N°5 | Scientific Letter

EDITO

ATLANCHIM PHARMA
Quand la chimie rejoint la biologie

Voici maintenant deux ans que la reprise d'ATLANCHIM PHARMA a eu lieu et nous sommes heureux de constater que grâce aux efforts et à la confiance de tous, nous poursuivons notre développement, malgré un contexte économique difficile.

En effet, si 2009 a été l'année des investissements en ressources humaines et matérielles, 2010 est une année charnière dans la vie de l'entreprise, avec pour changement majeur le déménagement du laboratoire. Ainsi, depuis le 6 avril 2010, ATLANCHIM PHARMA a rejoint l'équipe d'ATLANTIC BONE SCREEN (société mère) en intégrant le site de la Faculté de Médecine et de Pharmacie de Nantes.

Ce déménagement nous permet de disposer aujourd'hui d'une surface doublée (500 m² au total dont 200 m² pour ATLANCHIM PHARMA) et d'un meilleur accès aux équipements de laboratoire. De plus, le rapprochement des deux sociétés améliore l'interactivité entre celles-ci, permettant ainsi d'offrir des services personnalisés complémentaires (chimie médicinale et validation biologique des molécules synthétisées).

Dans cette nouvelle édition de notre lettre scientifique, nous vous présenterons le portrait de notre Responsable Juridique et Social, celui de notre Responsable de Production (Docteur en chimie), ainsi que le portrait d'une Assistante Chef de Projet (Ingénieur en biologie). Vous aurez également le plaisir de découvrir l'article scientifique intitulé « Cu-Catalyzed Azide-Alkyne Cycloaddition: "The Click Reaction" », rédigé par l'un de nos Directeurs Scientifiques, le Pr. Jacques LEBRETON.

Toute l'équipe d'ATLANCHIM PHARMA se joint à moi pour vous souhaiter une bonne lecture de notre lettre scientifique.

Ronan LE BOT
Directeur

ATLANCHIM PHARMA
When chemistry meets up biology

It has now been two years that ATLANCHIM PHARMA has been taken over and we are pleased to notice that thanks to the efforts and trust of all, we carry on with our development, despite a difficult economic environment.

Indeed, after a staff increase and equipment investments in 2009, 2010 is a pivotal year in corporate life with for major change, the laboratory moving. Thus, since 6 April 2010, ATLANCHIM PHARMA joined the team of ATLANTIC BONE SCREEN (parent company), by integrating the site of the Faculty of Medicine and Pharmacy of Nantes.

We thus benefit today from a doubled surface (500 m² in total among which 200 m² for ATLANCHIM PHARMA) and easier access to laboratory equipment. In addition, the gathering of the two companies enhances interactivity between them, thereby providing complementary personalized services (medicinal chemistry and biological validation of the synthesized molecules).

In this new edition of our scientific newsletter, we will present the portrait of our Legal and Human Resources Manager, our Head of Production (Doctor in Chemistry) and the portrait of an Assistant Project Manager (Engineer in biology). You will also be pleased to discover the scientific paper entitled « Cu-Catalyzed Azide-Alkyne Cycloaddition: "The Click Reaction" », written by one of our Scientific Directors, Prof. Jacques LEBRETON.

ATLANCHIM PHARMA team joins me in wishing you a nice reading of our Scientific Letter.

Ronan LE BOT
CEO

SOMMAIRE / *SUMMARY*

Cu-Catalyzed Azide-Alkyne Cycloaddition : "The Click Reaction"

While by no means all-inclusive, this letter does provide an overview of very recent applications of Cu-catalyzed azide-alkyne cycloaddition (Cu-AAC) over the past three years, in particular in medicinal chemistry.

Since the introduction of a Cu(I) catalyst by the Meldal[1] and Sharpless[2] groups independently in 2002, the Huisgen[3] 1,3-dipolar cycloaddition reaction between azides and terminal alkynes has greatly improved in terms of regioselectivity and rate of reaction (see Scheme 1). The thermally induced 1,3-dipolar azide-alkyne cycloaddition generally results in an approximately 1:1 mixture of 1,4- and 1,5-triazole isomers. It is worth noting that the use of a ruthenium catalyst, such as $CpRuCl(PPh_3)_2$, instead of Cu(I) results in the formation of the 1,5-regioisomer. Moreover, with this metal the click reaction can be extended to internal alkynes. In the context of click chemistry, Sharpless has defined click reactions as those that: *"are modular, wide in scope, high yielding, create only inoffensive by-products (that can be removed without chromatography), are stereospecific, simple to perform and that require benign or easily removed solvent".* It is clear that Cu-AAC fulfills these criteria and is a "click reaction": simplicity and efficiency.

Pr. Jacques LEBRETON
Scientific Director
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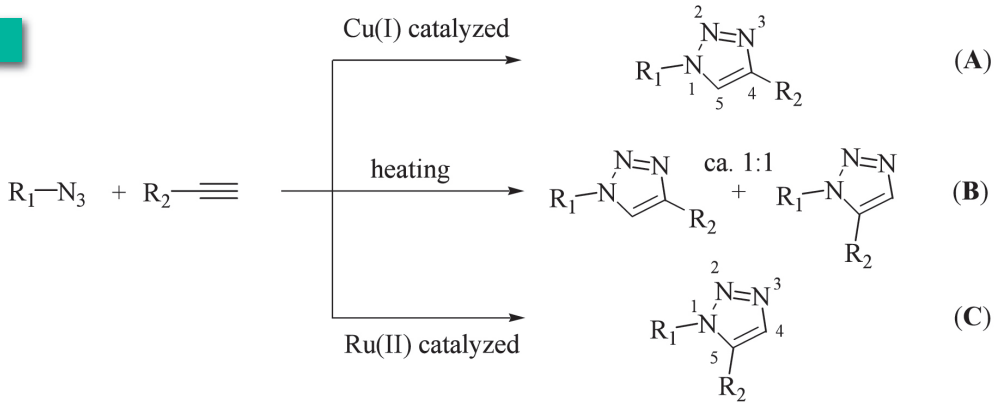
Scheme 1



Cycloaddition reactions between azides and alkynes: **(A)** copper(I)-catalyzed 1,3-dipolar cycloaddition reaction, **(B)** standard thermal 1,3-dipolar cycloaddition reaction, **(C)** ruthenium(II)-catalyzed cycloaddition.

In the past five years, interest in the use of Cu-AAC has considerably increased, not only in the agrochemical and medicinal fields as an important tool for drug discovery, but also in biology and material science.[4] Adding to the efficiency of Cu-AAC, the 1,2,3-triazole is an important core found in numerous synthetic pharmacologically significant heterocycles with a wide spectrum of biological activities including antiviral[5], antibacterial[6], antifungal[7], and antitubercular[8] activities. It is important to point out that the aromatic stabilized 1,2,3-triazole nucleus is very stable, being inert under various reductive and oxidative as well as acidic and basic conditions.

[1] (a) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057-3064. (b) Tornøe, C.W.; Meldal, M. *Peptidotriazoles: Copper(I)-catalyzed 1,3-dipolar cycloadditions on solid-phase, Peptides 2001, Proc. Am. Pept. Symp.*; American Peptide Society and Kluwer Academic Publishers: San Diego, 2001; pp 263-264.
[2] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002,** *41*, 2596-2599.
[3] For original [3+ 2] cycloaddition, see: R. Huisgen, *Angew. Chem. Int. Ed.* **1963**, *2*, 565-632.
[4] *QSAR Comb. Sci.* **2007**, *26* (11) is a special issue on click chemistry and its applications.
[5] I. Chaiken and Coll., *J. Med. Chem.* **2008**, *51*, 2638-2647.
[6] (a) X. Huang, A. Zhang, D. Chen, Z. Jia, X. Li *Bioorg. Med. Chem. Lett.* **2010**, *20,* 2859-2863. (b) S. Bera, G. G. Zhanel, F. Schweizer *Bioorg. Med. Chem. Lett.* **2010**, *20,* 3031-3035 and cited literature.
[7] J. N. Sangshetti, D. B. Shinde *Bioorg. Med. Chem. Lett.* **2010**, *20*, 742-745 and cited literature.
[8] C. Gill, G. Jadhav, M. Shaikh, R. Kale, A. Ghawalkar, D. Nagargoje, M. Shiradkar *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6244-6247 and cited literature.

Moreover, 1,4-substituted 1,2,3-triazoles are bioisosteres of the amide bond, which mimic the atom placement and electronic properties but without the same susceptibility to enzymatic cleavage.[9] Although the 1,2,3-triazole moiety is regarded as an inert link, it can participate in the interaction with the receptors by π-stacking interactions and/or hydrogen bonding, as will be mentioned in some examples below.

