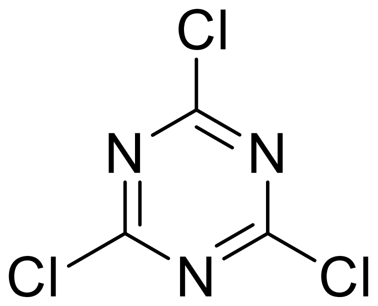
10.3390/ecsoc-24-08502

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

s-Triazine: A Multidisciplinary and International Journey



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[2]KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa
[3]Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

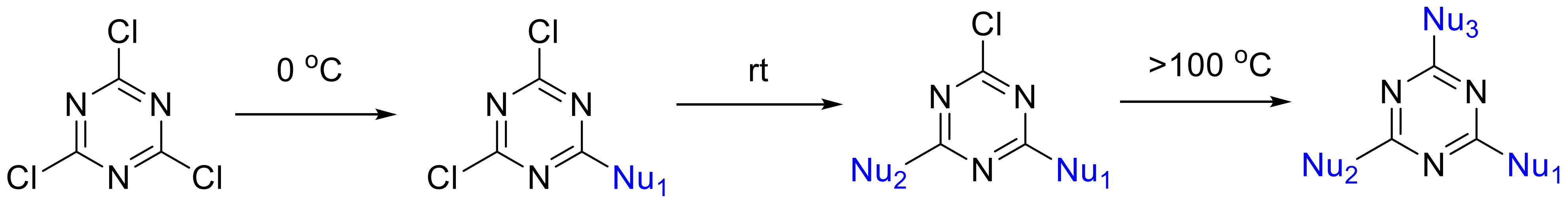
Abstract for 24th ECSOC

2,4,6-Trichloro-1,3,5-triazine (TCT) offers unique ability to undergo sequential nucleophilic substitution reaction using regular nucleophiles (first Cl replacement at 0 ºC, second at rt and third at > 90 ºC) making s-triazine a privileged scaffold finding application in drug development with an extension towards development of new materials.

This selective chemical property of TCT fulfills the goal of the chemists to control the organic structures and make it react in the required condition for achieving each objective. In this regard, orthogonality and chemoselectivity are two modern organic chemistry concepts which have been exploited in various areas of research ranging from supramolecular chemistry to organic/bioconjugation chemistry. We have demonstrated the fusion of these two concepts using TCT as "**Orthogonal Chemoselectivity**" and defined it **as discrimination between reactive sites in any order**. The usage of azide as one of the nucleophiles modulated the reactivity of s-triazine core for the last Cl replacement. This allowed us to overcome the barrier of higher temperature (> 90 ºC) for the last Cl replacement which happened at rt taking advantage of side chain of Cys, Tyr and Lys in biological context.

