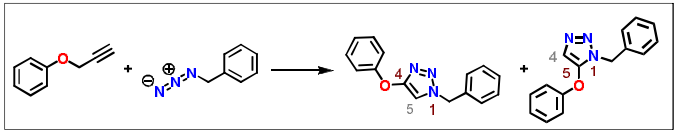
Comenius University, Faculty of Natural Sciences,
Department of Organic Chemistry, Bratislava, Slovakia

Click Chemistry in Drug Design

Andrej Boháč, 2015

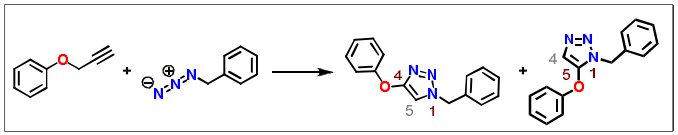
What is Click Chemistry?

joining molecules by an „***ideal chemical reaction***“



Requirements:

- **fast, irreversible** reaction, performed by **simple conditions**
- **starting materials** are readily **available**, **stable** and **biocompatible**
- **high yielding reaction**, **high atom economy**, wide application
- **insensitive** to water and oxygen
- **easy work-up** and **isolation**
- preferably **proceeding in water**

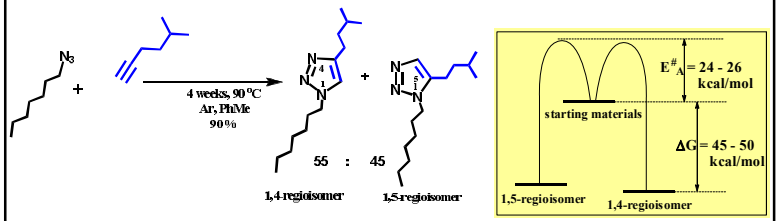


Alkynes and azides are stable across a broad range of organic reaction conditions and in biological environments. They are **highly energetic functional groups**.
Their **irreversible transformation to triazoles is highly exotermic**, albeit slow. It is a **modular reaction** (based on a fusion reaction of two universal components).

Catalysis allowes acceleration more than a million-fold giving almost **quantitative yields in water without** any need of **protection**.

Exploitation in material and life sciences.

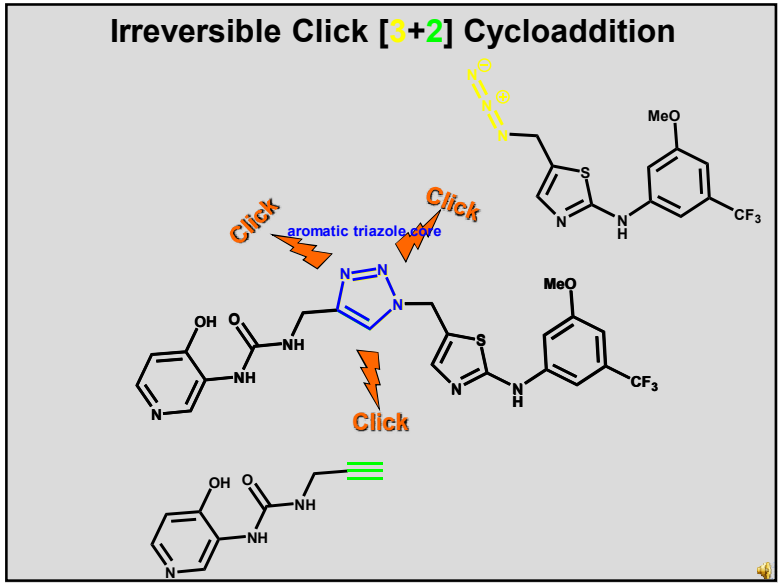
„*Ideal reaction*“ - *Huisgen* cycloaddition