For many biological applications, Cu-AAC is a powerful tool to functionalize macromolecules, viruses or cell surfaces with any kind of appropriate molecule such as fluorophores, chelates, radioisotopes and affinity tags.[10] In the field of materials, to combine various biomolecules with both biological and synthetic polymers, Cu-AAC offers an unprecedented and almost unlimited tool to prepare well-defined biohybrid polymers.[11] It is also possible to link together numerous biomolecules such as proteins, oligosaccharides, DNA and RNA. This covalent assembly between complex molecules and well-defined structures can be performed under physiological conditions. In addition, the fact that azides and terminal alkynes are extremely rare in living systems increases the chemoselectivity and therefore the applicability of this tool. Unfortunately, for some applications, the cytotoxicity of Cu(I) greatly hampers the potential of this method. Thus, to avoid copper contamination, alternative copper-free click reactions have been successfully developed using strained cyclooctyne derivatives, electron-deficient alkynes and substituted oxanorbornadiene derivatives (*vide infra*).

Azides[12] are easy to introduce as well as terminal alkynes mostly from aldehyde precursors with the Ohira-Bestmann[13] reagent. Azides are quite often used as precursors of amines and can be introduced by the nucleophilic displacement of a suitable nucleofuge by an azide ion or, less often, by conversion[14] of an existing amine by diazotransfer. Furthermore, azides are very resistant to many reaction conditions, although they are very sensitive to reduction.[15]

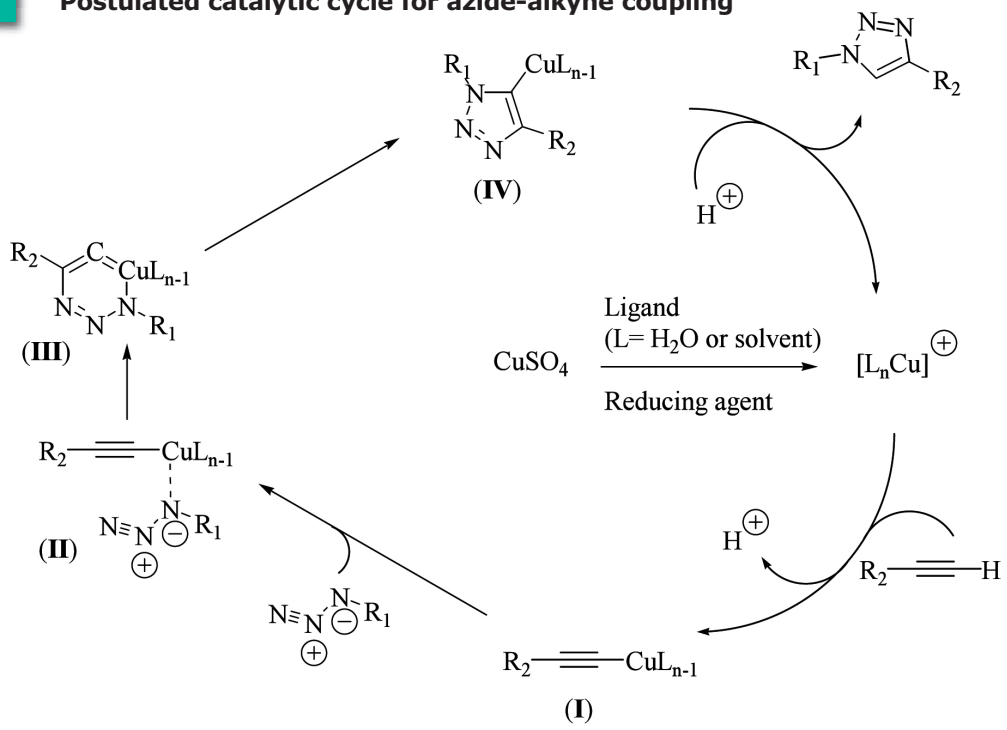
In general, Cu-AAC can be performed in various solvents, including water which is the ideal one, without special precautions over a wide temperature range. In addition, this amazing reaction is not only quite insensitive to pH (4 to 12) and tolerant of all functional and protecting groups tested so far but also the triazole derivatives are obtained in high yields without purification. It is worth mentioning that primary, secondary, and tertiary substituted azides as well as aromatic azides could be used. In general, Cu-AAC tolerated electron-withdrawing and electron-donating substituents placed on the azide and alkyne components. Electron depleted alkynes are the more reactive and can in some cases, react at ambient temperature without catalyst, as discussed in the last paragraph of this letter.

The postulated catalytic cycle for azide-alkyne coupling is outlined in Scheme 2.[16] At the initial stage, the reaction of an alkyne with Cu(I) affords the corresponding acetylide **I**. Density functional theory calculations ruled out the concerted [2+3] cycloaddition. A stepwise addition, starting from the copper(I) acetylide **I**, was proposed leading to the intriguing six-membered metallocycle intermediate **III**. By ring contraction, the latter intermediate leads to the triazolyl-copper derivative IV which, by protonolysis of the C-Cu bond, gives the desired 1,2,3-triazole adduct.

[9] B. H. M. Kuijpers, S. Groothuys, A. C. Soede, P. Laverman, O. C. Boerman, F. L. van Delft, F. P. J. T. Rutjes, *Bioconjugate Chem.* **2007**, *18*, 1847-1854.
[10] M. van Dijk, D. T. S. Rijkers, R. M. J. Liskamp, C. F. van Nostrum, W. E. Hennink, *Bioconjugate Chem.* **2009**, *20*, 2001-2016.
[11] M. G. Finn, V. V. Fokin, *Chem. Soc. Rev.* **2010**, *39*, 1231-1232.
[12] For an excellent review on azide chemistry and current developments, see: S. Bräse, C. Gil, K. Knepper, V. Zimmermann, *Angew. Chem. Int. Ed.* **2005**, *44*, 5188-5240.
[13] For a recent overview on the synthesis of the Ohira-Bestmann reagent, see: J. Pietruszka, A. Witt, *Synthesis* **2006**, 4266-4268.
[14] P. T. Nyffeler, C.-H. Liang, K. M. Koeller, C.-H. Wong, *J. Am. Chem. Soc.* **2002**, *124*, 10773-10778.
[15] For an overview on reduction of azides, see: J. Lebreton, *Scientific Letter AltanchimPharma,* **2008**, *1*, 2-11.
[16] For recent detailed mechanistic investigations, see: (a) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51-68. (b) C. Spiteri, J. E. Moses, *Angew. Chem. Int. Ed.* **2010**, *49*, 31-33. (c) B. R. Buckley, S. E. Dann, H. Heaney, *Chem. Eur. J.* **2010**, *16*, 6278-6284 and literature cited. (e) For experimental evidence concerning the formation of the cuprate-triazole intermediate **IV**, see: C. Nolte, P. Mayer, B. F. Straub, *Angew. Chem. Int. Ed.* **2007**, *46*, 2101-2103.

Scheme 2

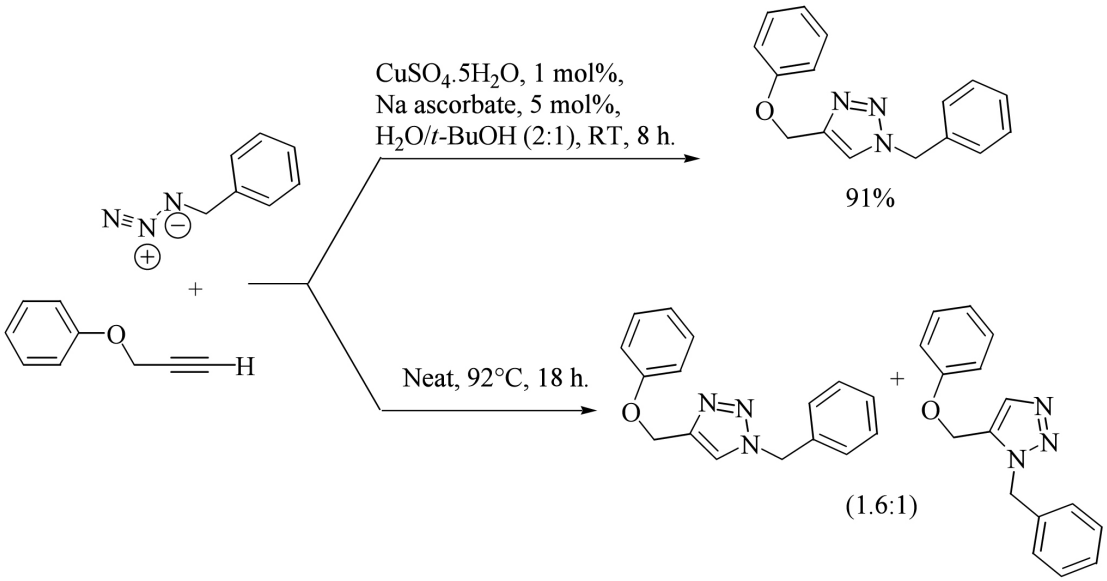
Postulated catalytic cycle for azide-alkyne coupling



