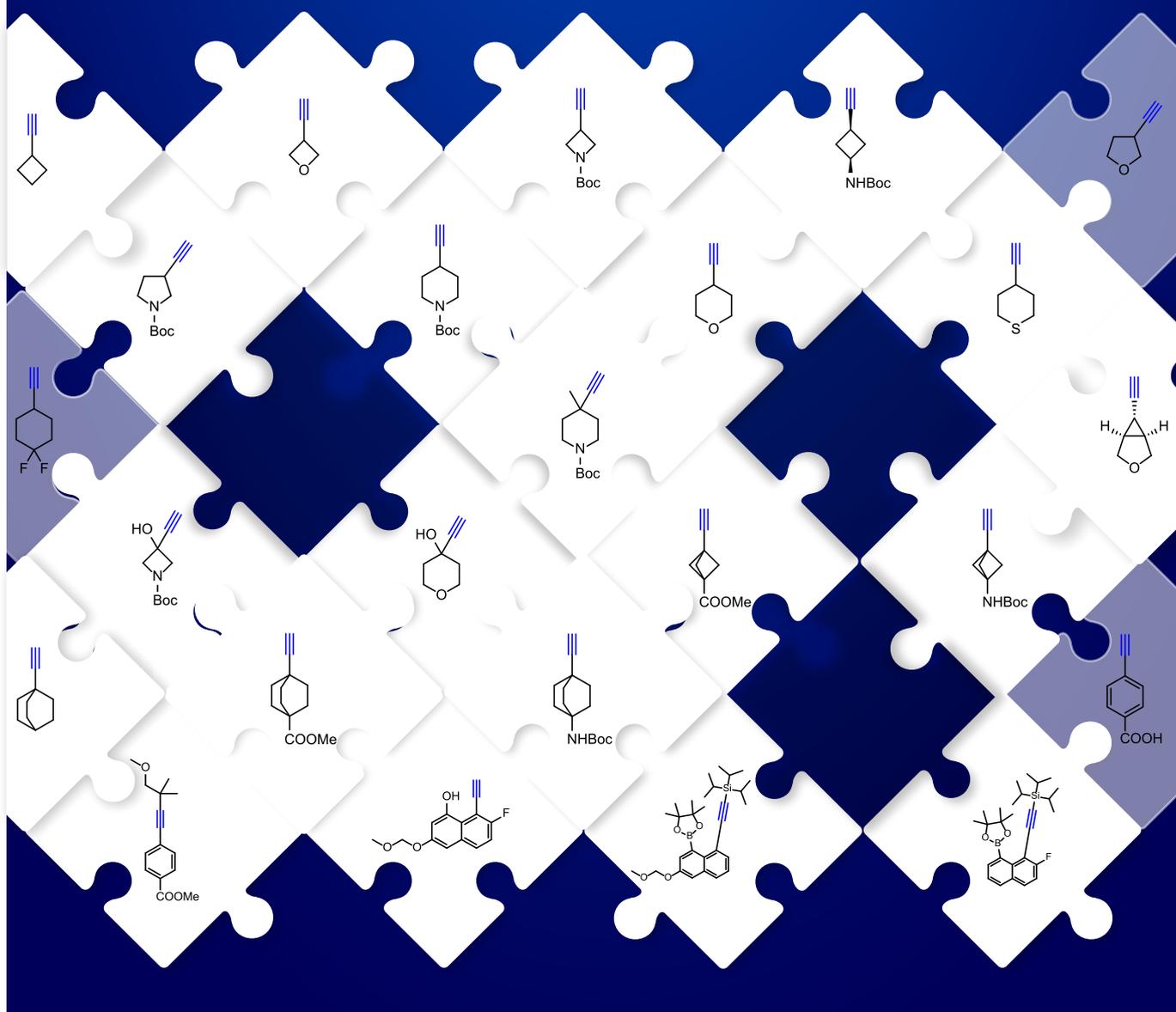


Application of Click Chemistry in Drug Discovery

Nov 2022



Introduction

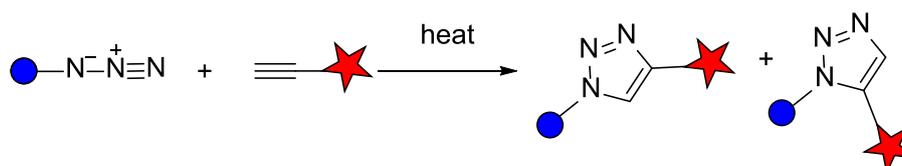
Click chemistry involves reactions that unite two synthetic molecules quickly and irreversibly. Some of these reactions can be performed inside living cells without disrupting biochemical processes, making them bioorthogonal. This approach is used to tag biomolecules with fluorescent probes, it also offers a way to produce antibody-drug conjugates. Click chemistry is even being directly applied inside patients, in ongoing clinical trials of a cancer therapy.

