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Alkyne-azide click reaction catalyzed by metallic copper under ultrasound

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This protocol is for the ultrasound-assisted 1,3-dipolar cycloaddition reaction of azides and alkynes using metallic copper as the catalyst. The azido group is a willing participant in this kind of organic reactions and its coupling with alkynes is substantially improved in the presence of Cu(I). The present protocol does not require additional ligands and proceeds with excellent yields. The copper-catalyzed azide-alkyne cycloaddition (CuAAC) is generally recognized as the most striking example of “click chemistry”. Reactions involving metals represent the favorite domain of sonochemistry because ultrasound favors mechanical depassivation and enhances both mass transfer and electron transfer from the metal to the organic acceptor. The reaction rate increases still further when simultaneous ultrasound and microwave irradiation are applied. The ultrasound-assisted click synthesis has been applied to the preparation of a wide range of 1,4-disubstituted 1,2,3-triazoles derivatives both from

small molecules and oligomers such as cyclodextrins. Using this efficient and greener protocol, all the adducts can be synthesized in 2-4 h (including work-up and excluding characterization). Click chemistry has been shown to be able to directly link chemistry to biology, becoming a true interdisciplinary reaction with extremely wide applicability.

[Green Chemistry; Click Reaction; Ultrasound; Sonochemistry; Microwaves.]

Ultrasound-assisted reactions: some experimental considerations

The origin of sonochemical effects lie in cavitation, a nonlinear phenomenon whose precise calculation involves some rather complicated mathematics. In a simplified picture, cavitation describes the rapid growth of microbubbles in a liquid when a large negative pressure (such as the acoustic pressure on the rarefaction cycle) is applied to it. In subsequent compression cycles these microcavities collapse violently and release enough kinetic energy to break chemical bonds. The accompanying shock waves and shear forces are also responsible of mechanical effects, thereby favoring especially heat and mass transfer in heterogeneous reactions. The widely-accepted hot-spot model suggests local temperatures exceeding 5000 K and pressures of several thousand atmospheres during collapse, with enormous cooling rates ($> 10^{10}$ K/s) as to consider cavitation a quasi-adiabatic process. Species trapped within the bubbles will preferably undergo excitation and homolytic cleavage, being delivered to the medium as radicals.

What must be borne in mind is that cavitation, and hence the actual ultrasonic energy entering the chemical system, depends largely upon the physical characteristics of the medium: temperature (i.e. vapor pressure), viscosity, and surface tension of the solvent. Since sonochemical efficiency is linked directly to the production of cavitation bubbles, dissolved gases will also have a major influence, with monoatomic gases as most effective (they have the highest ratios of specific heats).

Choosing a suitable ultrasonic system can be a puzzling question, although two major methods for transferring ultrasonic energy have been developed, ultrasonic baths and probes, both with both pluses and minuses. In general, probes (which combine a piezoelectric transducer and a sonic horn, a design originally developed for biochemistry as a cell disruptor) are highly recommended. In these systems the amount of ultrasonic power delivered by the horn is larger and directly related to the

magnitude of vibration of the tip. This does mean that precise and reproducible results can be achieved by controlling the power input to the transducer.

Choose a probe system according to the type of application as the primary concern. Numerous manufacturers now supply probe systems with variable power, pulse facilities, or flow cells for continuous processing, among other desirable features. If metal contamination is to be avoided, such as in bioassays or trace analysis, probes made of titanium alloy should be replaced by probes made of silica or polymeric materials which usually exhibit high chemical and temperature shock resistance.

Ultrasound intensity rapidly decreases both radially and axially from the probe. Accordingly, the space between the probe and the wall of the container must be kept to a minimum, while avoiding direct contact. Keeping dead zones to a minimum facilitates contact between the sample and the cavitation zones and can be attained by a judicious choice of the shape of the vessel.

Finally, accurate temperature control constitutes a major headache in sonochemical experiments. Any change in the reaction temperature as a result of prolonged sonication results in changes in cavitation and the optimum resonance frequency of the system. Cooling systems, commonly cells incorporating a water jacket, have been designed. Use of pulsed radiation also ensures dissipation of energy. An immediate strategy involves the use of an ice bath monitoring continuously the temperature within the vessel.

INTRODUCTION

Azide-alkyne coupling in a nutshell

The 1,3-dipolar cycloaddition of azides and alkynes has become the model for click reactions. This cycloaddition has been known for over 100 years and was studied extensively by Huisgen and co-workers in the 1960s¹. However, the conventional thermal protocol is often sluggish and usually results in a mixture of two regioisomeric triazoles². The resurgence of recent interest in the reaction has been stimulated by the discovery of the Cu(I)-catalyzed version of this cycloaddition (CuAAC) by the groups of Meldal³ and Sharpless⁴ independently, which increases the reaction rate by 3-4 orders of magnitude and produces the 1,4-disubstituted triazoles regioselectively. The success of this

reaction is determined by the excellent efficiency, selectivity and orthogonality that allow facile covalent links between building blocks⁵.

One appealing aspect of click chemistry is its application to label molecules of interest in complex biological samples⁶ and then detect those molecules with enormous sensitivity and almost complete orthogonality (i.e. not interfering with other chemical functionalities)⁷. Successful implementation of click chemistry labeling has been found in studies of proliferation⁸, DNA repair⁹, and cell signaling¹⁰, in imaging and quantification of RNA transcription¹¹, and for in-depth protein analysis as the azide- or alkyne-containing molecule can be incorporated into proteins, thereby enabling a radioisotope-free detection of post-translational modifications. Once labeled, the modified protein can be detected with the corresponding alkyne- or azide-containing fluorescent dye or hapten¹²⁻¹⁵. In addition, it has been recently shown that a methylene triazole fragment could replace the triphosphate backbone present in nucleotides, thereby adding extra motivation in medicinal chemistry and drug design¹⁶.

While the use of metallic copper as a catalyst dates back to the 19th century; copper-catalyzed clickable processes became to be an extremely powerful class of chemical reactions during last 6-8 years. However, the inherent toxicity of this heavy metal represents a major limiting factor of copper-based protocols in biological applications¹⁷. The innovative copper-free Huisgen cycloadditions partially overcome this limitation^{18,19}. Despite this, the most common catalyst systems for 1,3-dipolar cycloaddition employ water or alcohol as solvents and use a Cu(II) salt in the presence of a reducing agent (often sodium ascorbate or metallic copper) to generate the required Cu(I) catalyst *in situ*. The use of metallic copper as a source of the catalytic species was introduced as a promising candidate^{20,21}, although reaction time was longer (one day), the final product was clean and the work-up was a simple filtration of the copper turnings. Copper clusters²² have also been employed as precatalysts, and in some cases Cu(I) salts can be used directly. However, Cu(I) salts in strongly coordinating solvents (i.e. acetonitrile), usually require the presence of nitrogen or phosphorus ligands, to stabilize the Cu(I) oxidation state, and undesired alkyne-alkyne homocoupling products

are often observed under such reaction conditions²³. Volatile alkynes or azides (boiling point <70 °C) may represent a limitation for sonochemical conditions.