As pointed out by Sharpless and Coll.[2] in their seminal publication, the comparison of the thermal reaction between benzyl azide and phenyl propargyl ether with the copper-catalyzed reaction of the same substrates demonstrates the importance of copper catalysis : dramatic rate acceleration and high regioselectivity. It is worthy to mention that, under microwave irradiation, reactions times were dramatically reduced from 12-24 h to less than 1 h.[17]

Scheme 3

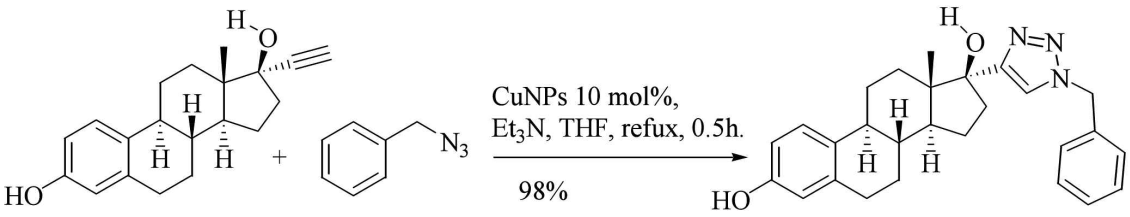
Thermal and Cu(I)-catalyzed 1,3-dipolar cycloadditions



[17] For a recent review on this topic, see: P. Appukkuttan, E. Van der Eycken, *Eur. J. Org. Chem.* **2008**, 1133-1155.

Screening of a wide range of Cu(I) sources using a variety of conditions has shown that aqueous conditions using $CuSO_4$ and sodium ascorbate[18], as reducing agent, provide the most efficient system allowing the formation of the desired 1,4-triazoles at room temperature in high yields with less than 5mol % catalyst loading. In these latter conditions, the catalytically active Cu(I) species are generated *in situ* by reduction of Cu(II) with ascorbate and immediately affords the key Cu-acetylide intermediates. It was also noticed that ascorbate prevents formation of the oxidative coupling products that are observed when Cu(I) is used directly. Various copper(I) salts are also used in the absence of a reducing agent. However, one equivalent of a nitrogen base (*N,N*-diisopropylethylamine (DIPEA) and Et_3N are commonly used) should be added and acetonitrile is needed as co-solvent. The base prevents the disproportionate oxidation of Cu(I) by coordinating the copper ion, and also accelerates the formation of the key Cu(I)-acetylene intermediate. In this case, the absence of oxygen also improves yields and purity. Very recently, in a nice study, Alonso, Yus and Coll.[19] used unsupported copper nanoparticles (CuNPs) instead of metal turnings or powder to reduce both the catalyst loading and the reaction times. The catalyst was prepared from $CuCl_2 \cdot 2H_2O$, lithium metal and a catalytic amount of 4,4'-di-*tert*-butylbiphenyl in THF. This procedure was found to be more efficient than that developed by Rieke. One representative example with the 17 α-ethynyloestradiol is outlined in Scheme 4.

Scheme 4

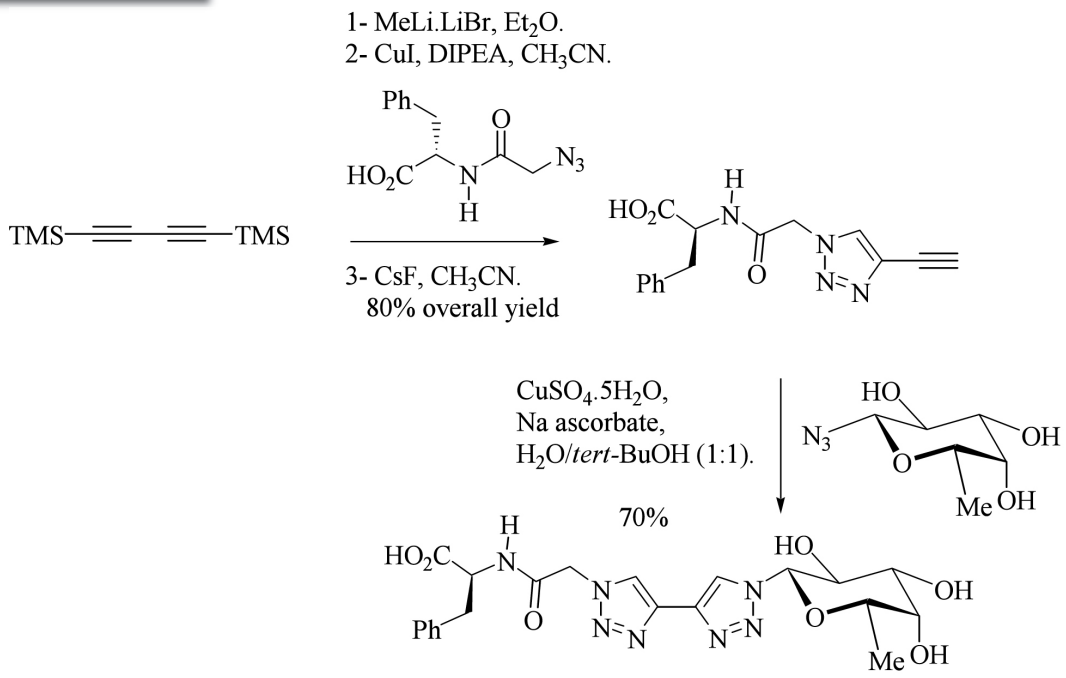


Applications of Cu-AAC in organic synthesis

In order to perform Cu-AAC selectively on diyne substrates, one terminal alkyne is temporarily masked with trimethylsilyl (or another silyl) group that can be easily cleaved after the first Cu-AAC. This modular approach, the so-called "double-click" strategy (or "click à la carte"[20]), is illustrated in Scheme 5 through the work of Aizpurua and Coll.[21] to prepare nonsymmetrical bis(1,2,3-triazole) derivatives. The last step of this latter sequence highlights the chemioselectivity of the triazole formation with CuAAC: both parents azide and alkyne are unprotected. In addition to this work, the same authors developed a complementary approach from the readily available 4-hydroxymethyl-1,2,3-triazole derivatives followed by a Swern oxidation/Ohira-Bestman alkynylation/Cu-AAC sequence.

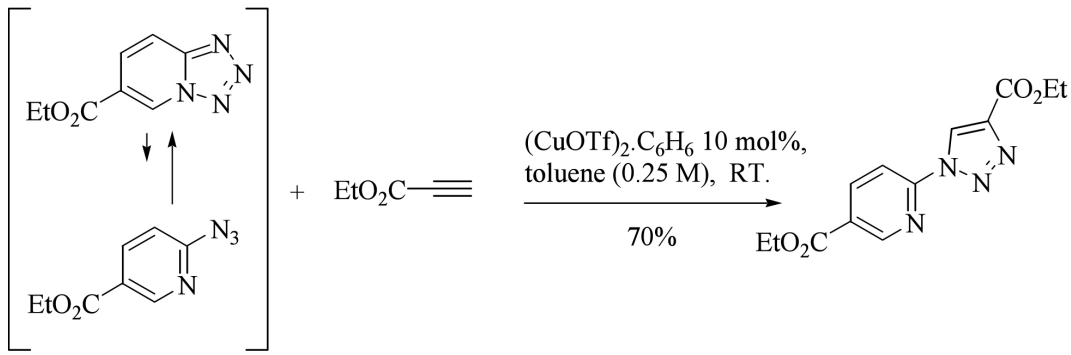
[18] For a recent overview concerning the use of (L)-ascorbic acid in organic synthesis, see: B. Füger, *Synlett* **2009**, 848-849.
[19] F. Alonso, Y. Moglie, G. Radivoy, M. Yus, *Eur. J. Org. Chem.* **2010**, 2395-2405.
[20] For another elegant work based on this strategy, see: (a) I. E. Valverde, A F. Delmas, V. Aucagne, *Tetrahedron* **2009**, 65, 7597-7602. (b) J. D. Megiatto, D. I. Schuster, S. Abwandner, G. de Miguel, D. M. Guldi, *J. Am. Chem. Soc.* **2010**, *132*, 3847-3861.
[21] J. M. Aizpurua, I. Azcune, R. M. Fratila, E. Balentova, M. Sagartzazu-Aizpurua, J. I. Miranda, *Org. Lett.* **2010**, *12*, 1584-1587.