The 2022 Nobel Prize in Chemistry was awarded to Carolyn Bertozzi, Morten Meldal and Barry Sharpless for their work on the development of click chemistry and bioorthogonal reactions.

More than 60 years ago, Rolf Huisgen discovered the 1,3-dipolar cycloaddition of an azide with a terminal or internal alkyne. It took place at high temperatures ($>80^{\circ}\text{C}$), typically giving a $\sim 1:1$ regioisomeric mixture of syn- and anti-1,2,3-triazoles (Figure 1a)^[1]. The application of this reaction was limited because of the reaction conditions and poor selectivity.

Two teams led by K. Barry Sharpless and Morten Meldal independently found that using a Cu(I) catalyst and catalytic amount of organic base (Et₃N, iPr₂NEt, or lutidine) could generate rapidly anti-1,2,3-triazoles as the sole products at room temperature in high yield. This is no longer a true concerted 1,3 dipolar cycloaddition, thus probably should not be termed a Huisgen cycloaddition. It has been suggested that this reaction be termed the Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC).

(a) Huisgen 1,3-dipolar cycloaddition



(b) Cu(I)-catalyzed azide-alkyne cycloaddition

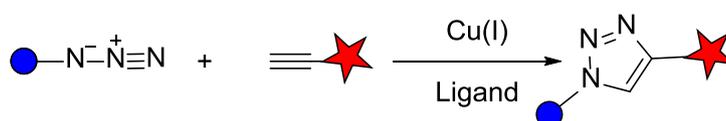
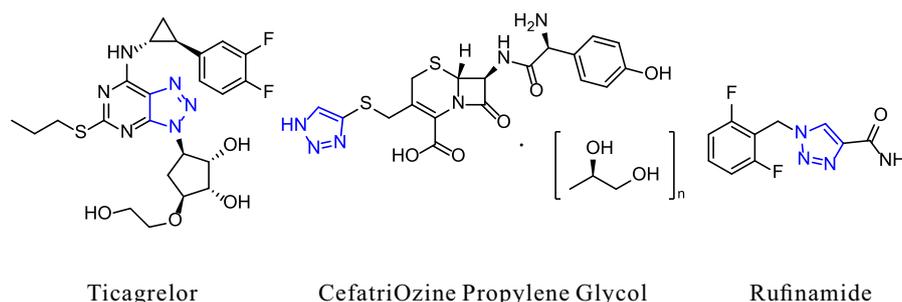


Figure 1. (a) Huisgen 1,3-dipolar cycloaddition
(b) Copper-catalyzed azide-alkyne cycloaddition^[1]

1,2,3-Triazole is a privileged scaffold in medicinal chemistry. Many molecules containing the triazole motif have been approved as therapeutic agents or advanced to human clinical trials (Figure 2)^[2]. Because of the mild conditions and high selectivity of the copper(I)-catalyzed azide-alkyne cycloaddition, compounds or libraries with 1,2,3-triazole can be easily generated. This reaction has been widely used in the synthesis of molecules with great complexity and variation.