Improved activation of copper-assisted alkyne-azide cycloaddition

To be practical in terms of reaction rate and regiospecificity, the alkyne-azide click reaction needs the addition of Cu(II) in the presence of a reducing agent to obtain *in situ* Cu(I) in a catalytic amount. Lipshutz *et al.* found that copper supported on charcoal (Cu(II)/C) was an efficient catalyst for the Huisgen cycloaddition²⁴; charcoal-supported Cu(II) or Cu(I) catalysts were also successfully employed by Cintas *et al.* in microwave-assisted protocols²⁵. The most attractive procedures to perform click reaction exploit the heterogeneous catalysis because the much easier work-up and purification. Metallic copper is the simplest and above all inexpensive solid catalyst. It is preferably used in aqueous solution, where water also acts as a ligand and metal wastes can easily be removed. Unfortunately, most of these methods require long reaction times, particularly in the case of bulky or poorly soluble substrates. Microwave (MW)²⁵⁻²⁷ and ultrasound (US)²⁸ irradiation has been used to promote this reaction. The latter is especially efficient in activating hard metal surfaces; the well-known cleaning effect of ultrasonic waves not only provides a de-passivated layer where the reaction partners interact with each other, but also facilitates the diffusion of the reagent from the solution to the metal, the electron transfer from the active surface to the reducible point of the organic substrate, and finally the extraction of an ion from the surface to generate a soluble product²⁹. It seems that cavitation energy (i.e. growth and violent collapse of microbubbles releasing enough kinetic energy that drives the process to completion) largely facilitates each of these steps as observed in the formation of common organometallics under sonication³⁰. Sreedhar reported a sonochemical CuI-catalyzed synthesis of 1,4-disubstituted 1,2,3-triazoles from terminal alkynes and alkyl/aryl azides, formed *in situ*. The specific advantages of MW³¹ and US³² in organic synthesis have been widely described. Their power to enhance reaction rates, yields and selectivity, may become additive when

they are used in combination, both sequential or simultaneous as revealed from several recent examples from the literature³³.

In a very recent work,³⁴ we described our study on the typical reaction of benzyl azide with phenylacetylene using metallic copper or Cu₂O as solid catalysts under different reaction conditions (conventional heating, MW heating, US and combined US/MW irradiation). The catalytic activity of de-passivated copper by washing with dil. HCl showed a dramatic drop (25% yield instead of 89%). This result supported our hypothesis that Cu(I) derives from the US-promoted red/ox between the copper metal and the copper oxide on the surface. Reactions catalyzed by Cu₂O powder required longer reaction times and gave somewhat lower yields. In the absence of any catalyst, a mixture of 1,4- and 1,5-disubstituted triazole isomers was obtained in very low yield (tot. 5%). The same comparison between different reaction conditions was applied to a series of other substrates (Table 1), among which was the 6-monoazido-6-monodeoxy- β -CD (β -CD) and its permethylated derivative (Scheme 1).

TABLE 1 | Synthesis of 1,2,3-triazole in the presence of copper turnings.

Reagents	Method	Time (h)	Yield (%)
1-azidoheptadecane/phenylacetylene	oil bath	6	34
	MW	3	16
	US	2	80
	US/MW	2	86
6-monoazido-6-monodeoxy- β -CD/ phenylacetylene	oil bath	10	64
	MW	3	55
	US	2	74
	US/MW	2	80
	oil bath	6	56

6-monoazido-6-monodeoxy-2,3,6- <i>O</i> -permethyl- β -CD/phenylacetylene	MW	3	68
	US	2	81
	US/MW	2	87

In the search for greener and more efficient procedures under heterogeneous catalysis, we propose a sustainable method catalyzed by metallic copper, avoiding any addition of ligands for the copper i.e. amines (Figure 1). Enhanced reaction rates relative to the conventional protocol can be obtained under sonication as well as the combined use of MW and US irradiations (Table 1). These constitute safe and innocuous activation techniques.

Everybody agrees that high-throughput MW and US applications definitively require flow reactors. Chemistry in flowing systems has become more prominent as a method of carrying out chemical transformations, ranging in scale from microchemistry up to kilogram-scale processes. Compared to classic batch US or MW reactors, flow reactors stand out for their greater efficiency and flexibility as well as lower energy consumption.

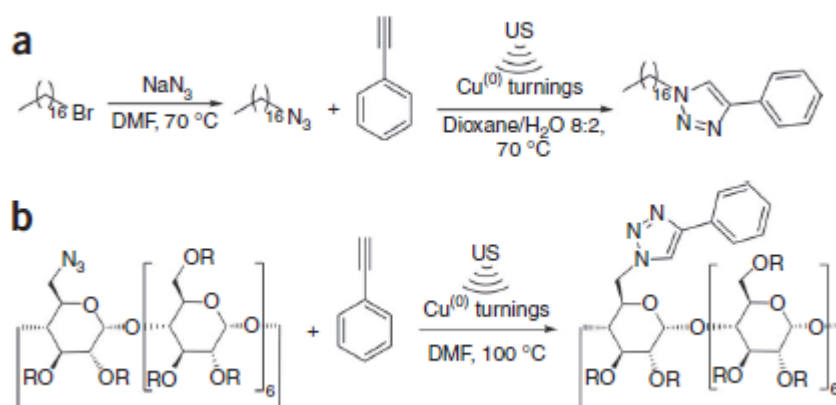


Figure 1. Example of copper-catalyzed azides/alkynes 1,3-dipolar cycloaddition under US irradiation. R = H (cyclodextrin), R'=phenyl (phenylacetylene), R=CH₃ (*O*-permethyl-cyclodextrin)

MATERIALS

REAGENTS

- Alkynes (commercially available from Sigma-Aldrich, Alpha Aesar, Acros Organics)

▲ **CRITICAL** Particular attention has to be paid for volatile alkynes (boiling point <70 °C)

- Azido derivatives were synthesized from the corresponding bromides (commercially available from Sigma-Aldrich, Alpha Aesar, Acros Organics) ▲ **CRITICAL** Particular attention has to be paid for volatile azides (boiling point <70 °C).