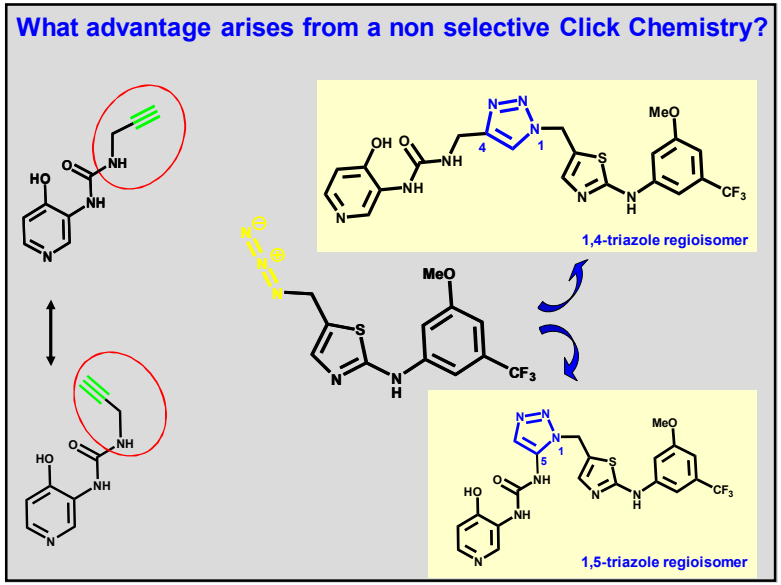
Azides and **alkynes**:

- **highly energetic species**
- **their reaction** ([3+2] cycloaddition) **is slow** due to the **high activation barrier** ($E^{\#}_A$ = 24 - 26 kcal/mol) but **highly exothermic** and **irreversible** due to the high thermodynamic driving force (ΔG = 45 - 50 kcal/mol)
- **inert** toward **water and oxygen**, **no protecting group** are needed
- **completely inert to biological molecules**

Irreversible Click [3+2] Cycloaddition

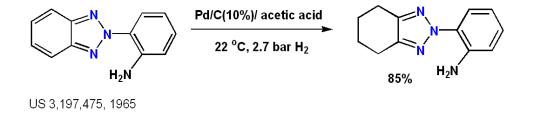


What advantage arises from a non selective Click Chemistry?

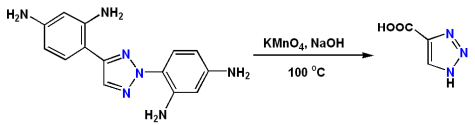


Hydrolysis, Reduction and Oxidation Conditions

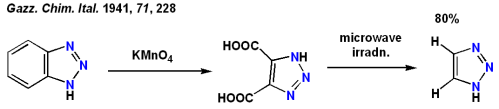
1,2,3-Triazoles:
Stable to Severe Reduction and Oxidation Conditions



US 3,197,475, 1965



Gazz. Chim. Ital. 1941, *71*, 228

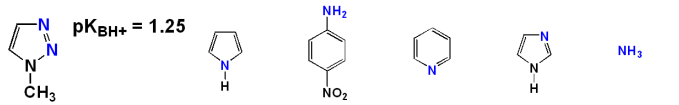


Jingxi Huagong 2003, *20*, 628

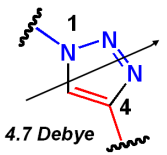
Triazole linkers can contribute to bioactivity

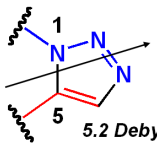
1,2,3-Triazoles:
Permanent Connectors with Pharmacophoric Properties

- Weakly basic. Hydrogen bond acceptors (N3 and N2):



- **Large dipole:**



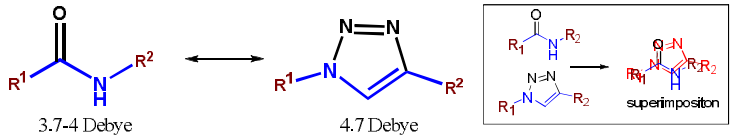


- hydrogen bonding
- dipole interactions
- pi-stacking int.
- „acidic" triazolic H-C= can form pseudo HB

1,2,3-Triazoles are bioisosteric to amides

Some peptidic groups were replaced with triazoles to improve stability against hydrolysis, but the activity of „protein" remained untouched

*(Org Biomol Chem 5 **2007** 971 – 75, TL 47 **2006** 6971-71)*



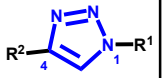
stable to hydrolysis, oxidation, reduction

Synthesis of 1,2,3-triazoles

➢ **Thermal Huisgen [3+2] cycloaddition**

1950-70 Huisgen • 80-120°C, 12-24h, both regioisomers ca 1/1
$E^{\#}_A$= **24-26 kcal/mol**

➢ **Cu(I) catalyzed** ($CuSO_4$ / sodium L-ascorbate)