Approved drugs



Drug Candidates

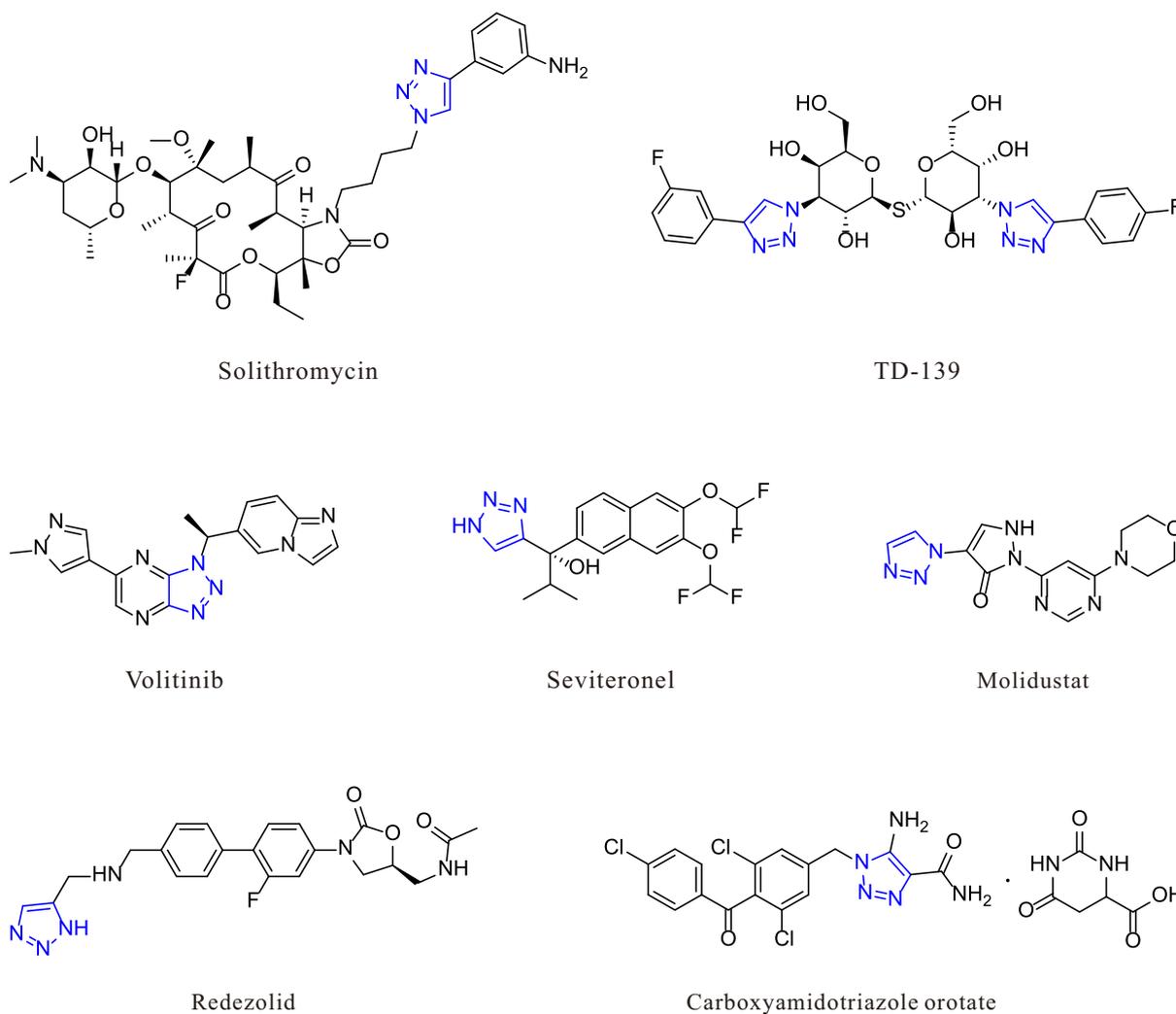


Figure 2. Approved drugs or Drug Candidates containing 1,2,3-triazole^[2]

The copper ions used in the classic click reaction are toxic to living cells, limiting the application of this particular reaction in biology. Bertozzi came up with a solution in 2004: a click reaction between an azide and a strained cyclooctyne that did not need a catalyst and therefore did not interfere with the biochemistry of a living cell (Figure 3). She used these bioorthogonal reactions successfully to map glycans on the surface of cells^[3].

Strain-promoted azide-alkyne cycloaddition

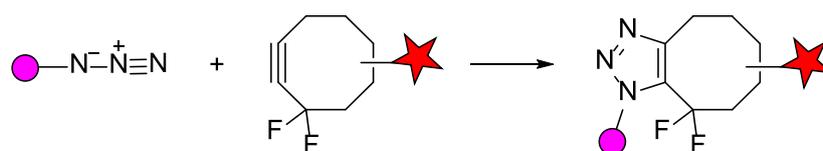


Figure 3. Strain-promoted azide-alkyne cycloaddition^[1]