The use of US-reactors that work under moderate pressure (up to 5-7 bar) would allow efficient cavitation at the required temperature even with low boiling reagents. Figure 2 shows the device developed in our laboratory in collaboration with Danacamerini sas (Torino, Italy), both horn and reactor chamber are made in titanium, the latter is cooled by flowing tap water or refrigerated fluids.



Figure 2a. A water cooled horn-type US-reactor to work under pressure (up to 7 bar).



Figure 2b. Horn and reaction chamber of the same reactor.

• **For the preparation of azido derivative: Please mention this in the Introduction and include the reaction in one of the Figures?]**

• 6-monoazido- β -cyclodextrin and 6-monoazido-2,3,6-permethyl- β -cyclodextrin were purchased from Cyclolab Ltd - Hungary, (code no. CY-2051 and CYL-3030 respectively; web site: www.cyclolab.hu)

• Sodium azide (Aldrich, cat. no. S2002) **! CAUTION** Explosive and toxic

• Anhydrous Na₂SO₄ (Carlo Erba, product code: 375709)

• DMF (Sigma-Aldrich, cat. no. 319937)

• Dioxane (Sigma-Aldrich, cat. no. 360481)

• Acetone (Carlo Erba, product code: 301506), ethyl acetate (Carlo Erba, product code: 341503), hexane (Carlo Erba, product code: 339852)

• Deionized water

• Copper turnings (in our case, copper reduced powder RPE from Carlo Erba, product code: 475330)

EQUIPMENT AND GLASSWARE

• Rubber septa (see EQUIPMENT SETUP)

• Three-necked pear-shaped flasks (see EQUIPMENT SETUP)

• One-neck round-bottomed flasks

• Glass stoppers

• Rotary evaporator

• Rotative pump

• Gas-tight glass syringes and micro-syringes (Hamilton)

• Spatula

• Erlenmeyer flasks

• Büchner, Hirsh, separatory and glass funnels

• Filtering paper

- Heating oil bath
- A digital thermocouple (*DIGITAL*-thermometer)
- Aluminum-backed silica gel 60 F₂₅₄ thin layer chromatography plates (Merck)
- High-power US probe system working at a frequency of 21 kHz (Danacamerini sas-Torino, Italy)
(see EQUIPMENT SETUP)
- A Microsynth multimode oven operating at 2.45 GHz equipped with a high-power US probe (Milestone, Italy) with optic fibre thermometer (see EQUIPMENT SETUP)
- Silica gel or RP18 flash-chromatography columns
- A device for automatic flash-chromatography with auto injection and fractions collector, UV detector (CombiFlash Rf[®] Teledyne ISCO) (see EQUIPMENT SETUP)
- Glass Test Tubes
- GC-MS instrument (see EQUIPMENT SETUP)

EQUIPMENT SETUP

- The rubber septa are designed specifically for a gas-tight setup (Danacamerini), this elastomeric material is extremely flexible, chemically inert and transparent to microwaves.

Figures 3

Rubber septum in which the horn probe is inserted and which is adjusted to the central neck of the flask (**a**);

second rubber septum through which the fiber optic (**b**) or the thermocouple

(**c**) is introduced into the side neck of the reaction vessel.

- The pear-shaped reaction flasks allow optimal cavitation.

Figure 4 | Three necked pear-shaped reaction vessel (100 mL).

- High-intensity US device: probe system with a titanium horn (frequency is 21 kHz). Every kind of commercially available horn-type US reactors working in the frequency range of 18-35 kHz may be used for this application. In our device the electronic generator acting on the oscillating circuit continuously adjusts the US frequency (± 0.5 kHz max variation) to the actual resonance value of the reaction system (which is a function of the viscosity, the dissolved gases, the acoustic impedance, etc.). This value correspond to the frequency that maximizes the US output for a given power setting (lock frequency system)³⁵.

Figure 5 | Complete assembling of high intensity US device (probe system with the titanium horn and the electronic generator) and a silicon oil bath on a heating plate. On the left the horn in evidence.

- US Pyrex[®] horn (in this case, 20.5 kHz working frequency) and a generator with a power and a frequency regulator (lock frequency system). Every kind of commercially available non-metallic US horn³³ working in the frequency range of 18-35 kHz may be used for this application.

a b

Figure 6a | Complete assembling of combined US/MW system with electronic generator and probe for US irradiation and a professional MW oven (Milestone MicroSYNTH).

Figure 6b | The Pyrex[®] horn to be inserted into the MW oven.

- GC-MS analyses are performed on an Agilent Technologies 6850 Network GC System with 5973 Network Mass Selective Detector using HP-5

MS fused silica column (length 30 m; i.d. 0.25 mm; film thickness 0.25 μm). Upon injection the oven temperature is maintained constant at 50 $^{\circ}\text{C}$ and hold at this value for additional 3 min. Follows a temperature ramp up to 80 $^{\circ}\text{C}$ over 10 min then up to 300 $^{\circ}\text{C}$ over 22 min and hold at 300 $^{\circ}\text{C}$ for other 10 min. GC analysis general conditions are: injection split 1:20, injector temperature: 250 $^{\circ}\text{C}$, detector temperature 280 $^{\circ}\text{C}$; He as carrier gas at 1.2 mL/min.

- For Flash Chromatography analyses, see **Table 2**. In general for small lipophilic molecules, the direct-phase method with hexane–EtOAc mixture as eluent is preferable, whereas for bigger or less lipophilic structures (e.g., permethylated CD) the mixture dichloromethane–methanol was
- the eluent of choice. For hydrophilic compounds, use the reverse-phase chromatography on RP18 and water–methanol mixture as eluent. As usually this reaction has an almost quantitative yield, the starting materials can be found only in traces (< 2–4%). Under our conditions no by-products were detected, apart from the traces of degradation products from phenylacetylene (yellow, strong UV absorption). TLC on silica gel plates showed for all the triazole derivatives from lipophilic molecules (small molecules and permethyl CD) a lower R_f compared with the starting materials. A reverse situation was observed for native CDs.

TABLE 2 | Analytical flash chromatography equipment and conditions for analyses of crude products.