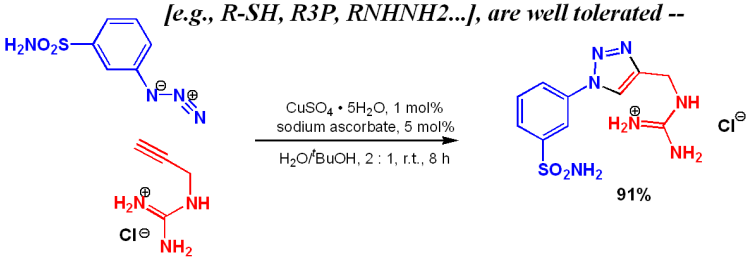
2002, Fokin, Sharpless Melda • only 1,4-regioisomer, high yield, rt, t-BuOH / water
$E^{\#}_A$= **15 kcal/mol** (10^6 times faster than Huisgen r.)

➢ **Ru catalyzed** ($Cp^*RuCl(PPh_3)_2$)

2005, Fokin Shrapless • mainly 1,5-regioisomer

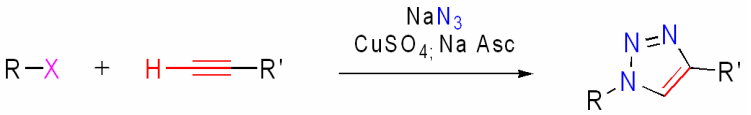
Cu(I)-catalyzed azide-alkyne cycloaddition

--- no know functional group restrictions:
all acidic and basic groups, as well as redox active groups
[e.g., R-SH, R3P, RNHNH2...], are well tolerated --



complete regiochemical control
***pH** does not matter*
***temperature** does not matter*
***solvent** does not matter*
***presence of other functional groups** does not matter*
*overall **yields can be >96%***
***purification** is not necessary*

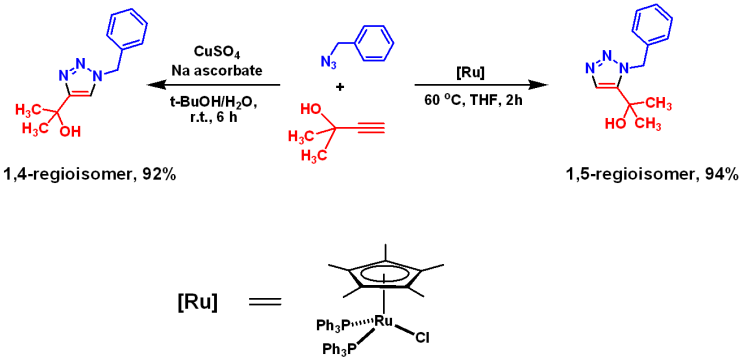
One-Pot Route



Since azide anion has no effect on the Cu-catalyzed ligation process, the azides are readily generated, and used in situ:

Alina K. Feldman, Benoît Colasson, and Valery V. Fokin*, Org. Lett., 2004

1,2,3-Triazoles: "The Other Regioisomer"

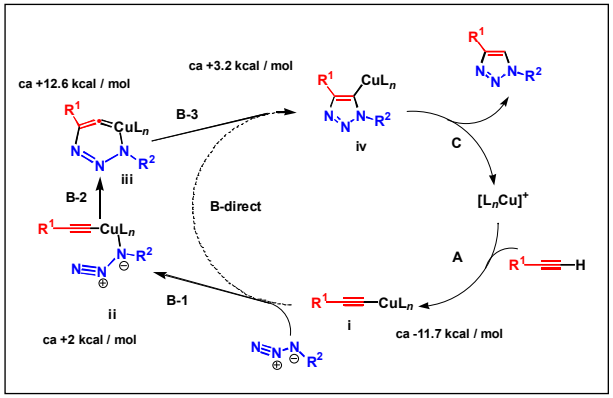


Weinreb et al. JOCH 71 2006 8680-8683.