Bioorthogonal chemistry illuminates the cell

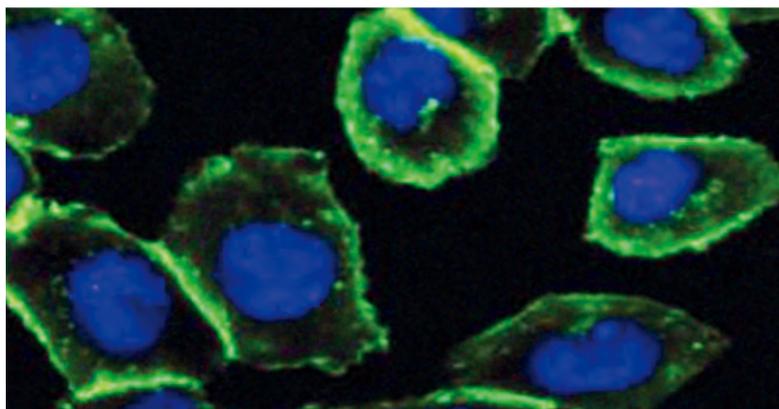
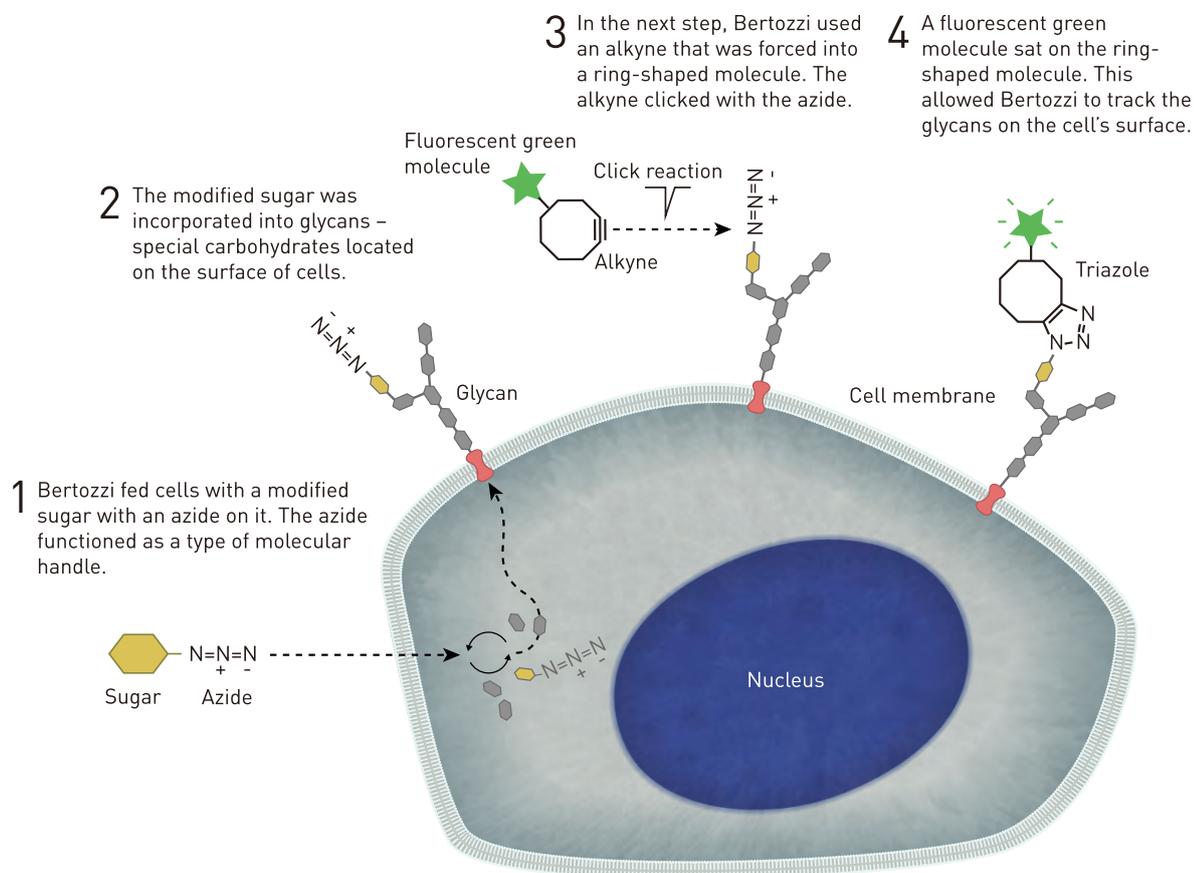


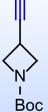
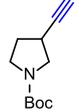
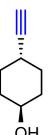
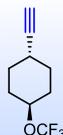
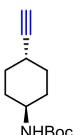
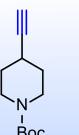
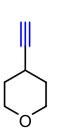
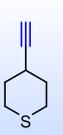
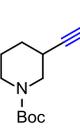
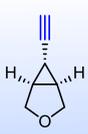
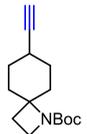
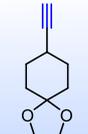
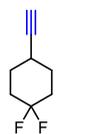
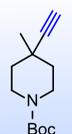
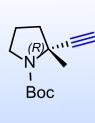
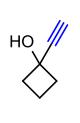
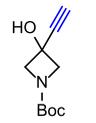
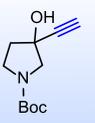
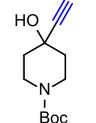
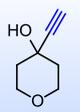
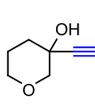
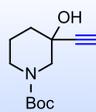
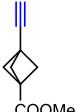
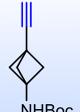
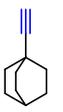
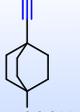
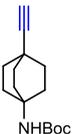
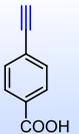
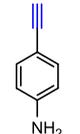
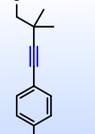
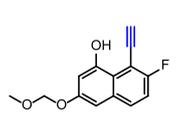
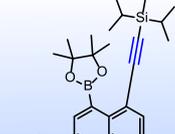
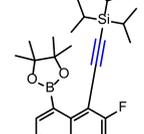
Image from *Proc Natl Acad Sci USA* (2007) 104:16793–16797

Bertozzi used the strain-promoted click reaction to track glycans. They have a green glow in the picture. The cell nucleus is coloured blue. Thanks to the glycans' green glow, Bertozzi was able to follow them in the cell.

Figure 4. The process of using the strain-promoted click reaction to track glycans^[4]

Click chemistry and bioorthogonal reactions are practical and efficient. They are now in widespread use and have proven extremely useful in drug development ranging from lead generation to discovery of new therapeutic targets and modalities.

