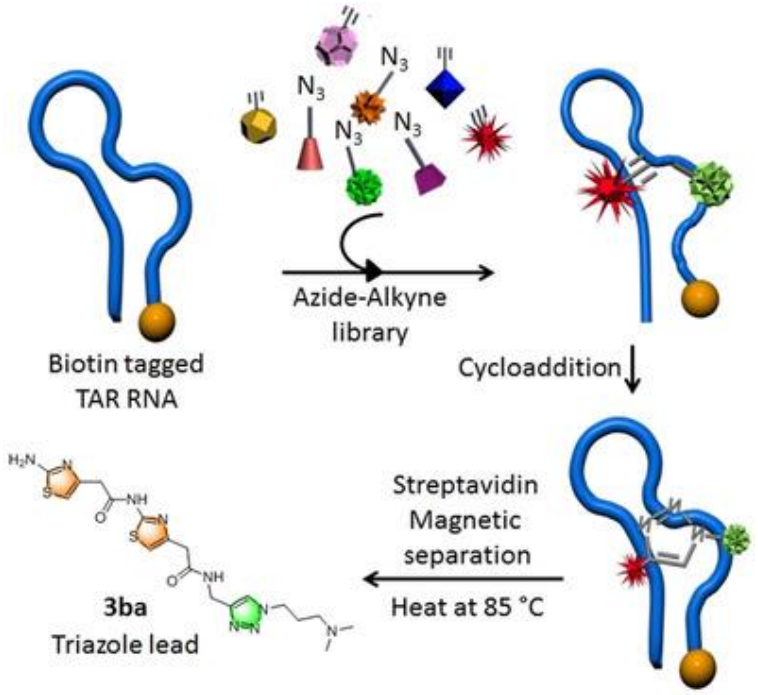

Figure 24. Identification of binding sites strategy (Reproduced with permission.[123] Copyright Year, Publisher).

The HIV-1 envelope gp120/gp41 glycoprotein complex plays a critical role in virus-host cell membrane fusion and has been a focus for the development of HIV fusion inhibitors. The synthesis of dimers of HIV fusion inhibitor peptides C37H6 and CP32M has been considered as a target to the trimeric gp41 in the pre-hairpin intermediate state, to inhibit membrane fusion. Reactive peptide modules were synthesized using native chemical ligation and then assembled into dimers with varying linker lengths using CuAAC click chemistry (Figure 25).

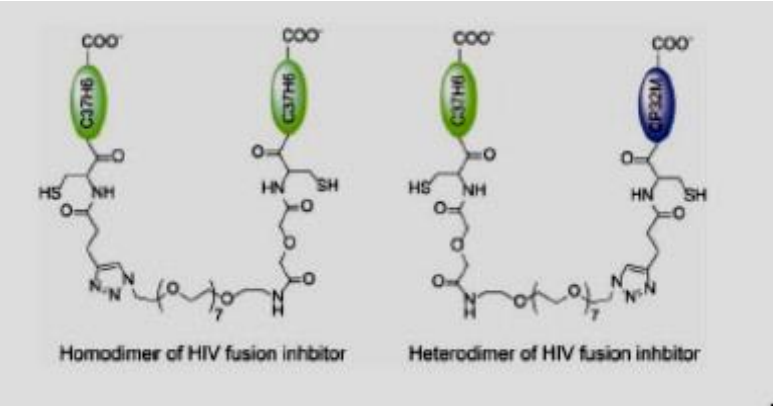

Figure 25. Structures of dimeric peptide derivatives (Reproduced with permission.[124] Copyright 2013, Elsevier).

Cell-cell fusion inhibition assays demonstrated that dimers with a (PEG)7 linker showed enhanced antiviral potency over the corresponding monomers. Moreover, the bio-orthogonal nature of the CuAAC reaction provides a practical way to assemble heterodimers of HIV fusion inhibitors. Heterodimers consisting of the T20-sensitive strain inhibitor C37H6 and the T20-resistant strain inhibitor CP32M were produced that may have broader spectrum activities against both T20-sensitive and T20-resistant strains.[124]