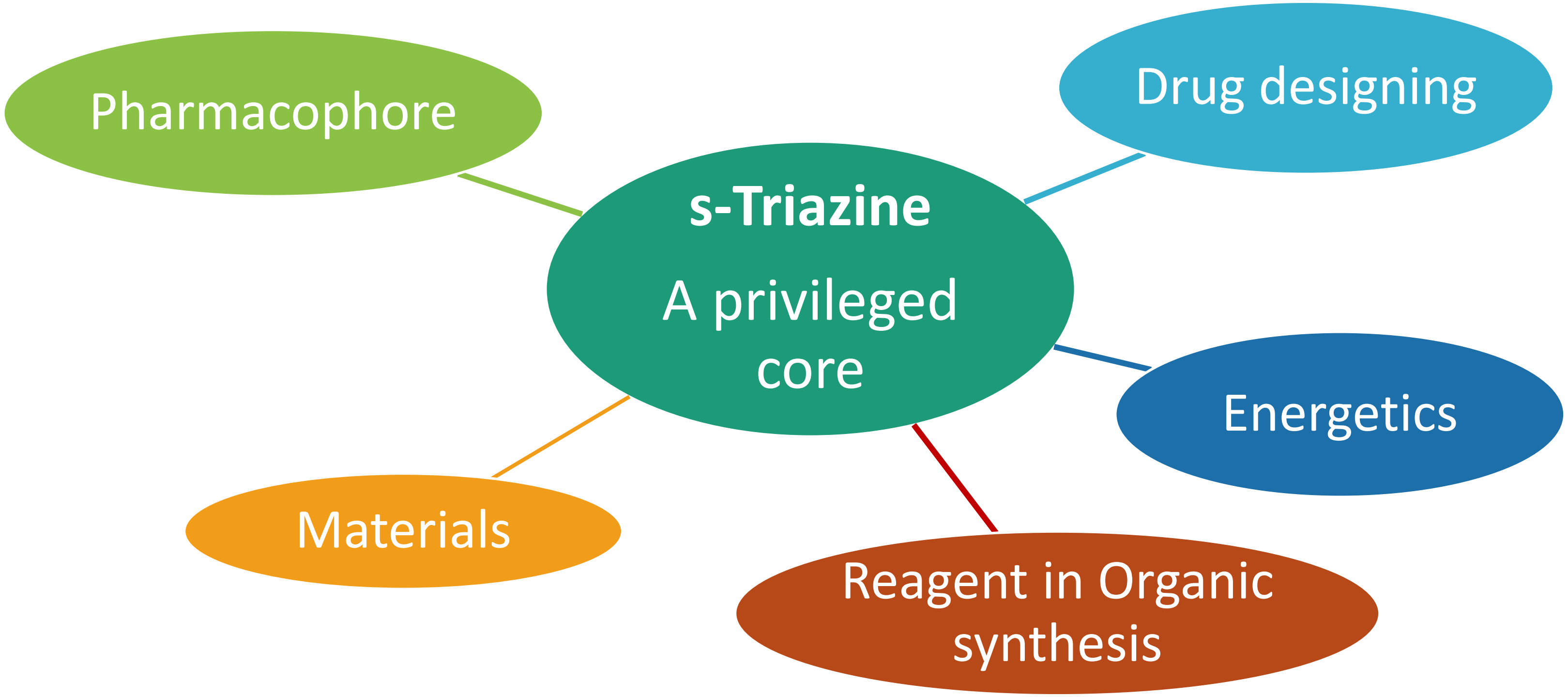
In this presentation, we revise the chemistry developed in our laboratories to manipulate the TCT core for application in our medicinal chemistry programs and in bioconjugation.

Reactivity of TCT and Applications of their derivatives



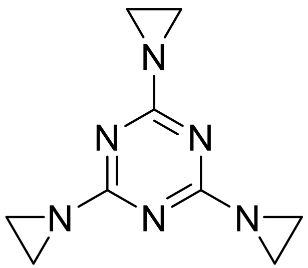
TCT

Nu = Nucleophile



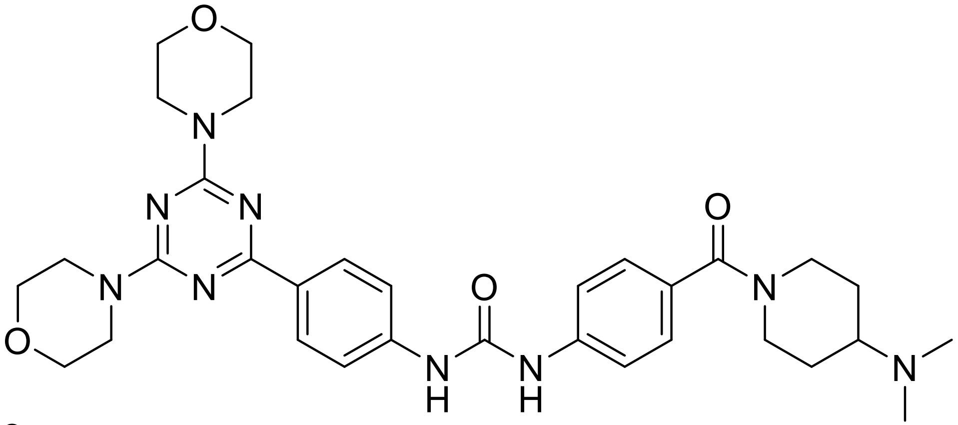
E.E. Simanek, H. Abdou, S. Lalwani, J. Lim, M. Mintzer, V.J. Venditto, B. Vittur, *Proc. R. Soc. A*, **2010**, 466, 1445.
P. Leriche, F. Piron, E. Ripaud, P. Frère, M. Allain, J. Roncali, *Tetrahedron Lett.*, **2009**, 50, 5673.