Building Blocks Containing Alkyne

						
PBL1731 50786-62-4	PBL1732 1290541-38-6	PB03363 73874-95-0	PBLG100390 1463502-31-9	PBN2011535 1100987-19-6	PB03364 73874-95-0	PBLJ0799 316141-37-4
						
PBZS3101 128073-39-2	PBJL100382 2231663-36-6	PB07179 937049-58-6	PB02531 73874-95-0	PB03365 73874-95-0	PB05304 128094-82-6	PB03366 73874-95-0
						
PBZ6231 1002108-56-6	PBU5750 1807022-76-9	PBLJ00038 1024589-68-1	PBLJ3614 68776-62-5	PBN2011051 610791-05-4	PBN20120071 1001907-60-3	PBN2011769 236406-22-7
						
PBZ2317 2168736-71-6	PB92675 2166232-54-6	PBU4874 98135-75-2	PBN20121281 1352492-38-6	PBN20120092 1001907-60-3	PBN20121231 275387-76-3	PBN20121232 275387-83-2
						
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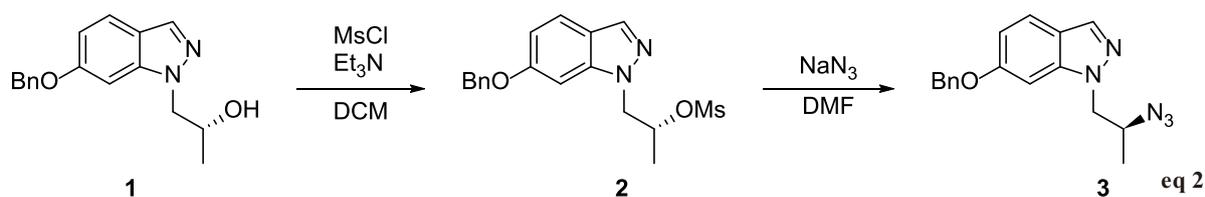
Stability Study of Organic Azide

Organic azides are considered as explosives whenever the azido content is remarkably high. Of course, there is no sharp threshold at which the explosive hazard starts. However, we can use the eq1 to help evaluate if your azide is stable enough to work with^[5].

(C+O)/N ≥ 3 (eq1)

- Organic azides with (C+O)/N ratio=3 (such as n-nonyl azide) can be isolated and stored in its pure form (up to 20 grams).
- Organic azides with (C+O)/N ratio between 1 and 3 can be synthesized and isolated, but should be stored below room temperature at no more than 1M concentration and at a maximum of 5 grams of material.
- Organic azides with (C+O)/N ratio < 1 should never be isolated. It may be synthesized if the azide is a transient intermediate species and the limiting in the reaction mixture and is limited to a maximum quantity of 1 gram.

(C+O)/N ratio of Diazidomethane is 1/6, so it exhibits a strong propensity to explode. Raymond E. Conrow^[6] reported an explosion caused by diazidomethane accumulated in the condenser. Herein we describe an explosion that occurred in the course of the reaction sequence of **eq 2**, which was carried out in a kilo laboratory using conventional glassware.



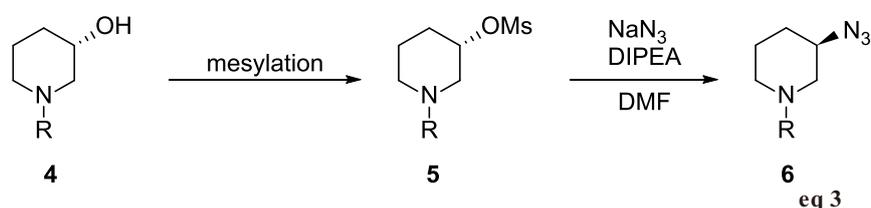
(R)-1-(6-(Benzyloxy)indazol-1-yl)-2-propanol (**1**, 1.26 kg, 4.45 mol) was converted to mesylate **2** under standard conditions as indicated. After an aqueous quench and phase separation the organic solution was concentrated on a rotary evaporator of 20-L capacity. The dual receiving flasks were then emptied. DMF (4 L) was added to the residue, and concentration (35 °C bath, ~20 Torr) was resumed until no further condensation was observed, the intention being to drive off DCM from the product. The residual solution of **2** was diluted with more DMF (12 L) and transferred to a reaction flask. Sodium azide (580 g, 8.9 mol) was added, and the stirred mixture was heated to 70 °C for 16 h, then cooled to rt and partitioned between diethyl ether and water. The organic extract was concentrated on the evaporator, and the receivers were then emptied.

The next morning it was noted that about 30 mL of a two phase liquid had collected in the glass crosspiece at the bottom of the condenser assembly. The distilling flask containing azide **3** was disconnected and set aside in a walk-in fume hood, and the PTFE stopcock leading to one of the receivers was then opened to drain the liquid. Within seconds a loud explosion occurred. The plastic-coated condenser and receiving flasks shattered. Larger glass fragments were propelled with sufficient force to break other glassware. A 2' square perforated metal ceiling vent cover above the evaporator was domed by pressure, while a 4 1/2' × 8' × 1/4" annealed glass window panel located 20' away was fractured by pressure or impact. Two chemists sustained noncritical lacerations but escaped more serious injury, principally by the proper wearing of safety glasses.