Substrate	Column	Solvents	Flow rate	Gradient Time (min)/B (% vol)	Injection
Small Lipophilic molecules	Silica, 12 g CV 16.8 mL	Hex/EtOAc	30 mL/min	3/0%, 1/10%, 5/10%, 1/20%, 5/20%, 1/30%, 5/30%, 5/100%, 2/100%	60 mg-1.2 g sample loading
Permethyl CD	Silica, 12 g CV 16.8 mL	$\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$	30 mL/min	2/0%, 2/2%, 5/2%, 5/5%, 5/5%, 2/10%, 5/10%, 5/50%, 4/50%	60 mg-1.2 g sample loading
Native CD	RP 18, 26 g CV 26 mL	$\text{H}_2\text{O}/\text{CH}_3\text{OH}$	22 mL/min	1.2/0%, 5.9/10%, 5.9/10%, 5.9/20%,	26-520 mg sample loading

				11.7/20%, 11.7/50%, 5.9/100%, 4/100%	
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Abbreviations: Hex: hexane, EtOAc: ethyl acetate.

PROCEDURE

Preparation of azido derivatives from the corresponding bromides • TIMING ~ 4-8 h

1| Weigh out the bromide derivatives (0.1 mol) on weighing paper if solid or pick them with a syringe if liquid. Introduce the reagents into a round-bottomed flask and add DMF (50-100 mL). Weigh out the NaN₃ (0.12 mol) on weighing paper and add to the reaction mixture.

2| Place the flask into a preheated oil bath at 70 °C and leave it to react for 1-4 h under vigorous magnetic stirring.

3| Monitor consumption of bromides and formation of azido derivatives by sampling the reaction mixture (by a syringe through the elastomeric septum every 10-15 min) for TLC/GC-MS analyses when the reaction is expected to be completed. For most substrates, a mixture of ethyl acetate in hexane should be a good solvent for TLC analysis. An optimal R_f value is between 0.3-0.4▲

CRITICAL STEP If the compounds are not well revealed on TLC by UV or any particular reagent sprayed (5% H₂SO₄ in ethanol or phosphomolybdic acid), use a GC-MS analysis to discriminate and quantify products and residual starting material.

4| Allow the reaction mixture to cool to room temperature (20-22 °C), filter off the precipitated salts (NaBr) on paper using a Büchner funnel into a vacuum Erlenmeyer flask (500 mL). Wash the reaction vessel and the paper filter with hexane (30 mL).

5| Pour the mixture in water (1 l) and extract with hexane (3x200 mL) in a separatory funnel, collect the organic layers in a Erlenmeyer flask, dry them few minutes over anhydrous Na₂SO₄ (25 g) and filter the salt on paper using a glass funnel. Collect the filtrate into a round-bottomed flask.

? TROUBLESHOOTING

6| Evaporate the organic layers under reduced pressure using a rotary evaporator (up to 50 °C at 5 mm Hg).

? TROUBLESHOOTING

■ **PAUSE POINT** Products can be stored in a glass bottle overnight at room temperature (RT) under air. **! CAUTION** Azido derivatives can be explosive if N/C ratio > 30%.

7| If necessary (for example in the case of...), purify the crude products by flash-chromatography on silica gel phase (see **Table 2** and ANTICIPATED RESULTS).

8| Collect the fractions which contain the pure product, as revealed by TLC, into a round-bottomed flask and evaporate to dryness the solvents using a rotary evaporator (RT to 40 °C at 5 mm Hg). Remove trace amount of solvents using high-vacuum pump.

■ **PAUSE POINT** Pure products can be stored in a glass bottle overnight at RT under air.

! CAUTION Azido derivatives can be explosive if N/C ratio > 30%.

9| Characterize the final product and evaluate its purity by NMR, IR, MS and, when suitable, by GC-MS.

● TIMING

Step 1: 15 min

Step 2: 1-4 h

Step 3: 10 min-1 h

Step 4: 30 min

Steps 5-6: 2 h

Steps 7-8: 2-3 h

Azido-alkyne 1,3-dipolar cycloaddition ● TIMING ~ 3-5 h

10| Weigh out the azido derivative (1 mmol) and the alkyne (1.05 mmol or 5 mmol if the azido derivative is a cyclodextrin) on weighing paper if solid or pick them with a syringe if liquid. Introduce the reagents into the reaction flask (the three-necked pear-shaped flask) and add the solvent mixture dioxane/water 8:2 (10 mL) or DMF (10 mL) when required by poor solubility. Weigh out the copper powder (50% mmol or 200% mmol if the azido derivative is a cyclodextrin) on weighing paper and add to the reaction mixture.

▲ CRITICAL STEP Since cyclodextrins are well known complexing agents, add 5 equivalents of alkynes to optimize yields. Complex and hindered structures such as cyclodextrins may require a reaction temperature of 100 °C. Therefore, chose DMF as reaction solvent either when the reagents are not soluble in dioxane/water or when a higher reaction temperature is required.

? TROUBLESHOOTING

11| The following steps can be performed in two different ways: option A (US procedure) and option B (US/MW procedure)

(A) US procedure

- (i)** Install the US probe connecting it to the power generator.
- (ii)** Put an oil bath under the US horn and heat it to 70 °C or 100 °C, when DMF is used as solvent.
- (iii)** Insert the horn into the septum and then into the central neck of the flask; introduce the thermocouple through the second septum into the side neck of the reaction vessel and close the last neck with a glass stopper.

▲ CRITICAL STEP Dip the horn in the reaction mixture not more than one third of the distance from the solvent level and the bottom of the flask. Keep the thermocouple as far as possible from horn tip to avoid damaging it

Figure 7 | Note the position of the titanium horn in the reaction vessel and the temperature probes on the left side.

- (iv) Immerse the flask into the preheated oil bath until the oil level in the bath corresponds with the level of the reaction mixture in the flask.

Figure 8 | Note the position of flask in the oil bath and the complete reaction setup (horn and septa position, internal and external temperature probes).

- (v) Sonicate the reaction for 2 h, adjusting the power for optimal cavitation without splashes of the liquid. Usually, the power per reaction volume is 2-3 W/cm³, *e.g.* with 10 mL reaction volume the power is about 25 W.

▲ **CRITICAL STEP** Make sure there is a vigorous mixing of reaction, in particular of copper powder as shown in picture b of **Figure 8**.

! CAUTION Although US lies beyond human hearing, it could be potentially harmful (only sound in the audible range having zero ear response means very low intensity). It should be noted that most ultrasonic devices irradiating a liquid medium give some audible sound. This is because the sound emitted is not composed exclusively of the transducer frequency, but also includes sub-harmonics in the audible region, which can be noisy indeed. Exposure for long periods to high-intensity probes without ear protection might result in damage. Permitted limits vary from country to country, but generally they lie between 85 and 90 dB (decibels) for a 8-h period. Any person working with ultrasonic equipment should wear either acoustic ear muffs or in-the-ear protectors. It is also a good practice to isolate the equipment in laboratories where access is limited (a closed door is a convenient sound barrier). In the chemical laboratory a fume hood usually represents a reasonable protection.