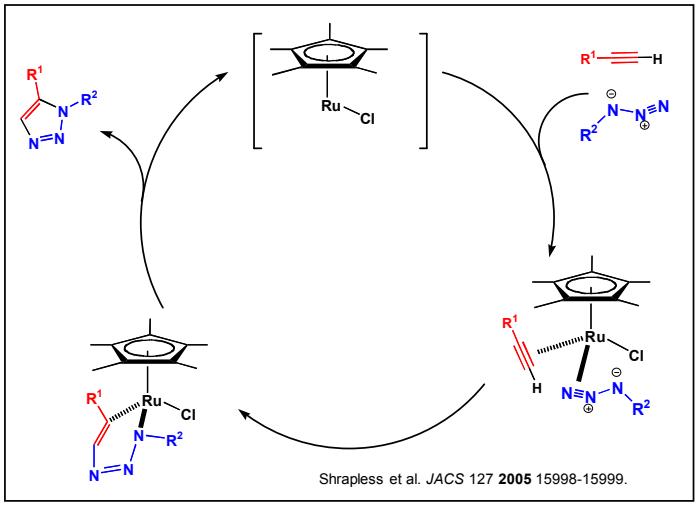
L. Zhang, G. Jia, V.V. Fokin et al. *JACS* 2005

Mechanism of Cu(I) catalysis

• H_2O / t-BuOH, **0.3 mol % CuSO4**, 3 mol % L-ascorbic acid, 20 h, rt, quant yield, **1,4-regioisomer only**



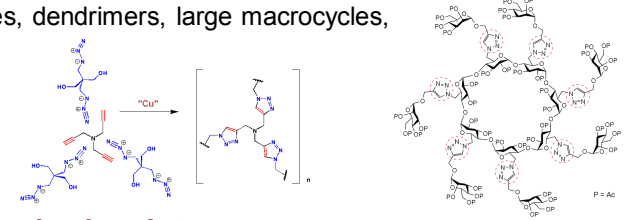
Mechanism of Ru catalysis (1 mol % Cp*RuCl(PPh3)2)



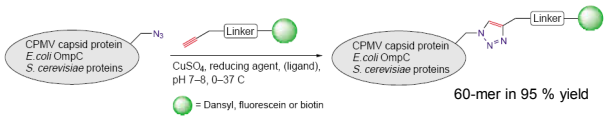
Shrapless et al. *JACS* 127 **2005** 15998-15999.

Click Chemistry Exploitation

➢ **Material sciences** (copolymers, functionalized surfaces, adhesives, dendrimers, large macrocycles,



➢ **Bioorganic chemistry** (biosensors, bioconjugates: tagging of proteins, nucleotides or in situ whole organisms)



➢ **Drug development – Medicinal Chemistry**

Click Chemistry SAR in Drug Development

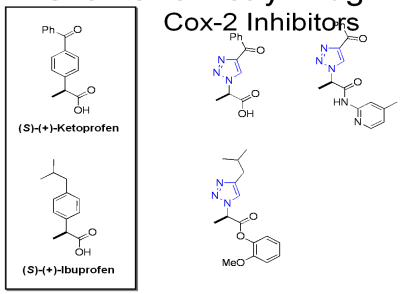
(**1/CC SAR**, 2/ In Situ CC, 3/ In Situ CC Screening)

1/ **Click chemistry as a tool for activity improvement by SAR**

- a drug aromatic core replacement by a triazole via Click chemistry

Click Chemistry Drug Mimics

Cox-2 Inhibitors

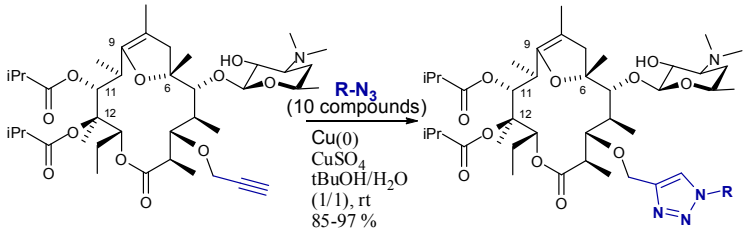


- **focused library construction**
- **cycloaddition**: thermal, Cu(I) o Ru accelerated
- **screening** (Click chemistry SAR)

Drugs for Resistant Bacterial Strains

macrolid antibiotics were found to be active against bacterial resistant strains:

staphylococcus aureus (MRSA)
vancomycin-resistant enterococcus (VRE)



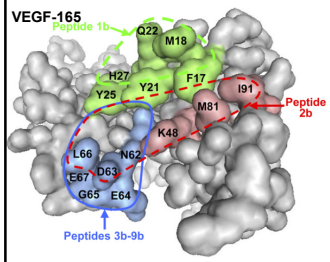
8,9-anhydroerythromycin A derivatives

SAR Click Chemistry R: adamantyl

BMCHL 17 2007 6340-44

VEGFR-1 inhibitor VEGF-A mimic

- AA residues important for receptor binding are colored, antagonists were determined by phage-display assay

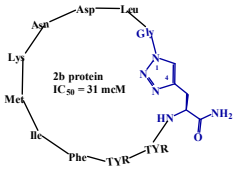


1,2,3-triazole is a peptide bond **isostere**.
Click reaction was usefull by **long chain cyclisation**.