In the course of the investigation, crude **3** was analyzed by ¹H and ¹³C NMR which revealed the presence of 8-10 mol % (i.e., 0.4 mol, 40 g) of diazidomethane. The persistent hazard of residual diazidomethane became evident when a piece of a receiver assembly exploded upon being set on the metal evaporator stand, resulting in a glass cut to the investigator's hand.

The residual DCM in compound **2** reacts with NaN₃ in the second step to generate diazomethane, so it is better to replace DCM with THF, Ethyl acetate, MTBE, toluene, etc. in the 1st step.

Merck^[7] also reported a proposed process made an azide compound as a process intermediate, but generated isopropyl azide with (C+O)/N ratio=2/3 as a reaction byproduct due to residual isopropanol mesylated in the first step (**eq 2**). Since the solvent used in step 2 was DMF, which has a relatively high boiling point, concerns were raised about potential condensation of isopropyl azide, which could be shock-sensitive.



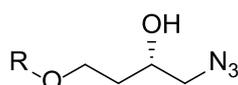
Organic compounds with high azido content are very sensitive to friction and impact, causing strong explosions. For example, cyanuric azide is very sensitive against mechanical stimulus and thus decomposes very easily by detonation. Although the initiation power of this detonation exceeds that of classical primary explosives no technical application has been found so far for this compound due to its high vapour pressure.

Organic azides also show remarkable lower ignition temperatures in comparison to inorganic metal azides. Most of the organic azides decompose at approx. 180 °C. Some organic azides also show light sensitivity^[8] and strong incompatibility with certain chemicals. Several examples have been published where azides exploded when they were brought in contact with sulfuric acid or other compounds^[9-11].

From our experience, it is essential to conduct sensitivity tests, thermoanalytical measurements and stability tests of energy-rich compounds at an early stage of every small-scale preparation. Naturally, such safety analysis is mandatory for the synthesis of new organic azides to decide whether the scale of synthesis can be enlarged and subsequent processing of this new compound is possible in a safe manner.

In addition to measuring the mechanical (Impact Sensitivity Testing, Friction Sensitivity Testing) and electrical (ESD Testing) sensitivity of energetic compounds, it is essential to also analyze their thermal and caloric properties thoroughly. The two most common thermoanalytical techniques to investigate thermal and caloric properties of energy-rich compounds are Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA).

Pharmablock also attaches great importance to process safety. For example, we studied the stability of the following Compound 7 during synthesis. The test results are as follows.