(Ref.: Mason, T. J. *Practical Sonochemistry. User's Guide to Applications in Chemistry and Chemical Engineering* 48-49 (Ellis Horwood, London, 1991).

? TROUBLESHOOTING

(B) US/MW procedure

- (i) Insert the US Pyrex[®] horn into the upper unit hole of the professional MW oven and connect it to a power generator.

! CAUTION To avoid microwave leakage use only professional MW ovens with an appropriate upper unit hole.

- (ii) Insert the horn into the septum and then into the central neck of the flask; introduce the fiber optic thermometer through the second septum into the side neck of the reaction vessel and close the third neck with a glass stopper.

▲ CRITICAL STEP Immerse the horn into the reaction mixture not more than one third of the distance from the solvent level and the bottom of the flask. Keep the temperature probe as far as possible from the horn tip to avoid damaging it.

Figure 9 | Note the complete reaction setup (horn, septa and internal temperature probe). In particular, note the position of the Pirex[®] horn in the reaction vessel and the lateral position of fiber optic.

- (iii) Set up the MW heating program using the temperature-controlled power method (See **Table 3** MW heating program).

- (iv) Irradiate the reaction with US for 2 h, adjusting the power in order to obtain the best cavitation conditions (the US power per reaction volume is 2-3 W/cm³).

▲ CRITICAL STEP Make sure there is a vigorous mixing of reaction, in particular of copper powder as shown in picture b of **Figure 10**.

? TROUBLESHOOTING

Figures 10a | The reaction *ab initio*: the copper powder is on the bottom of the flask.

Figures 10b | The reaction is ongoing and the copper powder is finely dispersed into the medium.

Figures 10c | The reaction is stopped, the copper powder still remains partially in suspension.

- (v) At the same time of the point (iv), irradiate the reaction with MW in order to reach and maintain the desired temperature (70 °C, when a mixture dioxane/water 8:2 is used as solvent or 100 °C, by using DMF).

? TROUBLESHOOTING

12| After about 30 min, when the reaction is expected to be close to completion, monitor the residual azido compound and the triazole derivative by sampling the reaction mixture for TLC/GC-MS analyses. For most substrates ethyl acetate/hexane mixtures can be used as eluent for TLC analysis. In the case of *O*-alkylated cyclodextrins, use methanol/dichloromethane mixtures (2-20% methanol), while for native cyclodextrin derivatives, use a mixture of isopropanol/ water/ethyl acetate/ammonium hydroxide 5:3:1:1. An optimal *R_f* value is between 0.3-0.5.

13| Allow the reaction mixture to reach RT, wash the horn and the temperature probe with acetone (10 mL) and filter off the copper powder on paper filter using placed on a Hirsh funnel and collect the filtrate in a vacuum Erlenmeyer flask (250 mL). Wash the reaction vessel and the paper filter with excess acetone (20 mL).

14| This procedure step depends on the reaction solvent used: in the case of DMF follow step A while step B with dioxane/water mixture.

(A) Evaporate the reaction solvents under reduced pressure using a rotary evaporator (up to 50 °C at 5 mm Hg).

(B) Evaporate DMF under higher vacuum using a rotative pump (up to 70 °C at 2 mm Hg).

■ **PAUSE POINT** Crude products can be stored overnight at RT under air.

15| Purify the crude products by flash-chromatography on direct phase (See **Table 2** and ANTICIPATED RESULTS).

16| Collect the fractions which contain the pure product, as revealed by TLC, into a round-bottomed flask and evaporate to dryness the solvents using a rotary evaporator (up to 40 °C at 5 mm Hg). Remove trace amount of solvents using a high-vacuum pump.

■ **PAUSE POINT** Pure products can be stored overnight at RT under air.

17| Check the purity and characterize the final product using NMR, IR, MS and, when suitable GC-MS.

● **TIMING**

Step 10: 15 min

Steps 11A (i-iv): 40 min

Step 11A (v): 2 h

Steps 11B (i-iii): 20 min

Steps 11B (iv-v): 2 h

Steps 12-13: 0.5-1 h

Step 14A: 20-40 min

Step 14B: 30 min

Steps 15-16: 2 h

? **TROUBLESHOOTING**

Troubleshooting advice can be found in **Table 4**.

TABLE 3 | MW heating program.

Step	Time	Temperature	Maximum power
1	4 min	70 °C/100 °C	200 W/300 W
2	2 h	70 °C/100 °C	80 W/100 W

TABLE 4 | Troubleshooting table.

Step	Problem	Possible reason	Solution
1	NaN ₃ is suspended in DMF	NaN ₃ is not completely soluble in DMF	It does not constitute a problem for the reaction yield, because it will be dissolved during the reaction
5	Residual product in water	The extraction volumes do not suffice	Extract another 2-3 times with hexane (200 mL)
6	High volume/weight of dried product due to residual DMF	Insufficient DMF partition in water/hexane, weak vacuum during evaporation	Repeat the work up extraction in water/hexane (small volumes) and evaporate under higher vacuum
10	Cu powder on the flask walls The product is suspended in the solvent	Splashes caused by US Scarce solubility of the product	Wash it with solvent, reduce the US power Use a better solvent (e.g. ...) and considering that the boiling point has to be at least 20 °C higher than the required reaction temperature
11 A. v.	The reaction volume decreases	The temperature is going over 70 °C or the flask is not tight enough	Control the temperature and check septa and glass stopper to make sure the system is closed

11 A. v., 11 B. iv.	The copper powder is not suspended	The US frequency is not well tuned or the power set is too weak	Check the position of horn tip, regulate the frequency and increase the power
11 B. v.	Weak dielectric heating, the temperature is lower than the program The reaction volume decreases The yield is lower than expected (<60%) The MW system indicate: CHECK SENSOR	The fiber optic cannot read the temperature or it is not well inserted into the liquid or the MW power is too low The temperature is over 70 °C or the system is not tighten One of reagents is volatile and the reaction vessel is not tight enough. One reagent is lost in the air. The reaction temperature is too low You are losing reagents or solvents in the oven, the MW power is too low, the fiber optic is not recording the temperature	Check the position of optical probe, and its correct functioning, finally increase the MW power limit Reduce the temperature by regulating the energy sources. Check the septa and the glass stopper to make sure the system is closed Check the septa and the glass stopper to make sure the system is tighten. Increase the temperature, paying attention to the boiling point of reagents and solvents Check the septa and the glass stopper to make sure the system is closed, check the correct functioning of the temperature probe and its position in the reaction, finally increase the MW power limit

Abbreviations: DMF, *N,N*-dimethylformamide.