Click reaction was performed by **Cu(I)** solid phase **catalysis**.

MIMICK OF $VEGF_{165}$: **L66-D63-N62-K48-M81-I91-F17-W21-Y25** (red)
in 3 letters **Leu-Asp-Asn-Lys -Met- Ile -Phe-Try - Tyr** (red)

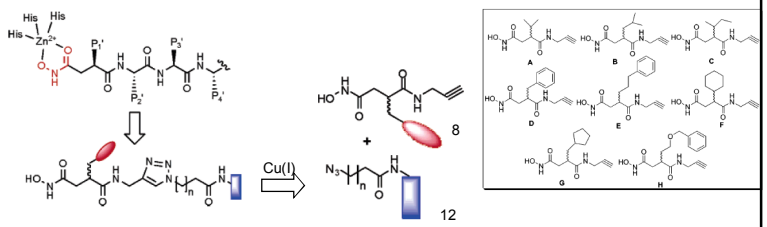
2a linear protein: N_3-Gly-**Leu-Asp-Asn-Lys-Met-Ile-Phe-Try-Tyr**-Gly-NH_2
IC_{50} = 23 mcM



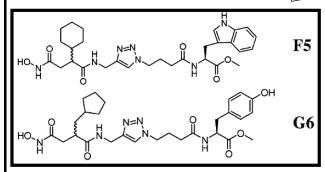
SP5.2 from phage-display VEGFR-1 specific antagonist IC_{50} = 28 mcM

BMCHL 17 **2007** 5590-4.

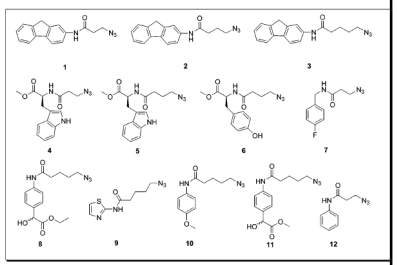
MMp selective inhibitors



8 x 12 x 2 = 96 x 2 = 192



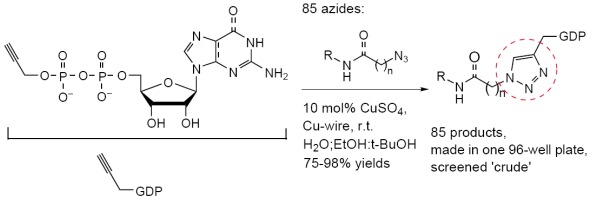
MMP7 selective, low mcM inhibitors



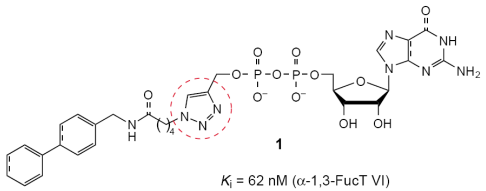
Org. Lett. 8 2006 3821-24

hu-Fucosyltransferase VI - nM inhibitor

Fucosyltransfera-se is responsible for **transfer of** an beta-L-**fucose** from GDP-fucose **to glycoprotein on the cell surface** that is important for **cell-cell recognition**, in immune response, cancer metastasis... Best old inhibitors were in mcM range.



(**note**: no protecting groups required)



1

K_i = 62 nM (α-1,3-FucT VI)
IC_{50} = 0.15 μM (α-1,3-FucT VI)

Wong et all. *JACS* 125 **2003** 9588-9589.

- **Carbonic anhydrase isozymes IX, XII and XIV**
  - BMCHL 17 **2007** 987-92.
- **Tacrine-melatonin hybrids**
  - JMCH 49 **2006** 459-62..
- **Protein tyrosine phosphatases**
  - Org Lett 8 **2006** 713-16, BMCH 15 2007 458-73.
- **Cyclic tetrapepdide**
  - Org Lett 8 **2006** 919-22.
- **Super-potent G-protein ligands**
  - J. Comb. Chem. 8 **2006** 252-61.
- **Zanamivir**
  - BMCHL 16 **2006** 5009-13.
- **Adenosine receptor agonists**
  - JMCH 49 **2006** 7373-83.
- **FAAH inhibitors**
  - Chem Biol 12 **2005** 1157-58.