Compound 7

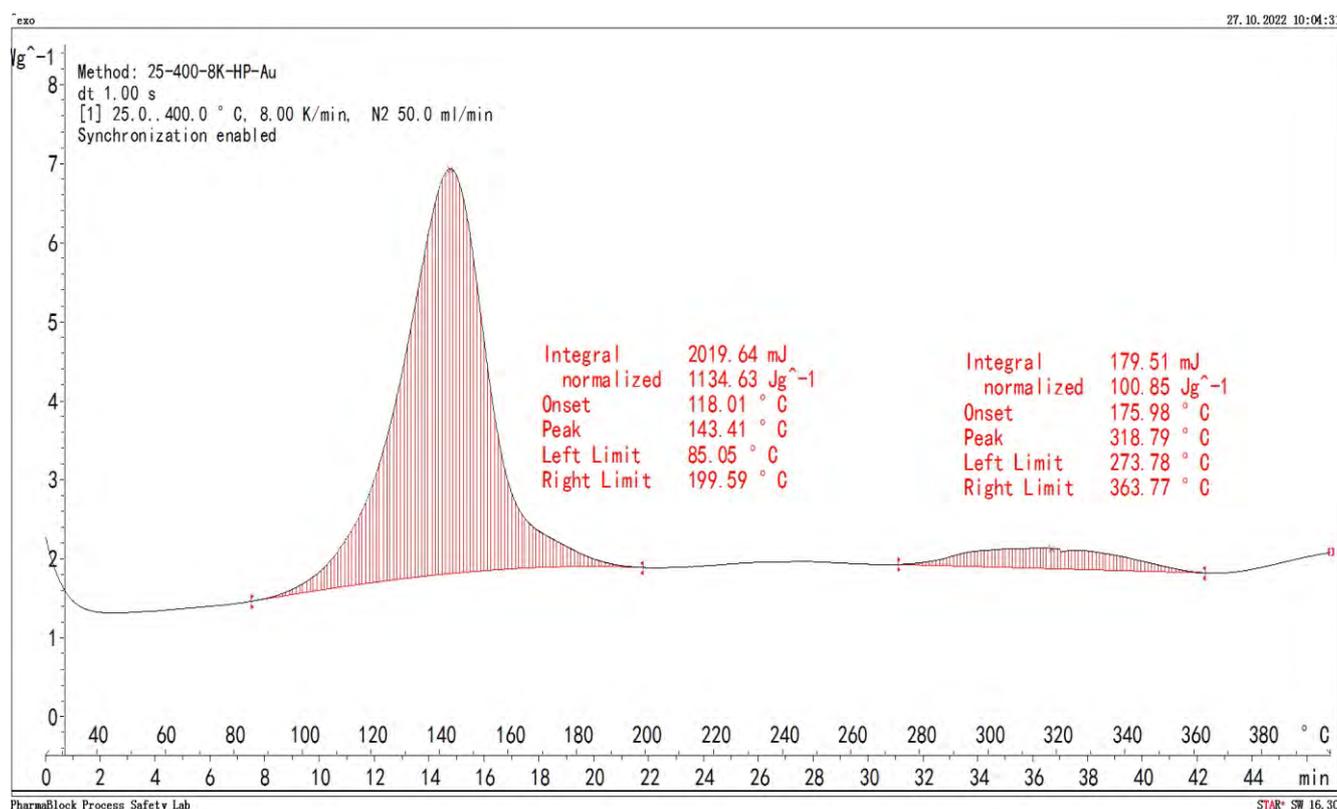


Figure 5. DSC Curve of Compound 7

Use a differential scanning calorimeter (model: Mettler DSC 3+) to test the thermal stability of the sample. The test mode is dynamic mode, the heating rate is 8K/min, and the sample mass is 1.78 mg. Use a high-pressure sealed golden crucible. The test protective gas is high-purity nitrogen (flow rate: 50 mL/min). The heat flow curve is as shown in Figure 5. The results show that the sample has two exothermic peaks within the range of 25~400 °C. The T_{onset} of the main exothermic peak is 118.01 °C, the T_p (peak temperature) is 143.41 °C, and the decomposition heat is calculated to be 1134.63 J/g. When the temperature rises to more than 273.78 °C, the sample continues to undergo decomposition exothermic, and the decomposition heat is about 100.85 J/g. According to the test results, it can be seen that the sample has lower initial decomposition temperature, higher exothermic and higher risk of thermal explosion.