Some Commercial s-Triazine Derivatives in the Drug Market



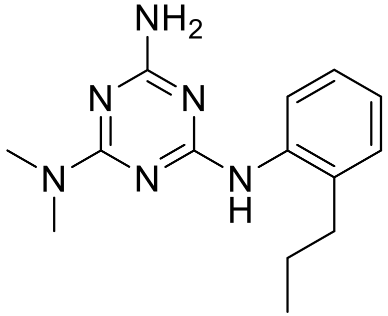
Tretamine

Cause chromatid aberrations



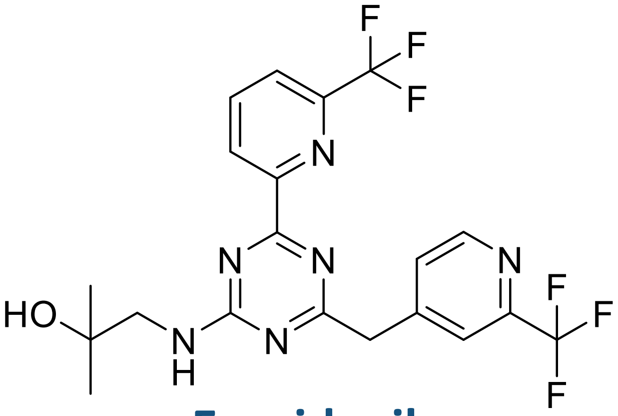
Gedatolisib

Targets PI3K/mTOR



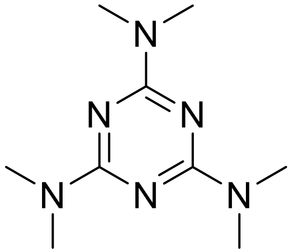
HL010183

Inhibits proliferation and invasion of Hs578T triple-negative (TN) breast cancer cells