ANTICIPATED RESULTS

Using the classic procedure for nucleophilic substitution of alkyl bromides, 1-bromoheptadecane (10 g, 31.3 mmol) and NaN₃ (2.14 g, 32.9 mmol) in DMF (50 mL) were reacted at 70 °C for 1 h, providing

8.02 g (91%) of 1-azidoheptadecane as a transparent oil. The analytical data are consistent with literature²⁵. ¹H NMR (300 MHz, CDCl₃): δ 3.26 (t, *J* = 6.6, 2H, H-1), 1.53-1.67 (m, 2H, H-2), 1.18-1.41 (m, 28H, CH₂ aliphatic), 0.887 (t, *J* = 6.9, 3H, H-17). IR (neat, cm⁻¹): 2924, 2855, 2094 (N₃), 1687, 1466, 1259. MS (EI, *m/z*): 252, 112, 98. MS (CI, *m/z*): 282 [M + H]⁺, 254. Anal. Calcd. for C₁₇H₃₅N₃ (281.3): C 72.54%, H 12.53%, N 14.93%. Found: C 72.48%, H 12.21%, N 14.74%.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US irradiation, 1-azidoheptadecane (281 mg, 1 mmol), phenylacetylene (115 μl, 1.05 mmol), Cu powder (32 mg, 0.5 mmol) and dioxane/water 8:2 (10 mL) were sonicated at 70 °C for 2 h. Flash chromatography on silica gel (hexane/ethyl acetate gradient, see **Table 2**) afforded 306 mg (80%) of 1-heptadecyl-4-phenyl-*IH*-1,2,3-triazole as a white powder. The analytical data are consistent with literature²⁵. ¹H NMR (300 MHz, CDCl₃): δ 7.88 (d, *J* = 7.2, 2H, H-2',6' Ph), 7.74 (s, 1H, H-5), 7.21-7.45 (m, 3H, H-3',4',5' Ph), 4.34 (t, *J* = 7.2, 2H, H-1'), 1.88 (m, 2H, H-2'), 1.52-1.63 (m, 2H, H-3'), 1.13-1.42 (m, 26H, CH₂ aliphatic), 0.88 (t, *J* = 6.9, 3H, H-17'). IR (KBr disk, cm⁻¹): 2918, 2941, 2847, 2096 (N₃), 1464, 1217, 1080, 1053, 976, 912, 841, 726, 734, 694. M.p. 94 °C. MS (EI, *m/z*): 383 [M]⁺, 354, 172, 145. MS (CI, *m/z*): 384 [M + H]⁺. Anal. Calcd. for C₂₅H₄₁N₃ (383.3): C 78.27%, H 10.77%, N 10.95%. Found: C 78.15%, H 10.52%, N 11.10%.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US/MW irradiation, 1-azidoheptadecane (281 mg, 1 mmol), phenylacetylene (115 μl, 1.05 mmol), Cu powder (32 mg, 0.5 mmol) and dioxane/water 8:2 (10 mL) were simultaneously irradiated with US and MW at 70 °C for 2 h. Flash chromatography in direct phase (hexane/ethyl acetate gradient, see **Table 2**) provided 325 mg (85%) of 1-heptadecyl-4-phenyl-*IH*-1,2,3-triazole as a white powder.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US irradiation, 6-monoazido-6-monodeoxy-β-cyclodextrin (300 mg, 0.259 mmol), phenylacetylene (184 μl, 5 eq, 1.295 mmol), Cu powder (33 mg, 2 eq, 0.518 mmol) and DMF (10 mL) were sonicated at 100 °C for

2 h. Flash chromatography on reverse phase (RP18, water/methanol, see **Table 2**) provided 242 mg (74%) of 6-monodeoxy-6-mono(4-phenyl-*IH*-1,2,3-triazol-1-yl)- β -cyclodextrin as a white powder. The analytical data are consistent with literature²⁵, however a more detailed characterization follows. R_f (*i*-PrOH/H₂O/EtOAc/NH₃ 5/3/1/1) = 0.50. ¹H NMR (DMSO, 300 MHz): δ 8.51 (s, 1H, H-5 *triaz*), 7.78 (d, J = 6.9 Hz, 2H, H-2,6 *Ph*), 7.41 (dd, J = 7.2, 6.9 Hz, 2H, H-3,5 *Ph*), 7.31 (t, J = 7.2 Hz, 1H, H-4 *Ph*), 6.00-5.50 (overlapped signals, 14H, 2,3-OH), 5.05 (d, J = 3.3 Hz, 1H, H-1), 4.92 (m, 1H, H-6'a), 4.80-4.70 (m, 6H, H-1), 4.60 (m, 1H, H-6'b), 4.70-4.41 (overlapped signals, 5H, 6-OH), 4.25 (t, J = 6.3 Hz, 1H, 6-OH), 4.13 (m, 1H, H-5'), 3.70-3.48 (overlapped signals, 23H, H-3,5,6), 3.48-3.10 (overlapped signals, 14H, H-2,4), 2.90 (br, 2H, H-6). ¹³C NMR (DMSO, 75 MHz): δ 130.7 (C1 *Ph*), 129.0 (C3,5 *Ph*), 127.8 (C4 *Ph*), 125.1 (C2,6 *Ph*), 122.0 (C5 *Triaz*), 102.0 (C1), 81.5 (C4), 74-71 (C2,3,5), 70 (C5'), 60-58 (C6), 50.8 (C6'). IR (KBr disk, cm⁻¹): 3420, 2926, 2104 (N₃), 1655, 1458, 1367, 1333, 1157, 1097, 1028, 947, 756. ESI-MS: Calculated for C₅₀H₇₅N₃NaO₃₄ [M + Na]⁺ 1284.41, found 1284.30. Anal. Calcd. for C₅₀H₇₅N₃O₃₄ (1262.13): C 47.58%, H 5.99%, N 3.33%. Found: C 47.79%, H 5.72%, N 3.40%. M.p. = >235 °C (dec.).