- **Spiramycin**
  - Heterocycles 69 **2006** 55.
- **Inhibitor of STAT3**
  - BMCHL 17 **2007** 3939-42.
- **Podophyllotoxin and steganacin analogues**
  - BMCH 15 **2007** 6748-57.
- **Ceramide**
  - BMCHL 17 **2007** 4584-87.
- **F-18 fluoro** (PET marked proteins)
  - TL 47 **2006** 6681-84,
    Lett in Drug Des Disc 4 **2007** 279-85.
- **Alpha-GalCer immunostimulant**
  - JMCH 50 **2007** 585-89.
- **Leishmania beta-1,2-mannosyltransferases**
  - ChemBiochem7 **2006** 1384-91.
- **DNA methyltransferase**
  - Org Lett 7 **2005** 2141-44.

In Situ Click Chemistry (**TDS**) target driven synthesis

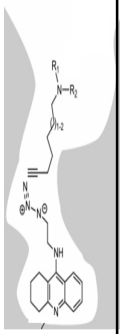
reduces the number of inactive compounds

compensate the lack of precision in the predictive ability of in Silico chemistry

Click chemistry is **completely biocompatible**, uses irreversible reaction to **unite reagents inside the protein´s binding pocket**

target itself will pick up the best fitting ligands from diverse sets of chemical building blocks

Significant portion of the reaction activation barrier is entropic (pieces have to approach each other in precisely the right orientation), pre-assembly of building blocks on the target active site can **accelerate cycloaddition.**



DDT 9 2004 348.

Click Chemistry in Drug Development

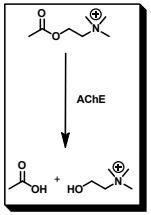
(1/Drug D&I, 2/ **In Situ CC**, 3/ In Situ CC Screening)

In Situ Click Chemistry (*AChE-2002, HIV-1 protease-2006*)

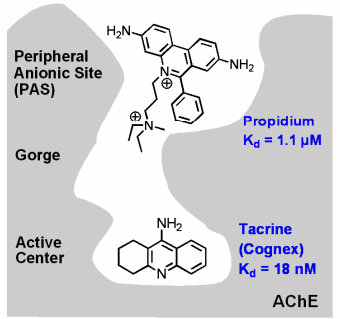
- ligands are incubated with biological target that catalyses the reaction
- only the best fitting ligands from combinatorial library are connected to form product
- both regioisomers can be formed by **orthogonal cycloaddition**
- the best inhibitor will be created **(nM - fM)**
- direct LC-MS-SIM identification (MS fragments and retention time)
- synthesis and bioactivity evaluation

Acetylcholine Esterase Inhibition

Neurological Diseases (Alzheimer...)



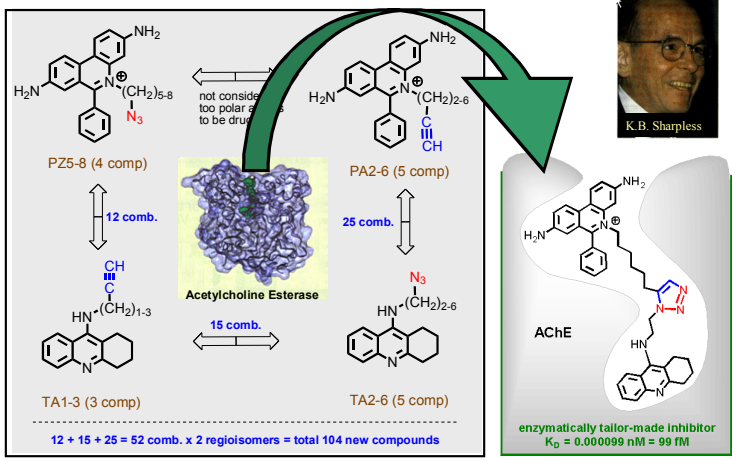
Acetylcholinesterase



- Terminates neurotransmission through hydrolysis of the neurotransmitter ACh
- AChE Inhibitors:
  - Alzheimer drugs (e.g. tacrine, Cognex™)
- Two distinct binding sites at opposite ends of a 20Å deep gorge: PAS and active center