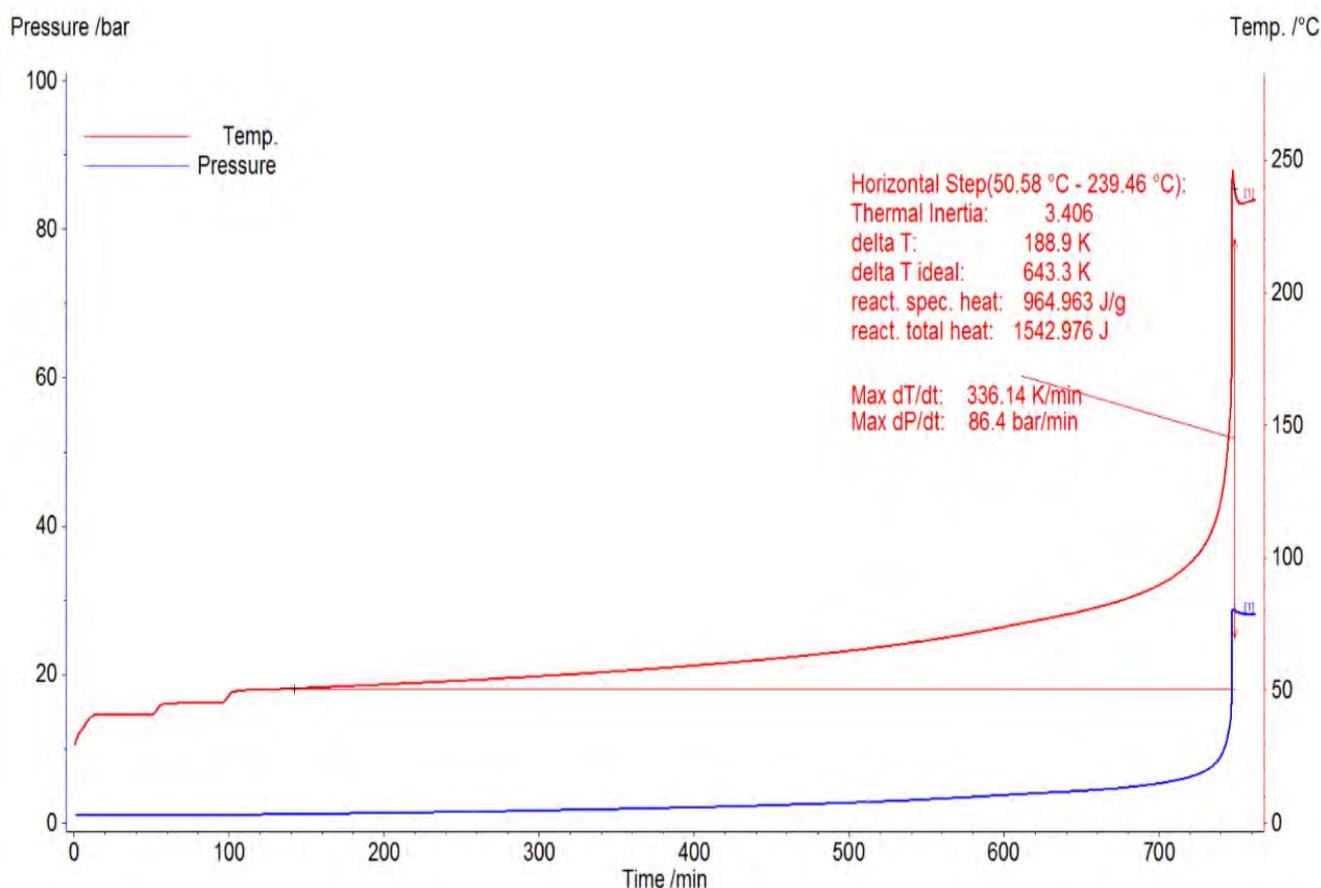


Figure 6. ARC of Compound 7

In order to further determine the thermal explosion risk of the sample, an adiabatic accelerated calorimeter (Netzsch ARC254) is now used for thermal stability test. The test temperature range is 35~300 °C, the waiting time is 15min, the seek time is 15min, the test vessel is titanium alloy test cell (Ti, 10g), the sample mass is 1.6g, and the thermal inertia of the test system is 3.406. The temperature and pressure curves during the test are shown in Figure 6. According to the test results, the initial decomposition temperature of the sample during the test is about 50.58 °C, the corrected adiabatic temperature rise is 643.3 °C, the decomposition heat is calculated to be 964.96 J/g, and the maximum temperature rise rate and maximum pressure rise rate are 336.14 °C/min and 86.4 bar/min respectively. Compared with the DSC test results, the initial decomposition temperature of the sample is significantly reduced, which is mainly due to the different test modes and the more sample quality used in the adiabatic test. According to the adiabatic heat test results, the initial decomposition temperature of the sample is low, the heat release is large, and the temperature and pressure in the process of decomposition heat release have increased rapidly, so the sample has a high risk of thermal explosion.

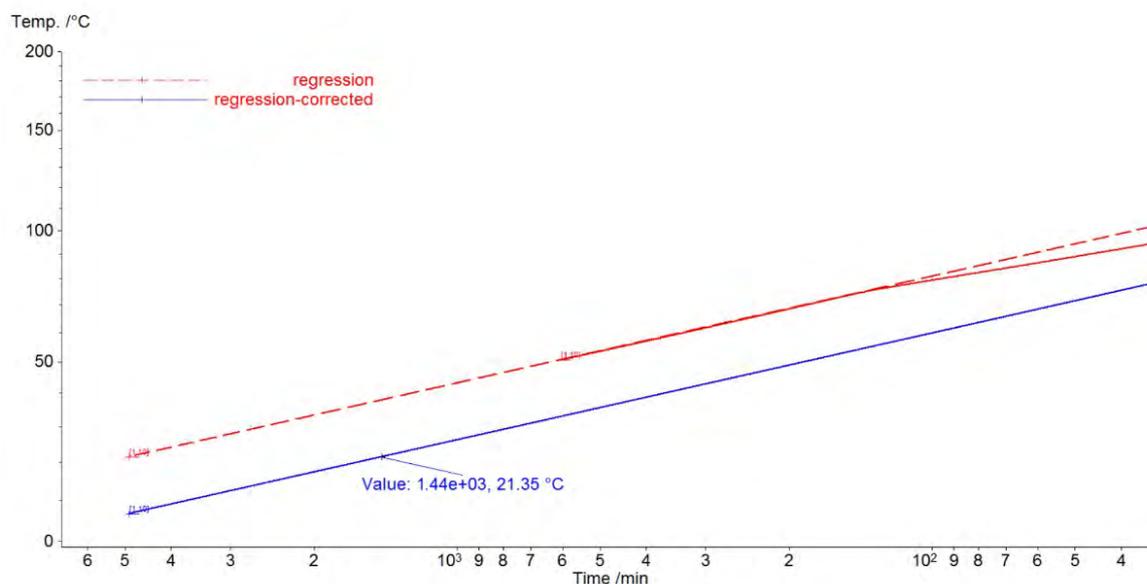


Figure 7. TMRad Calculation Results of Compound 7

Use the exothermic section data to predict the time to maximum reaction rate under adiabatic condition (TMRad) of the sample (see Figure 7). The results are shown in the Figure 7. According to the prediction results, the TD24 of the sample is 21.35 °C.

When our reaction substrate contains azide group, for safety concerns, we can consider applying flow chemistry instead of batch approach. Many references^[12-15] have used flow technology for reactions involving organic azides.

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