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US/MW irradiation, 6-monoazido-6-monodeoxy- β -cyclodextrin (300 mg, 0.259 mmol), phenylacetylene (184 μ l, 5 eq, 1.295 mmol), Cu powder (33 mg, 2 eq, 0.518 mmol) and DMF (10 mL) were simultaneously irradiated with US and MW at 100°C for 2 h. Flash chromatography on reverse phase (RP18, water/methanol, see **Table 2**) provided 262 mg (80%) of 6-monodeoxy-6-mono(4-phenyl-*IH*-1,2,3-triazol-1-yl)- β -cyclodextrin as a white powder.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US irradiation, 6-monoazido-6-monodeoxy-2,3,6-*O*-permethyl- β -cyclodextrin (300 mg, 0.208 mmol), phenylacetylene (148 μ l, 5 eq, 1.04 mmol), Cu powder (26 mg, 2 eq, 0.416 mmol) and DMF (10 mL) were sonicated at 100 °C for 2 h. Flash chromatography on direct phase (dichloromethane/ methanol, see **Table 2**) provided 259 mg (81%) of 2,3,6-*O*-permethyl-6-monodeoxy-6-mono(4-phenyl-*IH*-

1,2,3-triazol-1-yl)- β -cyclodextrin as a white powder. R_f (CHCl₃/MeOH 95/5) = 0.46. ¹H NMR (300 MHz, CDCl₃): δ 7.88 (s, 1H, H-5 triaz), 7.83 (d, J = 6.9 Hz, 2H, H-2,6 Ph), 7.44 (dd, J = 7.2, 6.9 Hz, 2H, H-3,5 Ph), 7.31 (t, J = 7.2 Hz, 1H, H-4 Ph), 5.27 (d, J = 3.6 Hz, 1H, H-1), 5.20-5.05 (overlapped signals, 7H, H-1,6'a), 4.57 (dd, J = 7.8, 7.5 Hz, 1H, H-6'b), 4.19 (m, 1H, H-5'), 4.02-3.75 (overlapped signals, 10H, H-5,6), 3.70-3.40 (overlapped signals, 62H, H-3,4,6, CH₃O-2,3), 3.40-3.00 (overlapped signals 25H, H-2, CH₃O-6), 3.10 (br, 2H, H-6). ¹³C NMR (CDCl₃, 75 MHz): δ 130.7 (C1 Ph), 129.0 (C3,5 Ph), 128.3 (C4 Ph), 125.7 (C2,6 Ph), 122.0 (C5 Triaz), 99.7-98.5 (C1), 83.5-81.3 (C2,3), 80.5-79.8 (C4), 71.6-71.4,70.6 (C6), 71.2-70.6 (C5), 61.9-61.4 (3-OCH₃), 59.3-58.7 (2,6-OCH₃), 51.8 (C6'). IR (KBr disk, cm⁻¹): 2930, 2836, 2101 (N₃), 1655, 1458, 1367, 1333, 1157, 1097, 1028, 947, 756. MS ESI (m/z): Calculated for C₇₀H₁₁₅N₃NaO₃₄ [M + Na]⁺ 1564.72, found 1564.70 [M + Na]⁺. Anal. Calcd. for C₇₀H₁₁₅N₃O₃₄ (1542.66): C 54.50%, H 7.51%, N 2.72%. Found: C 54.62%, H 7.42%, N 2.83%. Mp = 116 °C.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US/MW irradiation, 6-monoazido-6-monodeoxy-2,3,6-*O*-permethyl- β -cyclodextrin (300 mg, 0.208 mmol), phenylacetylene (148 μ l, 5 eq, 1.04 mmol), Cu powder (26 mg, 2 eq, 0.416 mmol) and DMF (10 mL) were simultaneously irradiated with US and MW at 100°C for 2 h. Flash chromatography on silica gel (dichloromethane/methanol, see **Table 2**) provided 277 mg (87%) of 2,3,6-*O*-permethyl-6-monodeoxy-6-mono(4-phenyl-*1H*-1,2,3-triazol-1-yl)- β -cyclodextrin as a white powder.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US irradiation, 6-monoazido-6-monodeoxy- β -cyclodextrin (400 mg, 0.345 mmol), 1,3-bis(propargyloxy)benzene (POB)²⁵ (32 mg, 0.172 mmol), Cu powder (44 mg, 0.69 mmol) and DMF (10 mL) were sonicated at 100 °C for 2 h. Flash chromatography on reverse phase (RP18, water/methanol, see Table 1) provided 605 mg (70%) of 1,3-bis((1-(6-deoxy- β -CD-6-yl)-*1H*-1,2,3-triazol-4-yl)methoxy)benzene as a white powder. Analytical data were in accordance with reported values³⁴.

According to general procedure for azides/alkynes 1,3-dipolar cycloaddition under US/MW irradiation, 6-monoazido-6-monodeoxy- β -cyclodextrin (400 mg, 0.345 mmol), 1,3-bis(propargyloxy)benzene (POB)²⁵ (32 mg, 0.172 mmol), Cu powder (44 mg, 0.69 mmol) and DMF (10 mL) were irradiated with combined US/MW at 100 °C for 2 h. Flash chromatography on reverse phase (RP18, water/methanol, see Table 1) provided 639 mg (74%) of 1,3-bis((1-(6-deoxy- β -CD-6-yl)-*IH*-1,2,3-triazol-4-yl)methoxy)benzene as a white powder. Analytical data were in accordance with reported values³⁶.

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COMPETING INTERESTS STATEMENT

G.C. declare that the authors have no competing interests as defined by Nature Publishing Group, or other interests that might be perceived to influence the results and/or discussion reported in this article.

1. Huisgen, R. 1,3-Dipolar cycloaddition. Introduction, survey, mechanism. In *1,3-Dipolar Cycloaddition Chemistry* (ed. Padwa, A.) 1-176 (Wiley, New York, 1984).
2. Padwa, A. Intermolecular 1,3-dipolar cycloaddition. In *Comprehensive Organic Synthesis* (eds. Trost, B.M & Fleming, I.) Vol. 4, 1069-1109 (Pergamon, Oxford, UK, 1994).