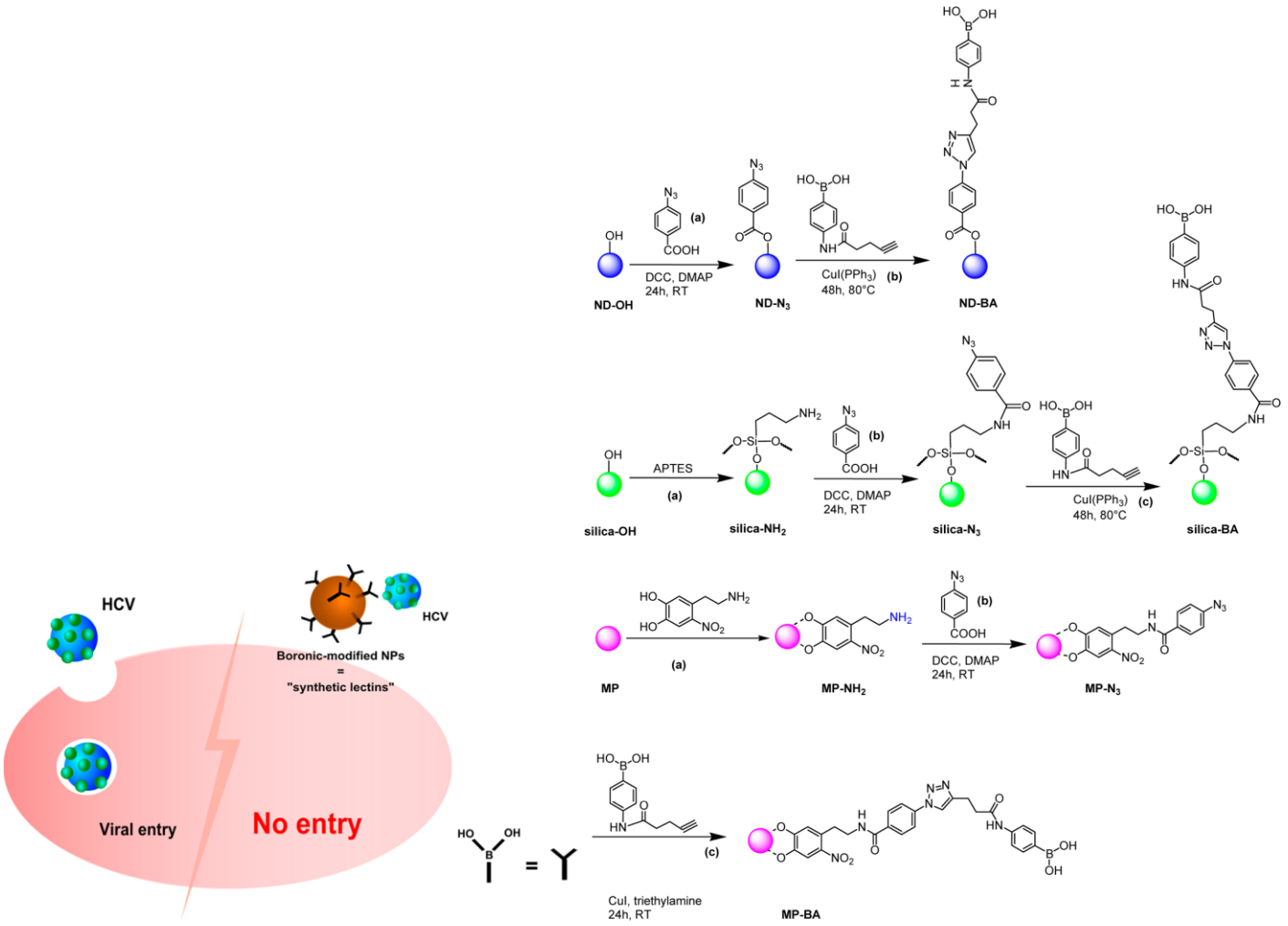

Figure 26. Mode of action of boronic-modified NPs (Reproduced with permission.[125] Copyright 2013, ACS).

To conclude, we wish to cite first the preparation of phenylboronic-acid-modified nanoparticles (NPs) for biological and biomedical applications. A convenient and general protocol for attaching multiple copies of para-substituted phenylboronic acid moieties onto either iron-oxide-, silica- or diamond-derived NPs has been developed through the functionalization of NPs fabricated by first modifying the surface of each particle type with 4-azidobenzoic ester functions. These azide-terminated nanostructures were then reacted with 4-[1-oxo-4-pentyn-1-yl) amino]phenylboronic acid units via a Cu(I) catalyzed Huisgen cycloaddition to furnish the corresponding boronic-acid modified NPs (or "borono-lectins", Figure 26).