Scheme 5



Very recently, Gevorgyan and Coll.[22] have shown that various fused tetrazole derivatives (pyrido-, quinolino-, pyrazino-, and quinoxalinotetrazoles) could serve as masked azides in a Cu-AAC, as outlined in Scheme 6. Around 50 examples are presented and $(CuOTf)_2.C_6H_6$ was found to be the best source of Cu(I). It is important to point out that the present methodology permits to generate *in situ* the 2-azidopyridine derivatives, which exist in equilibrium between the azide and the tetrazole forms. Surprisingly enough, both forms are inert under standard Cu-AAC conditions.

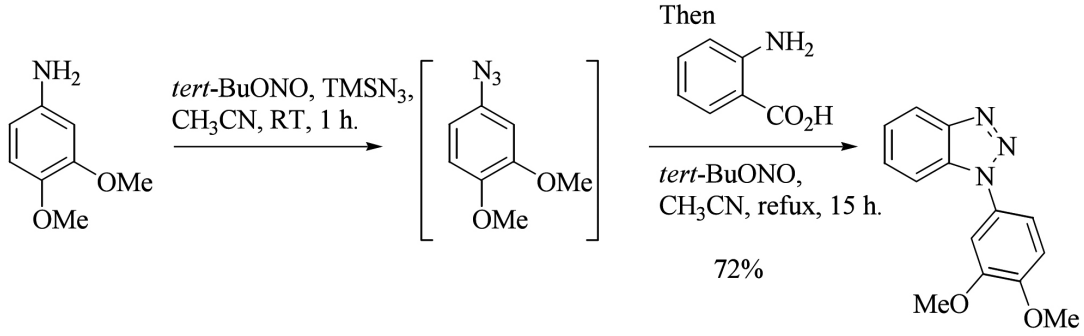
Scheme 6



An elegant one-pot synthesis of various substituted benzotriazoles has been developed by Zhang and Moses[23] based on catalyst-free Huisgen 1,3-dipolar cycloaddition. Both aromatic azides and benzyne[24] intermediates are generated sequentially with tert-butyl nitrite from amines and anthranilic acid derivatives, respectively (see Scheme 7). Anthranilic acid is a precursor of benzyne by conversion *in situ* to the corresponding diazonium salt, which spontaneously loses CO_2 and N_2. Moreover, benzyne, as all other strained internal alkynes, readily reacts with azides without Cu(I)-catalyze.

[22] B. Chattopadhyay, C. I. Rivera Vera, S. Chuprakov, V. Gevorgyan, *Org. Lett.* **2010**, *12*, 1584-1587.
[23] F. Zhang, J. E. Moses, *Org. Lett.* **2009**, *11*, 1587-1590.
[24] For a review on benzyne chemistry, see: H. Pellissier, M. Santelli, *Tetrahedron* **2003**, *59*, 701-730.

Scheme 7

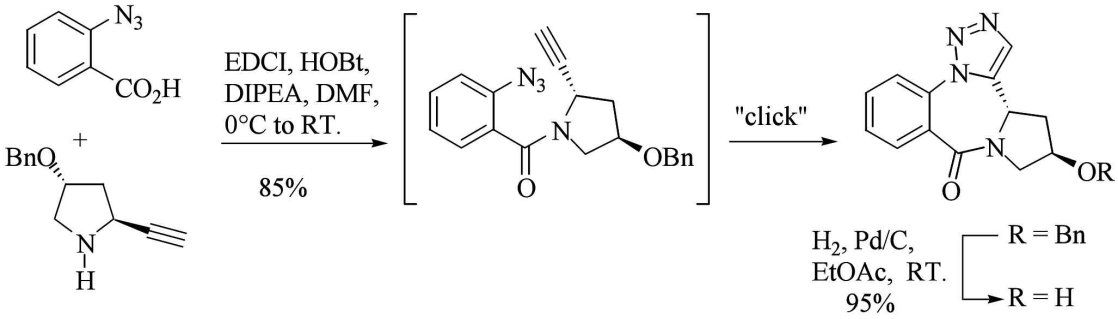


Applications of Cu-AAC in medicinal chemistry

The versatility of Cu-AAC to generate a triazole nucleus has been utilized for various aspects of drug discovery and some relevant applications are outlined in this part.

The tetrahydro-1,4-benzodiazepine scaffold, a rigid heterocycle based structure, is an important class of prototypical "privileged" structures associated with a wide range of biological activities and found in various marketed drugs such as diazepam (Valium®). A one-pot synthesis based on an intramolecular 1,3-dipolar cycloaddition without catalyst was published by Mohapatra and Coll.[25] and provides an efficient entry to new chiral tetracyclic triazole fused benzodiazepine derivatives, as illustrated in Scheme 8. It is important to pointed out that this intramolecular cycloaddition afforded the corresponding 1,5-triazole isomer.

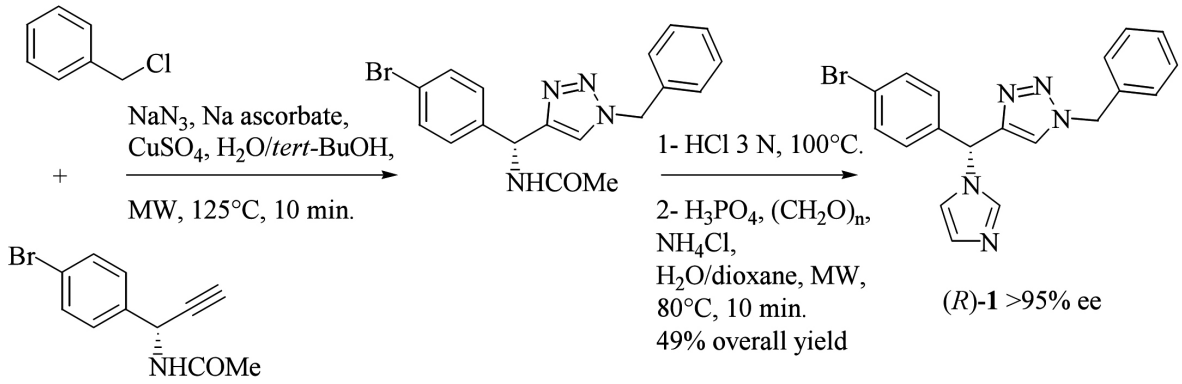
Scheme 8

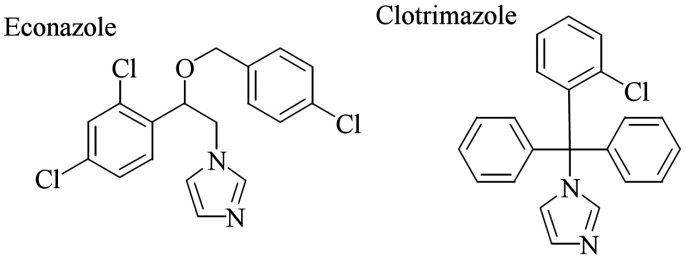


In the context of the development of new therapeutic agents against tuberculosis, new azole derivatives with a triazole motif have been prepared using click chemistry and evaluated by Botta and Coll.[26] (see Scheme 9). The key click reaction was carried out under microwave irradiation in a one-pot sequence from benzyl chloride in the presence of sodium azide to form the desired partner in situ. The enantiomerically pure compound (*R*)-**1** exhibited a similar biological profile against *Mycobacterium tuberculosis* (the main human pathogenic mycobacteria responsible for tuberculosis) comparable to the azole antifungal drugs econazole and clotrimazole.