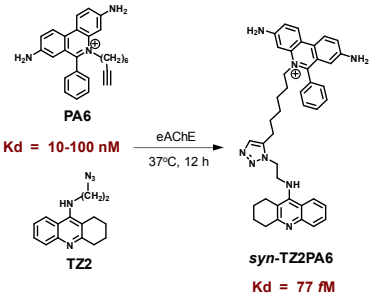
Orthogonal In Situ Click Chemistry

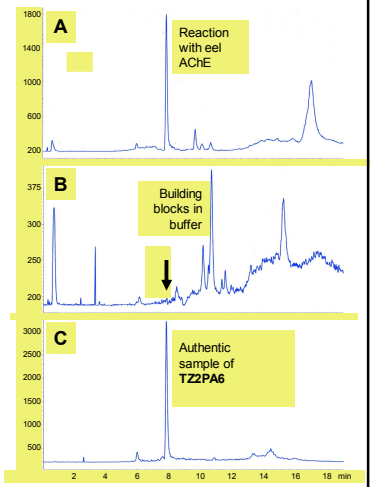
The enzyme AChE catalyzes the formation of its own femtomolar inhibitor.



Sharpless et al. *Angew. Chem. IE* 41 **2002** 1053-1057. *JACS* **2004**, 126, 12809 - 12818.

Screening: LC/MS-SIM





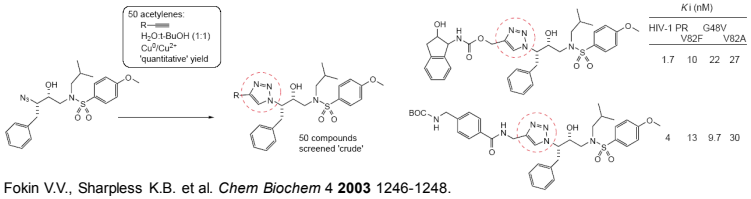
Great sensitivity
- Short reaction times, fast screening throughput
- Small reagent amounts

Reliable product identification
- HPLC separation of components
- ID by molecular weight & retention time

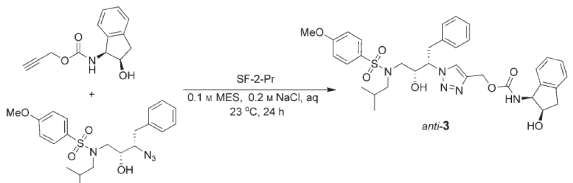
K. B. Sharpless, H. C. Kolb et al. *JACS* **2004**, *126*, 12809.

HIV protease nM inhibitors

HIV protease is responsible for virus maturation in AIDS disease. Because of fast virus mutation, new drugs are needed. Starting scaffold was inspired by Glaxo´s drug Amprenavir. Reaction in water, screened as crude products against wild type and mutants of HIV.



Fokin V.V., Sharpless K.B. et al. *Chem Biochem* 4 **2003** 1246-1248.



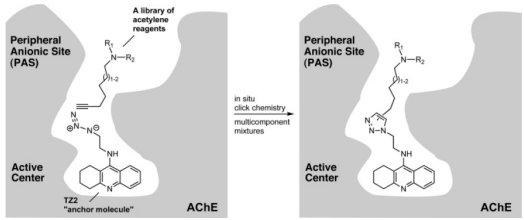
Sharpless K.B., Elder J.H., Fokin V.V. et al. *Angew Chem IE* 45 **2006** 1435-1439.

Click Chemistry in Drug Development

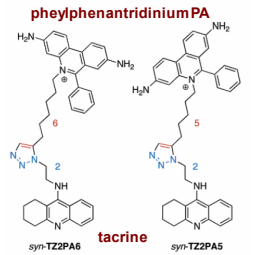
(1/Drug SAR, 2/ In Situ CC, 3/ In Situ CC Screening)

In Situ Click chemistry Screening (AChE-2005, bCA-II-2005)

- library with **one anchor ligand** and other ligands with unknown activities (*in situ CC screening*)
- **target** itself can **assemble the combinations** between the anchor compound and other best fitting ligands
- **new inhibitors could be easily identified** by in situ Click chemistry screening