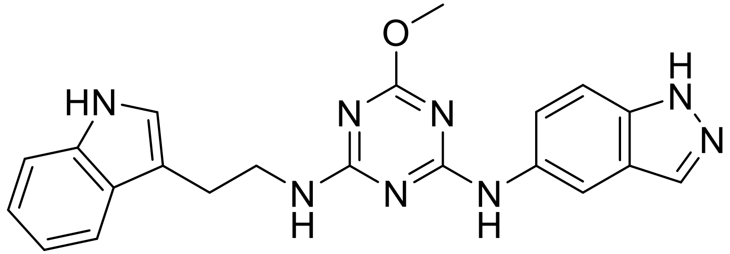
Enasidenib

Isocitrate dehydrogenase inhibitor



Altretamine

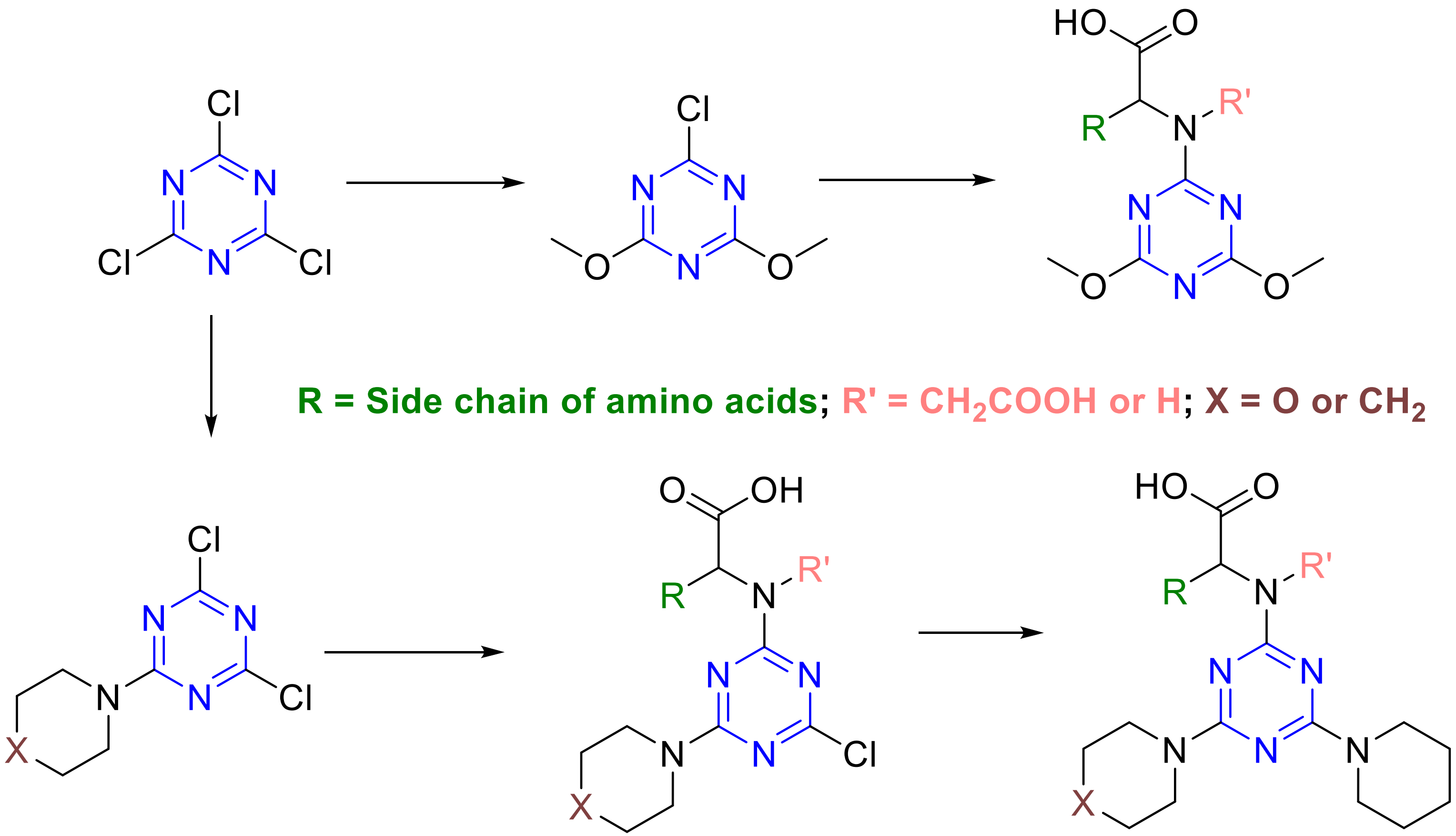
Alkylating antineoplastic agent



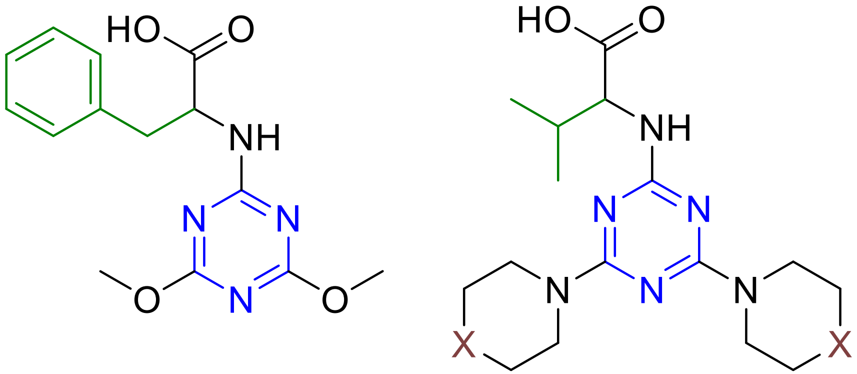
KY-04031

PAK4 inhibitor

Our Journey with s-Triazine – MAO-A inhibitors



Total 21 derivatives synthesized