[25] D. K. Mohapatra, P. K. Maity, M. Shabab, M.I. Khan, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5241-5245.
[26] D. Castagnolo, M. Radi, F. Dessì, F. Manetti, M. Saddi, R. Meleddu, A. De Logu, M. Botta, *Bioorg. Med. Chem. Lett.* **2009**, *19, 2203-2205.*

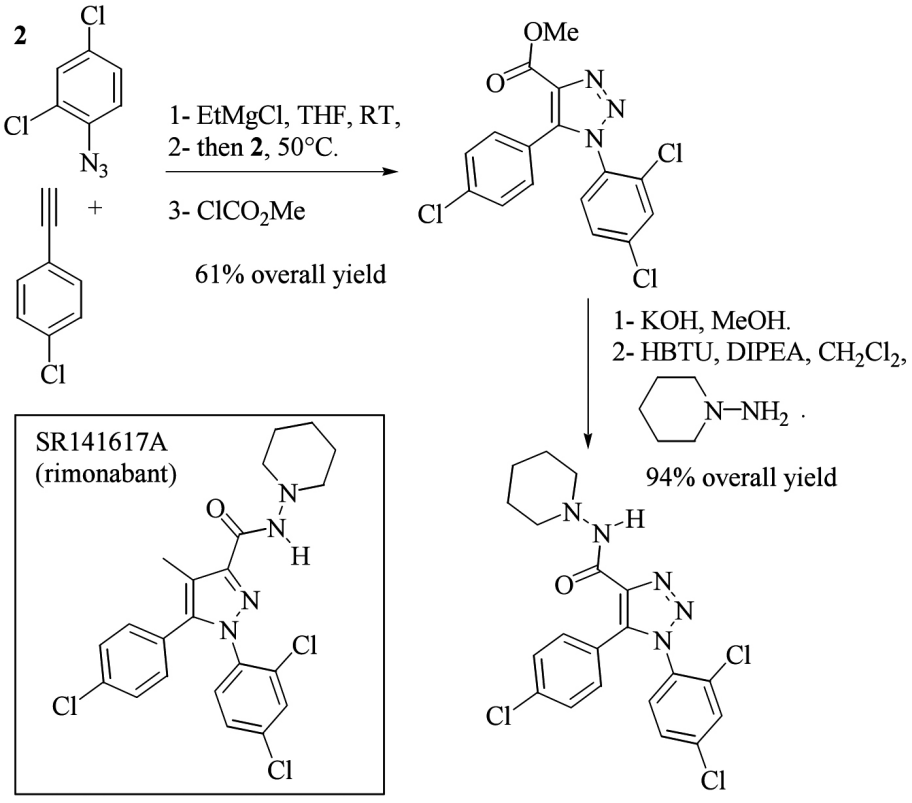
Scheme 9





Cannabinoid type 1 receptor antagonists such as SR141617A (rimonabant) represent a novel approach to treat obesity and related metabolic disorders. This type of receptor also has a pivotal role in the regulation of various psychological functions; therefore it is not surprising that active research is ongoing to find new potent and selective ligands. In this way, a set of analogues of SR141617A[27] has been efficiently prepared thanks to the condensation of a magnesium acetylide[28] with an azide to afford the corresponding 1,5-disubstituted-4-magnesio-1,2,3-triazole intermediate which was trapped with methyl chloroformate as electrophile (see Scheme 10). This methodology provided an efficient access to 1,4,5-trisubstituted-1,2,3-triazoles in complementary way to the Cu-AAC version.

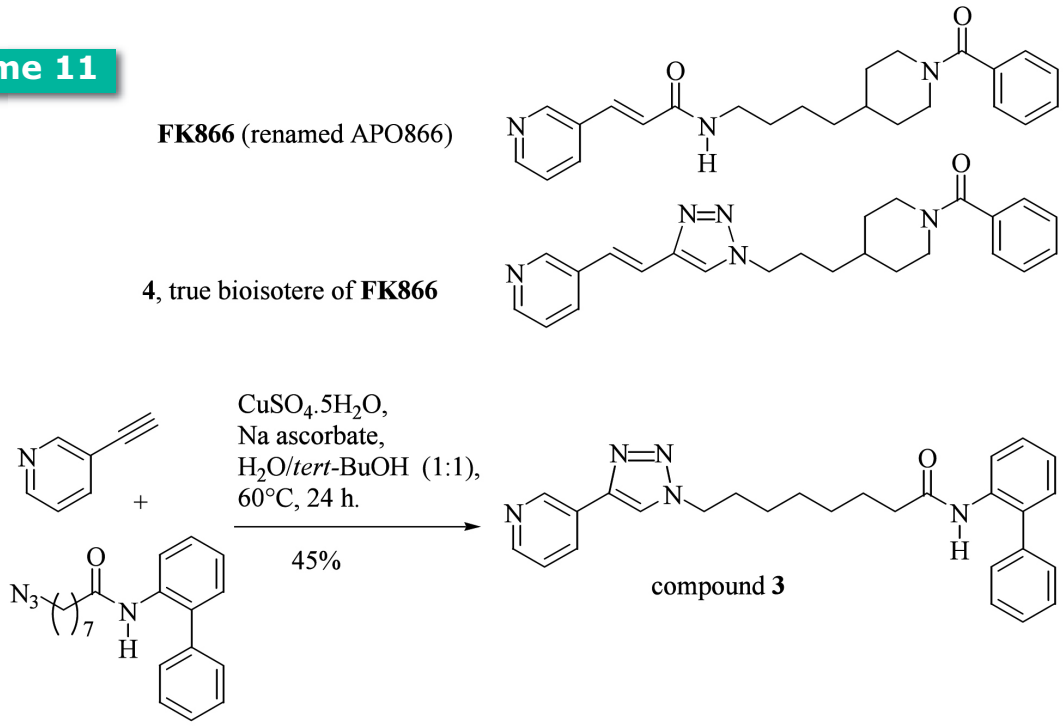
Scheme 10



[27] H. Shu, S. Izenwasser, D. Wade, E. D. Stevens, M. L. Trudell, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 891-893.
[28] A. Krasinski, V. V. Fokin, K. B. Sharpless, *Org. Lett.* **2004**, *6*, 1237-1240.

Inhibition of nicotinamide phosphoribosyltransferase (NMPRTase), which has a crucial role in the salvage pathway of nicotinamide adenine dinucleotide (NAD^+) biosynthesis, could reduce cellular NAD^+ levels and induce apoptosis in tumors and, therefore, presents a novel target for anticancer agents. Using a Cu-AAC, Tron and Coll.[29] have reported the design and synthesis of a library containing 186 analogues of **FK866** (now renamed **APO866**), an anticancer drug candidate that has recently entered phase II clinical trials. Scheme 11 illustrates the synthesis of the more active compound **3**, which has a similar mechanism of action compared to **FK866**. Surprisingly, compound **4**, the true bioisostere of **FK866**, was found to be less active than **FK866**, from nanomolar range instead of micromolar range.

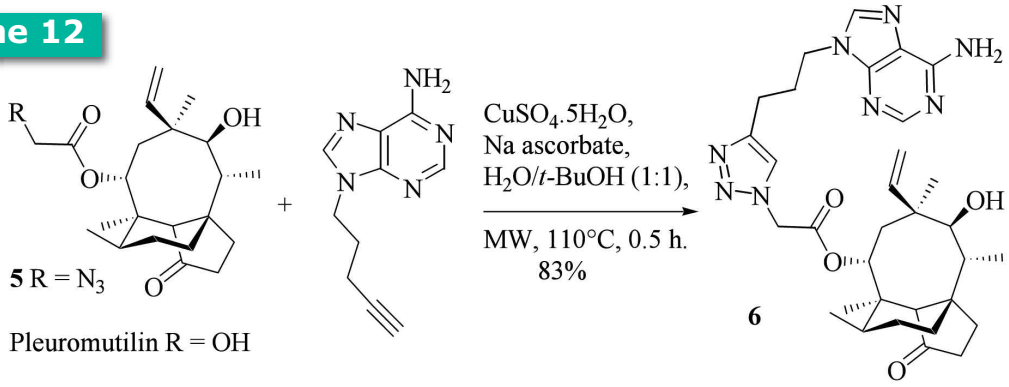
Scheme 11



Recent investigations into the nature of the interaction between pleuromutilin antibiotics and the peptidyl transferase active site of the ribosome were a starting point for the design of conjugated derivatives with nucleosides or acyclic nucleosides. It was postulated that the introduction of a moiety containing a nucleobase using a Cu-AAC could increase not only the binding to the target region by inherent H-bonding properties as well as stacking properties, but also water solubility. The overall pharmacological data show that all the pleuromutilin derivatives synthesized (around 20 compounds with a triazole linker) bind, in most cases, the targeted region of the peptidyl transferase center in the ribosome with higher affinity, in most cases, compared to the natural pleuromutilin antibiotic. This trend is markedly reinforced with the adenine-pleuromutilin derivative **6** (see Scheme 12) which presents the highest affinity and significantly better bonding properties.