The potential of these novel "borono-lectins" as antiviral inhibitors was investigated against the Hepatitis C virus (HCV) exploiting a bioassay that measures the potential of drugs to interfere with the ability of cell-culture-derived JFH1 virus particles to infect healthy hepatocytes. The novel viral entry activity demonstrated, and the fact that the described boronic-acid-functionalized NPs all display much reduced cellular toxicities compared with alternate NPs, sets the stage for the support of NP-derived borono-lectins as a potential therapeutic method for blocking viral entry of HCV.[125]

The ability of modified peptide triazole inhibitors to target HIV-1 gp120 and disrupt virus particles in the absence of host cells has been reported. Under conditions similar to those at which the peptide triazole KR13 inhibits HIV-1BaL pseudovirus infection of HOS.T4.R5 cells, they also cause release of HIV-1 gag p24 when incubated with virus alone. Both inhibition of cell infection and p24 release are enhanced substantially by

multivalent display of KR13 on gold nanoparticles. Virucidal function of the modified peptide triazoles argues for their potential use as microbicidal and therapeutic agents to suppress the progression and spread of HIV-1 infection. The results also suggest that ligand-specific pathogen rupture may be possible for other viruses, such as influenza, Ebola and Dengue, which contain metastable prefusion surface protein complexes.[126]

Conclusions

We wish to complete this first part dealing with azides by citing two reviews on azide applications to the synthesis of antiviral compounds. The synthesis of 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) 2'-deoxyribonucleosides, including β-D- and β-L-enantiomers, fluoro derivatives, and 2',3'-dideoxyribonucleosides has been reviewed by a German group. The work covers the various aspects of convergent nucleoside synthesis. Stereochemically defined α-D and α-L 2'-deoxyribonucleosides as well as sugar derivatives were prepared by nucleobase anion glycosylation. This glycosylation reaction is regioselective for the pyrrole nitrogen and stereoselective for β-nucleoside formation. Common glycosylation protocols lead to 7-deazapurine 2'-deoxyribonucleosides with unusual glycosylation sites. 7-Deazapurine 2',3'-dideoxyribonucleosides were also obtained from 2'-deoxy- or 3'-deoxyribonucleosides by Barton-McCombie deoxygenation, by elimination of sugar hydroxyl groups or by anion glycosylation. Furthermore the review covers the functionalization of pyrrolo[2,3-*d*]pyrimidine nucleosides. A broad range of reporter groups were introduced by the Sonogashira cross coupling or the copper(I)-catalyzed Huisgen-Meldal-Sharpless "click" reaction. The application of 7-deazapurine nucleosides as antiviral or anticancer agents, and the use of 7-deazapurine nucleoside triphosphates in the Sanger dideoxy DNA-sequencing are also reported.[127]

We have seen that the 1,2,3-triazole ring is a major pharmacophore system among nitrogen-containing heterocycles and that the five-membered heterocyclic motif with three nitrogen heteroatoms can be prepared easily using "click" chemistry with copper- or ruthenium-catalysed azide-alkyne cycloaddition reactions. The linker property of 1,2,3-triazoles was demonstrated, and a novel class of 1,2,3-triazole-containing hybrids and conjugates was synthesized and evaluated as lead compounds for diverse biological targets. These lead compounds have been demonstrated as anticancer, antimicrobial, anti-tubercular, antiviral, antidiabetic, antimalarial, anti-leishmanial, and neuroprotective agents.

The cited review summarizes advances in lead compounds of 1,2,3-triazole containing hybrids, conjugates, and their related heterocycles in medicinal chemistry published in 2018. This review will be useful to scientists in research fields of organic synthesis, medicinal chemistry, phytochemistry, and pharmacology.[128]

This review has been dedicated to azides and, not surprising, these 1,3-dipoles hold a double role in the chemistry ay hand. First, they are 1,3-dipoles allowing for the construction of triazole or even tetrazole rings when the dipolarophiles permits; these heterocyclic rings can occur in drug synthesis at different frequency. In fact, tetrazole is the 17th ring system found in small molecule drugs listed in the FDA Orange Book[129] while triazole is only the 98th. Second, azides are extremely popular as the 1,3-dipoles of election to perform "click chemistry" processes.[130] This can be a limitation in the use of these reactive compounds since they behave just "linkers" between two parts of a larger molecule and in some case they also behave as "innocent by-standers" from the biological point of view. We have described both the approaches trying to concentrate the attention to those cases where the role is primarily oriented to the enhancement of the biological activity of the molecules prepared according to these synthetic strategies.

Acknowledgements

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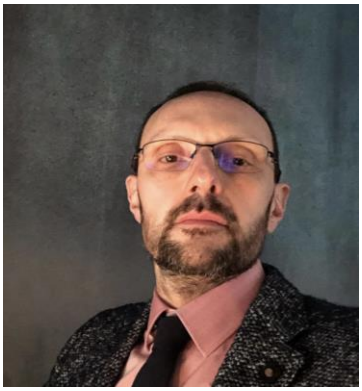

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