3. Rostovtsev, V.V. *et al.* A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective “Ligation” of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* **41**, 2596-2599 (2002).
4. Tornøe, C. W., Christensen C. & Meldal, M. Peptidotriazoles on Solid Phase: 1,2,3-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.* **67**, 3057-3064 (2002).
5. Inglis, A. J. *et al.* Ultrafast Click Conjugation of Macromolecular Building Blocks at Ambient Temperature. *Angew. Chem., Int. Ed.* **48**, 2411-2414 (2009).
6. Kele, P. *et al.* Dual labeling of biomolecules by using click chemistry: a sequential approach. *Angew. Chem. Int. Ed.* **48**, 344-347 (2009).
7. Sletten, E. M. & Bertozzi, C. R. Biorthogonal chemistry: fishing for selectivity in a sea of functionality. *Angew. Chem. Int. Ed.* **48**, 6974-6998 (2009).
8. Kharas, M. G. *et al.* Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL + leukemia cells. *J. Clin. Invest.* **118**, 3038-3050 (2008).
9. Limsirichaikul, S. *et al.* A rapid non-radioactive technique for measurement of repair synthesis in primary human fibroblasts by incorporation of ethynyl deoxyuridine (EdU). *Nucleic Acids Res.* **37**, e31 (2009).
10. Chehrehasa, F. *et al.* EdU, a new thymidine analogue for labeling proliferating cells in the nervous system. *J. Neurosci. Methods* **177**, 122-130 (2009).
11. Jao, C. Y. & Salic, A. Exploring RNA transcription and turnover in vivo by using click chemistry. *Proc. Natl. Acad. Sci. USA* **105**, 15779-15784 (2008).
12. Kostiuk, M. A. *et al.* Identification of palmitoylated mitochondrial proteins using a bio-orthogonal azido-palmitate analogue. *FASEB J.* **22**, 721-732 (2008).

13. Martin, D. D. *et al.* Rapid detection, discovery, and identification of post-translationally myristoylated proteins during apoptosis using a bio-orthogonal azidomyristate analog. *FASEB J.* **22**, 797-806 (2008).
14. Agnew, H. D. *et al.* Iterative in situ click chemistry creates antibody-like protein-capture agents. *Angew. Chem. Int. Ed.* **48**, 4944-4948 (2009).
15. Wang, Z. *et al.* Site-specific GlcNAcylation of human erythrocyte proteins: potential biomarker(s) for diabetes. *Diabetes* **58**, 309-317 (2009).
16. Rowan, A.S. *et al.* Nucleoside triphosphate mimicry: a sugar triazolyl nucleoside as an ATP-competitive inhibitor of *B. anthracis* pantothenate kinase. *Org. Biomol. Chem.* **7**, 4029-4036 (2009).
17. Le Droumaguet, B. & Velonia, K. Click Chemistry: A Powerful Tool to Create Polymer-Based Macromolecular Chimeras. *Macromol. Rapid Commun.* **29**, 1073-1089 (2008).
18. Agard, N. J., Prescher, J. A. & Bertozzi, C. R., A Strain-Promoted [3+2] Azide-Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems. *J. Am. Chem. Soc.* **126**, 15046-15047 (2004).
19. Becer, C.R. Hoogenboom, R. & Schubert, U.S. Click chemistry beyond metal-catalyzed cycloaddition. *Angew. Chem. Int. Ed.* **48**, 4900-4908 (2009).
20. Himo, F. *et al.* Copper(I)-Catalyzed Synthesis of Azoles. DFT Study Predicts Unprecedented Reactivity and Intermediates *J. Am. Chem. Soc.*, **127**, 210-216 (2005).
21. Gommermann, N.; Gehrig, A. & Knochel, P. Enantioselective Synthesis of Chiral α -Aminoalkyl-1,2,3-triazoles Using a Three-Component Reaction. *Synlett* 2796-2798 (2005).
22. Pachòn, L. D., van Maarseveen, J. H. & Rothenberg, G. Click chemistry: Copper clusters catalyse the cycloaddition of azides with terminal alkynes. *Adv. Synth. Catal.* **347**, 811-815 (2005).
23. Moses, J. E.; Moorhouse, A. D. The growing applications of click chemistry. *Chem. Soc. Rev.* **36**, 1249-1262 (2007).

24. Lipshutz B. H., Taft B. R. Heterogeneous Copper-in-Charcoal-Catalyzed Click Chemistry. *Angew. Chem. Int. Ed.* **118**, 8415- 8418 (2006).
25. Cintas, P. et al. Improved Protocols For Microwave-Assisted Cu(I)-Catalyzed Huisgen 1,3-Dipolar Cycloadditions *Collect. Czech. Chem. Commun.*, **72**, 1014-1024 (2007).
26. van Dijk, M. et al. Synthesis and Characterization of Biodegradable Peptide-Based Polymers Prepared by Microwave-Assisted Click Chemistry. *Biomacromolecules* **9**, 2834-2843 (2008).
27. Appukkuttan, P. et al. A microwave-assisted click chemistry synthesis of 1,4-disubstituted 1,2,3-triazoles via a copper(I)-catalyzed three-component reaction. *Org. Lett.* **6**, 4223-4225 (2004).
28. Sreedhar, B. & Surendra, Reddy P. Sonochemical Synthesis of 1,4-Disubstituted 1,2,3-Triazoles in Aqueous Medium. *Synth. Commun.* **37**, 805- 812 (2007).
29. Cintas, P. & Luche, J.-L. Organometallic sonochemistry. In *Synthetic Organic Sonochemistry* (ed. Luche, J.-L.) 165-234 (Plenum Press, New York, 1998).
30. Tuulmets, A., Kaubi, K. & Heinoja, K. Influence of sonication on Grignard reagent formation. *Ultrason. Sonochem.* **2**, 75-78 (1995).
31. Kappe, C.O., Dallinger, D. & Murphree S. S. *Practical Microwave Synthesis for Organic Chemists*. Strategies, Instruments, and Protocols (Wiley-VCH, Weinheim, Germany, 2009).
32. Cravotto, G. & Cintas, P. Power ultrasound in organic synthesis: moving cavitation chemistry from academia to innovative and large-scale applications. *Chem. Soc. Rev.* **35**, 180-196 (2006).
33. Cravotto, G. & Cintas, P. The Combined Use of Microwaves and Ultrasound: New Tools in Process Chemistry and Organic Synthesis. *Chem. Eur. J.* **13**, 1902-1909 (2007).
34. Cravotto, G., Fokin V. V., Garella, D., Binello, A., Boffa, L. & Barge, A. Ultrasound-Promoted Copper-Catalyzed Azide-Alkyne Cycloaddition. *J. Comb. Chem.* DOI.
35. Cravotto, G., Omiccioli, G. & Stevanato, L. An Improved Sonochemical Reactor. *Ultrason. Sonochem.* **12**, 213-217 (2005).

36. Mourer M., *et al.* Click chemistry as an efficient tool to access β -cyclodextrin dimers.
Tetrahedron **64**, 7159-7163 (2008).