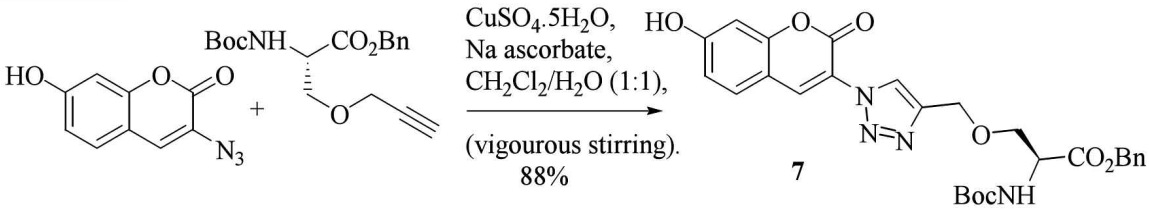
[29] G. Colombano, C. Travelli, U. Galli, A. Caldarelli, M. G. Chini, P. L. Canonico, G. Sorba, G. Bifulco, G. C. Tron, A. A. Genazzani, *J. Med. Chem.* **2010**, *53*, 616-623.

Scheme 12



A set of new amino ester derivatives containing different fluorophores in their side-chain, such as fluorescein, dansyl, 4-nitro-2,1,3-benzoxadiazole, coumarin and benzothiadiazole, has been prepared based on a Cu-AAC by Deprez and Xie.[30] All these new fluorescent amino esters presented spectroscopic properties similar to their native fluorophores, with high quantum yields and fluorescence in the visible region (420–520 nm). The preparation of these fluorescent labeling of biomolecules is illustrated through the coumarin-substituted amino ester **7** (see Scheme 13), which appeared to be very sensitive to the solvent proticity and pH value, affording a potential pH-sensitive amino acid for probing the local pH in cell compartments.

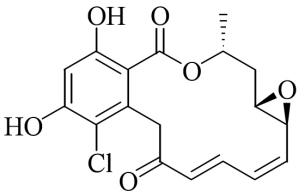
Scheme 13



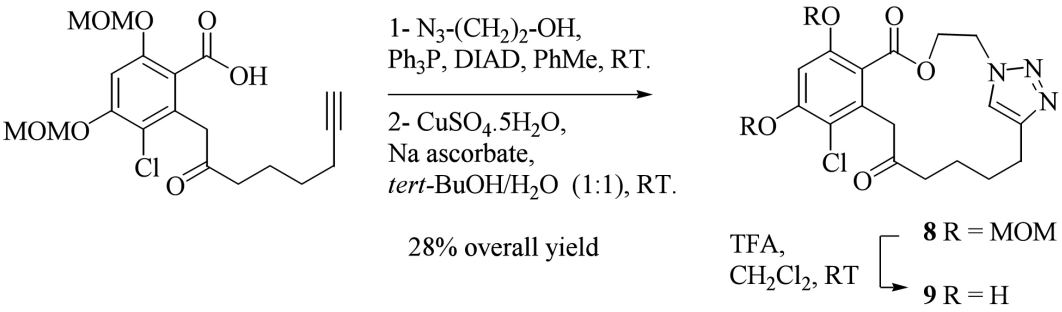
The molecular chaperone, Heat shock protein 90 (Hsp90), is capable of binding and hydrolyzing ATP in a deep pocket region in its *N*-terminal domain. Radicicol, a macrocyclic anti-fungal antibiotic, is able to inactivate this essential molecular chaperone by occupying this nucleotide binding site. This important cellular protein in eukaryotic cells has become a promising anticancer drug target. Various analogues of radicicol have been prepared and evaluated as potential selective Hsp90 inhibitors and several of them are currently in clinical trial.[31] A set of analogues has been recently obtained by Moody and Coll.[32] using "click chemistry" as outlined in Scheme 14 with the preparation of triazole-macrocyclic compound 9, which was found to be about 600 times less potent than radicicol itself.

[30] C. Li, E. Henry, N. Kumar Mani,. J. Tang, J.-C. Brochon, E. Deprez, J. Xie, *Eur. J. Org. Chem.* 2010, 2395-2405.
[31] C. Prodromou, J. M. Nuttall, S. H. Millson, S. M. Roe, T. S. Sim, D. Tan, P. Workman, L. H. Pearl, P. W. Piper, *ACS Chem. Biol.* **2009**, *4*, 289-297.
[32] J. E. H. Day, S. Y. Sharp, M. G. Rowlands, W. Aherne, P. Workman, C. J. Moody, *Chem. Eur. J.* **2010**, *16*, 2758-2763.

Scheme 14

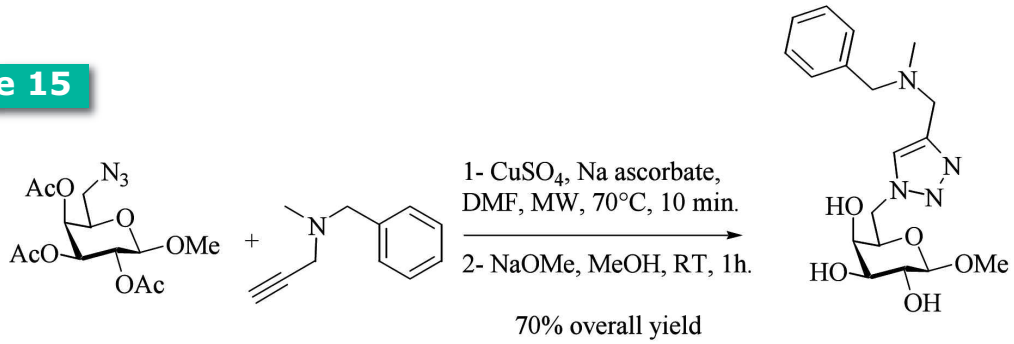


Radicicol



Trypanosoma cruzi, the protozoan responsible for Chagas disease, expresses on its surface a *trans*-sialidase which is able to transfer sialic acid residues from host glycoconjugates to its surface. Inhibition of this enzyme has emerged as a promising drug target to develop novel, effective and selective drugs. Moreover, previous investigations suggested that galactose could be an excellent scaffold. Based on this assessment, a small library of galactoses, modified at either the C-1 or C-6 positions with 1,4-disubstituted 1,2,3-triazole moieties, was prepared as exemplified through the best candidate from the 46 triazole derivatives obtained (see Scheme 15).[33] This compound exhibited a moderate *trans*-sialidase inhibitor activity: 50% of parasites killed at around 200 μM.

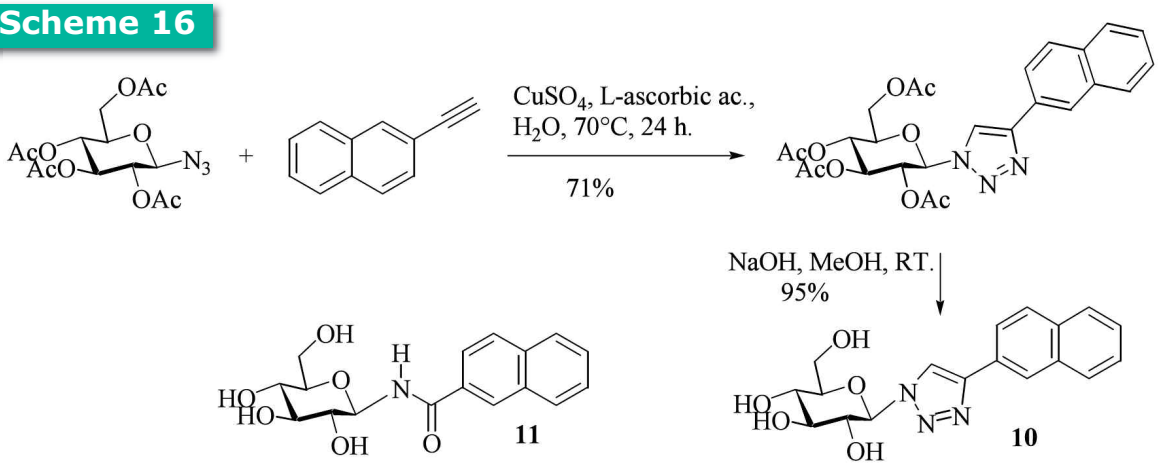
Scheme 15



In previous work, the Somsák[34] group published the preparation of an *N*-(β-D-glucopyranosyl) 3-(2-naphthyl)-propenoic amide as an inhibitor of glycogen phosphorylase, a key target for new therapies in the treatment of type 2 diabetes mellitus. Based on the evidence that 1,4 disubstituted 1,2,3-triazole rings are effective bioisosteres for *cis*-amide bonds in peptidomimetics, they prepared the 1-(D-glucopyranosyl)-1,2,3-triazole analogue 10 (see Scheme 16), which presented remarkably similar inhibition properties compared to its amide parent 11.[35]