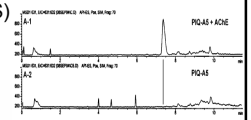


In Situ Click Chemistry Screening

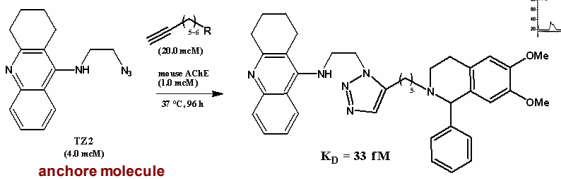


ACH IE 41 **2002** 1053-57, JACS 126 **2004** 2809-18.

from potentially 104 products, only 2 femtomolar inhibitors (1,5-triazoles) were assembled inside AChE both having 99fM activities. Triazoles were 2 methylene away from tacrine. From X-ray: **triazoles contribute to bioactivity** (enzyme accelerates cycloaddition by lowering the energy of TS)



23 PA com. mimics



10 alkines at once crude to LC/MS-SIM

Two enantiomers 33 and 36 fM, 3 x more active and better pharmacophoric properties

JACS 127 **2005** 6686-6692.

Carbonic Anhydrase Inhibitors

In Situ Click chemistry Screening

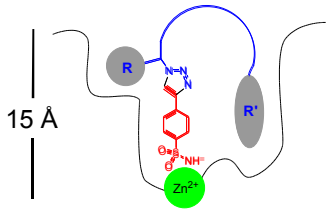
Carbonic anhydrase

- **catalyzes the interconversion of HCO_3^- and CO_2**
- **involves in key biological processes**
  - **respiration and transport of CO_2/HCO_3^-**
  - **acid secretion and pH control**
  - **bone resorption and calcification**
  - **glaucoma, tumorigenicity …**
- **Inhibitors: Ar-SO_2NH_2 (Anchore)**
- **CA-IX & XII overexpressed in tumors**

Test Case for Validation Purposes:

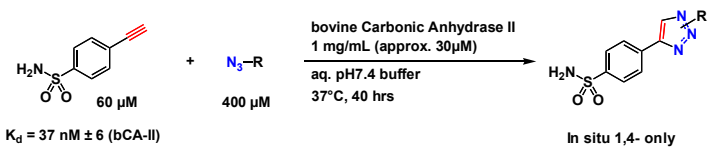
- **Carbonic Anhydrase-II**
  - **Expressed in erythrocytes, lung, stomach, kidneys**

15 Å



V.P. Mocharla, K.B. Sharpless, H.C. Kolb, *et al. Angew. Chem. IE 44*, **2005**, 116-120.

Carbonic Anhydrase: Binding Affinities



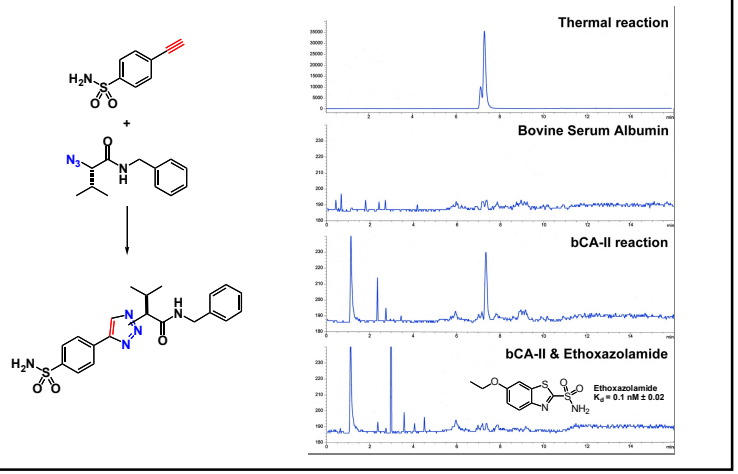
K_d = 37 nM ± 6 (bCA-II)

In situ 1,4- only

- No 'false positives' (no enzyme no product)
- Some 'false negatives' (some active 1,4-triazoles not formed in situ)
- *In situ* hits are the most potent compounds (triazol not contributes)

9 in situ hits	1 in situ hit	1 in situ hit	No in situ hits	No in situ hits	No in situ hits
K_d =					
0.2 – 2.4 nM	5 nM	7 nM	inactive	1.3 & 9 nM	8 nM
185 – 15 x	7.4 x	5.2 x		28 & 4 x	4.6 x

Carbonic Anhydrase: Hit Discovery & Validation



Thank you for your attention