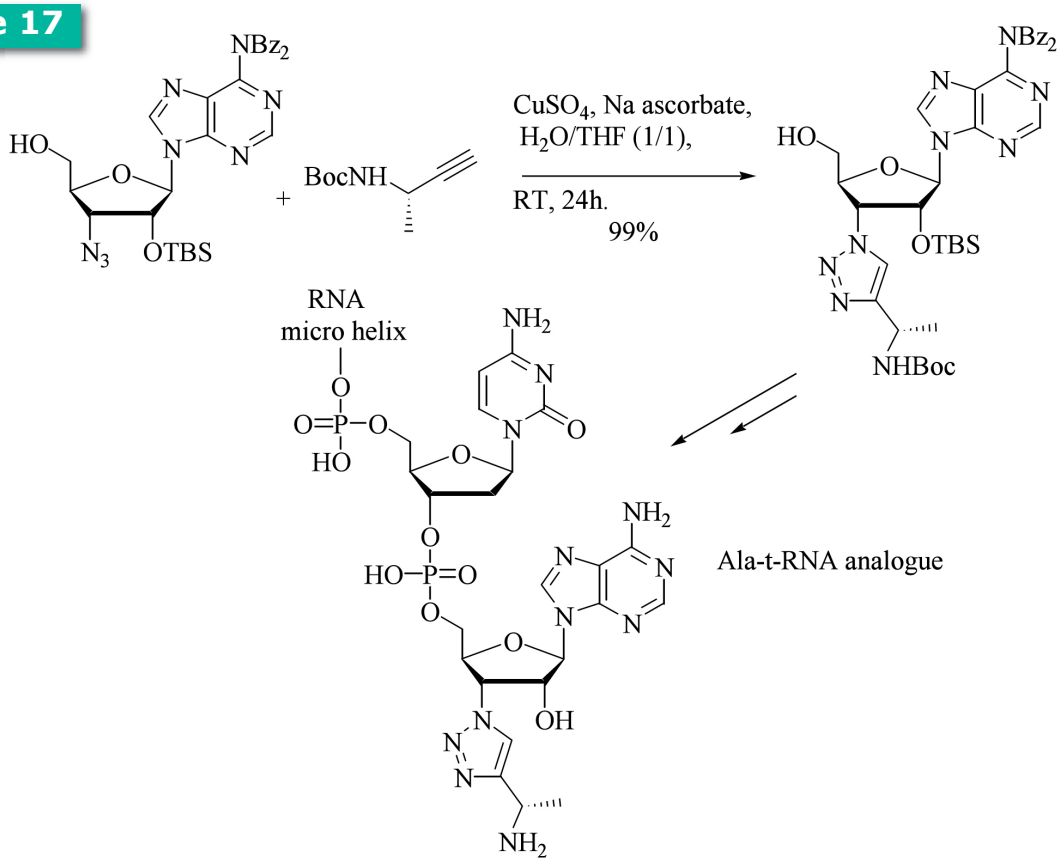
[33] I. Carvalho, P. Andrade, V. L. Campo, P. M. M. Guedes, R. Sesti-Costa, J. S. Silva, S. Schenkman, S. Dedola, L. Hill, M. Rejzek, S. A. Nepogodiev, R. A. Field, *Bioorg. Med. Chem.* **2010**, *18*, 2412-2427.
[34] Z. Györgydeák, Z. Hadady, N. Felföldi, A. Krakomperger, V. Nagy, M. Tóth, A. Brunyánszki, T. Docsa, P. Gergely, L. Somsák, *Bioorg. Med. Chem.* **2004**, *12*, 4861-4870.
[35] E. Bokor, T. Docsa, P. Gergely, L. Somsák, *Bioorg. Med. Chem.* **2010**, *18*, 1171-1180.

Scheme 16



In the field of nucleoside and nucleotide chemistry, Cu-AAC has provided attractive possibilities for bioconjugation reactions and the synthesis of various analogues. Etheve-Quelquejeu and Coll.[36] have synthesized an aminoacyl-tRNA analogue containing a triazole linkage instead of an ester function as depicted in Scheme 17. It is worth mentioning that the same sequence using the $CpRuCl(PPh_3)_2$ catalyst in refluxing benzene on a 6-N-benzoyl adenosine derivative gave the 1,5-triazole linkage in modest yield (37%). After incorporation into aminoacyl-tRNAs, the analogue containing the 1,4-triazole core was found to be slightly more active compared to the 1,5-triazole analogue.

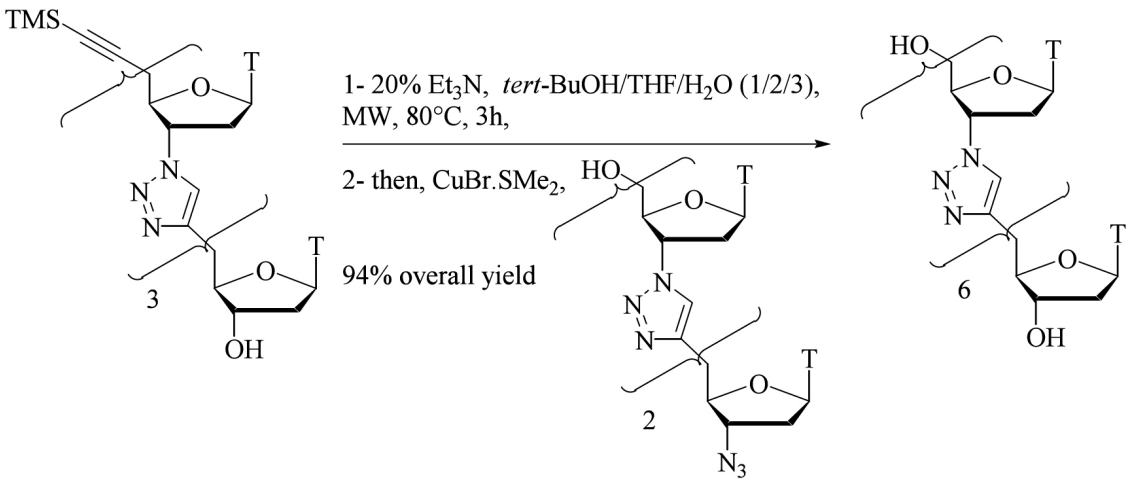
Scheme 17



[36] M. Chemama, M. Fonvielle, M. Arthur, J.-M. Valéry, M. Etheve-Quelquejeu, *Chem. Eur. J.* **2009**, *15*, 1929-1938.

Oligonucleotides, modified to form stable duplexes for antisense[37] or RNA interference[38] approaches, have now been used for many years to alter the expression of specific genes both *in vivo* and *in vitro*. Although replacement of the phosphodiester bond by other internucleoside linkages greatly increases resistance to nuclease degradation, destabilization of the corresponding duplexes with DNA and/or RNA is unfortunately also induced in many cases. Isobe and Coll.[39] have described a convergent route for the solution-phase synthesis of fully modified 7-mer and 8-mer triazole-linked DNA strands using a one-pot procedure for desilylation of masked acetylene followed by a Cu-AAC as depicted in Scheme 18. No data on duplex formation ability are yet available for this type of modification.

Scheme 18

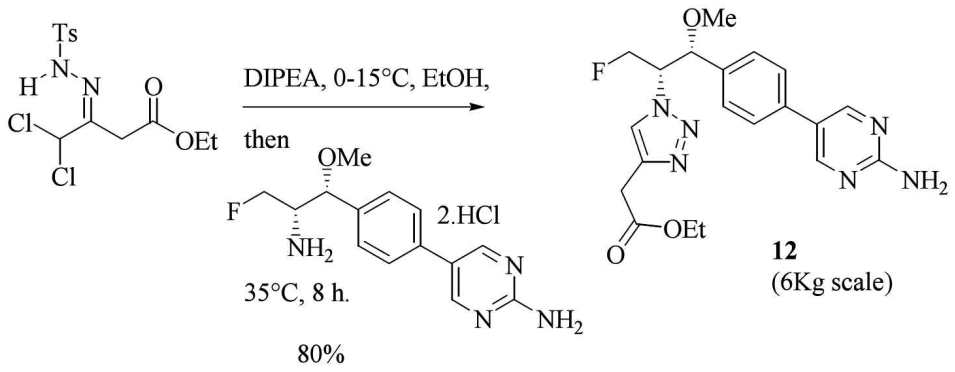


Although the preparation of azide compounds is well documented in the literature and ensures rapid access to reasonable amounts of desired material, the thermal instability associated with this class of derivatives remains a hindrance in large-scale preparations and has prompted some industrial researchers to investigate alternative approaches to Cu-AAC.[40]

In the course of the development of a scalable preparation of a new antibacterial macrolide, Hanselmann and Coll.[41] from Rib-X Pharmaceuticals synthesized the key triazole intermediate **12** using a safer alternative to Cu-AAC. They constructed the heterocyclic core of the compound **12** based on a previous method published by Sakai and Coll.[42] more than 20 years ago (see Scheme 19).

[37] T. Aboul-Fadl, *Curr. Med. Chem.* **2005**, *12*, 763-791.
[38] S. Shukla, C. S. Sumaria, P. I. Pradeepkumar, *ChemMedChem* **2010**, *5*, 328-349.
[39] T. Fujino, N. Yamazaki, H. Isobe, *Tetrahedron Lett.* **2009**, *50*, 4101-4103.
[40] For an interesting discussion on the explosive potential of organic azides and the "azidophobia", see: R. E. Conrow, W. D. Dean, *Org. Process Res. Dev.* **2008**, *12*, 1285-1286.
[41] R. Hanselmann, G. E. Job, G. Johnson, R. Lou, J. G. Martynow, M. M. Reeve, *Org. Process Res. Dev.* **2010**, *14*, 152-158.
[42] K. Sakai, N. Hida, K. Kondo, *Bull. Chem. Soc. Jpn.* **1986**, *59*, 179-183.

Scheme 19



Copper-free Cu-AAC

In order to circumvent the use of copper ions under physiological conditions,[43] Bertozzi and Coll.[44] have designed a strain-promoted [3+2] cycloaddition reaction that involves azides and a strained cyclooctyne derivative (**A**) (see Figure 1). Nevertheless, compared to Cu-AAC, this first generation of cyclooctynes suffers from low reactivity towards the azide partners. Fortunately, this group[45] has developed a second generation of difluorinated cyclooctynes (**B**) which presents similar reaction kinetics to Cu-AAC. In parallel with this work, the groups of Boons[46] and Rutjes[47] explored different strategies toward copper-free Cu-AAC with 4-dibenzocyclooctynol (**C**) and trifluoromethyl-substituted oxanorbornadiene (**D**) derivatives, respectively. Thus, in the context of copper-free Cu-AAC, the difluorinated cyclooctyne-based ligations appear to be an important tool in biology, even if they are rather difficult to synthesize. Furthermore, the low water solubility of this latter class of strain-alkynes has been improved with a new generation of hydrophilic azacyclooctyne probes (**E**) published by Bertozzi and Coll.[48]

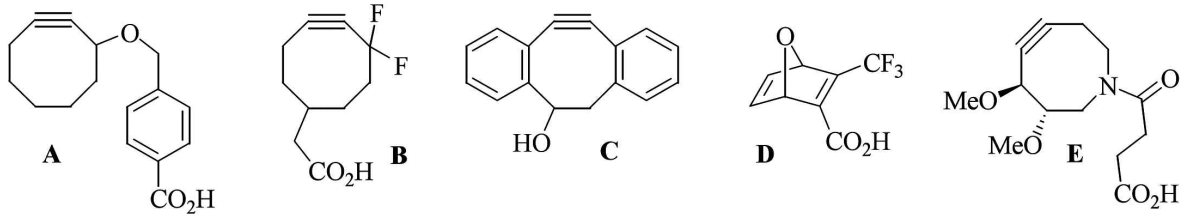


Figure 1. Reagents for labeling azido-containing biomolecules using copper-free Cu-AAC

[43] For an excellent discussion on copper-free Cu-AAC and perspectives, see: J.-F. Lutz, *Angew. Chem. Int. Ed.* **2008**, *47*, 2182-2184.
[44] N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046-15047.
[45] (a) J. A. Codelli, J. M. Baskin, N. J. Agard, C. R. Bertozzi, *J. Am. Chem. Soc.* **2008**, *130*, 11486-11493. (b) N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo, C. R. Bertozzi, *ACS Chem. Biol.* **2006**, *1*, 644-648.
[46] X. H. Ning, J. Guo, M. A. Wolfert, G. J. Boons, *Angew. Chem. Int. Ed.* **2008**, *47*, 2253-2255.
[47] S. S. van Berkel, A. J. Dirks, M. F. Debets, F. L. van Delft, J. J. L. M. Cornelissen, R. J. M. Nolte, F. P. J. T. Rutjes, *ChemBioChem* **2007**, *8*, 1504-1508.
[48] E. M. Sletten, C. R. Bertozzi, *Org. Lett.* **2008**, *10*, 3097-3099.

Outlook

In less than a decade, Cu-AAC has found a plethora of applications in various aspects of chemistry, biotechnology and material science, and this will continue in the future. We should also anticipate that the copper-free version of Cu-AAC, due to its compatibility with biological environments, could provide a powerful tool to study biological processes *in vitro* and *in vivo* in direct relevance to drug discovery.[49]

I would like to thank my colleagues, Dr. André Guingant, Dr. Fabrice Dénès and Dr. François-Xavier Felpin for fruitful discussions and invaluable comments.

[49] (a) T. Böttcher, S. A. Sieber, *J. Am. Chem. Soc.* **2010**, *132*, 6964-6972. (b) J. C. Jewett, E. M. Sletten, C. R. Bertozzi, *J. Am. Chem. Soc.* **2010**, *132*, 3688-3690.




Presentation of a chemistry biology team

Cécile CARYDIS est Responsable Juridique et Social pour le compte d'ATLANTIC BONE SCREEN et d'ATLANCHIM PHARMA depuis novembre 2009. Elle est titulaire d'un DEA en Droit de la Santé et d'une Maîtrise en Droit des Affaires. Après 6 ans passés dans l'Industrie Pharmaceutique et 2 ans à l'Université dans la cellule de Valorisation de la Recherche, Cécile CARYDIS a réintégré le secteur privé. Depuis elle s'occupe de la mise en place de la politique sociale, gère les ressources humaines et les aspects juridiques des deux sociétés.

Cecile CARYDIS *is Legal and Human Resources Manager at ATLANTIC BONE SCREEN and ATLANCHIM PHARMA since November 2009. She holds a postgraduate degree in Health Law and a Master in Business Law. After six years in Pharmaceutical Industry and 2 years at University in the office of technology transfer, Cecile CARYDIS came back to the private sector. Since then, she deals with the implementation of the social policy, manages human resources and legal aspects of both companies.*

Le **Dr. Bertrand COTTINEAU**, Responsable de Production, a intégré ATLANCHIM PHARMA en 2004. Titulaire d'un Doctorat en chimie fine avec une spécialisation en synthèse organique, il a réalisé un post-doctorat en Irlande et un post-doctorat en France. Le Dr. COTTINEAU synthétise et optimise des contrats industriels de recherche en synthèse à façon de molécules complexes et est également en charge de l'organisation de la production au sein du laboratoire.

Dr Bertrand COTTINEAU*, Head of Production, integrated ATLANCHIM PHARMA company in 2004. Holder of a Ph.D. in fine chemistry with a specialization in organic synthesis, he achieved one post-doctoral in Ireland and one post-doctoral in France. Dr. COTTINEAU synthesizes and optimizes industrial contracts of research in custom-chemical synthesis of complex molecules and he is also in charge of organizing Production in the laboratory.*

Tiphaine DAVID, Assistante Chef de Projet chez ATLANTIC BONE SCREEN depuis octobre 2007, est titulaire d'un Master 2 en Biologie et Pharmacologie du vieillissement et d'un BTS Biochimie et analyses biologiques. Après de nombreuses expériences dans des laboratoires de Recherche & Développement publics (INRA, ENVN, INSERM) et privés (NUTRINOV, VIVALIS), elle obtient le niveau I d'expérimentation animale. Au sein d'ATLANTIC BONE SCREEN, elle conçoit des plans et rapports d'études et réalise des protocoles en culture cellulaire, biologie moléculaire, histologie et expérimentation animale pour les études clients.

Tiphaine DAVID*, Assistant Project Manager at ATLANTIC BONE SCREEN since October 2007, has a postgraduate degree in Biology and Pharmacology of aging and a Biochemistry and biological analysis BTS. After several experiences in public Research & Development laboratories (INRA, Nantes Veterinary School, INSERM) and private companies (Nutrinova, VIVALIS), Tiphaine DAVID obtained the Level I of animal experiment. Within ATLANTIC BONE SCREEN, she designs study reports and study plans, and implements protocols in cell culture, molecular biology, histology and animal testing for customers' studies.*

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