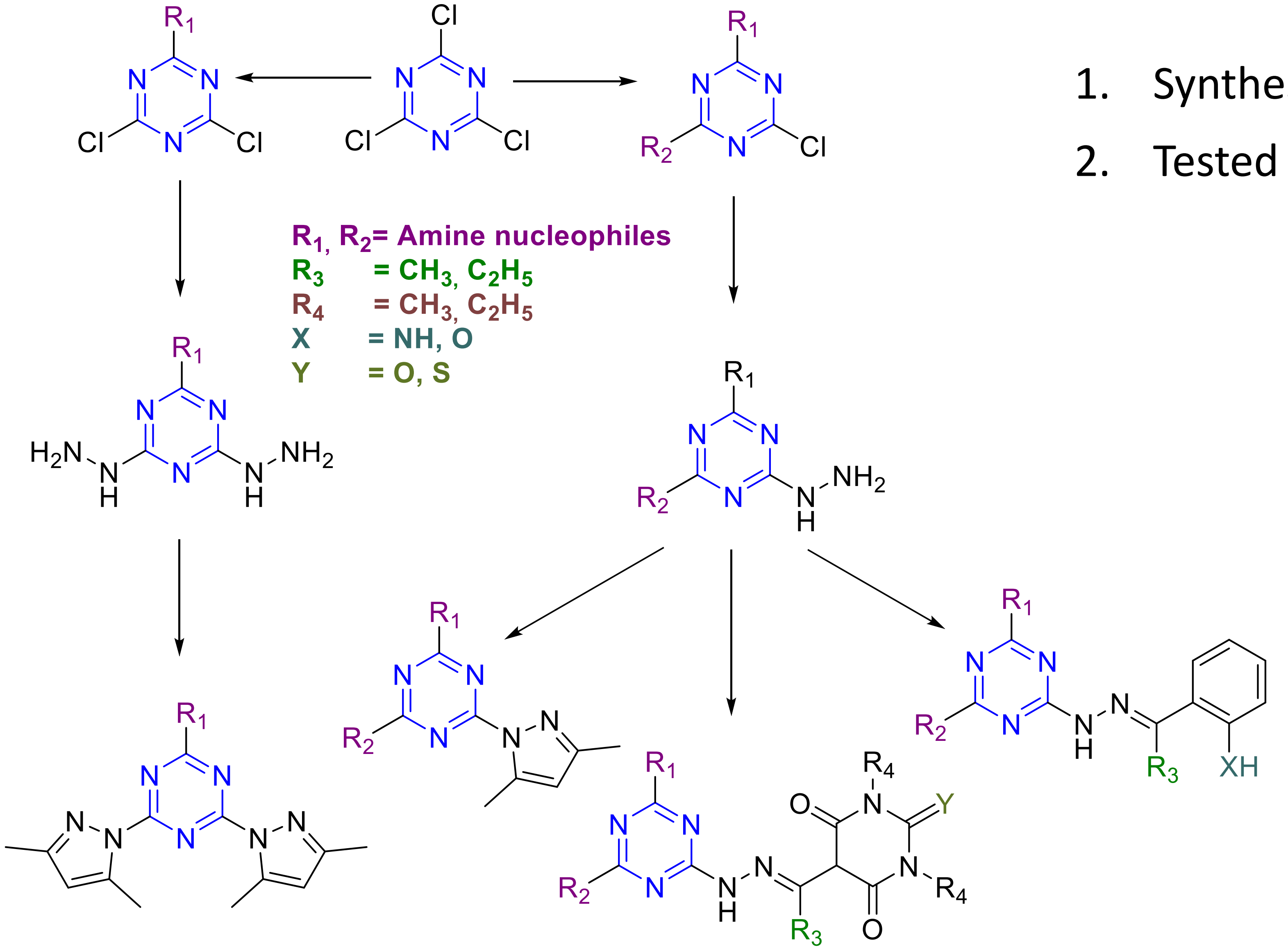


Potential MAO-A inhibitors

Comparable to reference Clorgyline

S.N. Khattab, H.H. Khalil, A.A. Bekhit, M.M. El-Rahman, A. El-Faham, F. Albericio, *Molecules*, **2015**, 20, 15976.

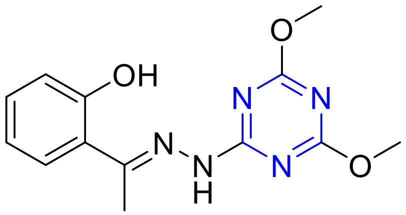
Our Journey with s-Triazine – Antiproliferative/Anticancer agents



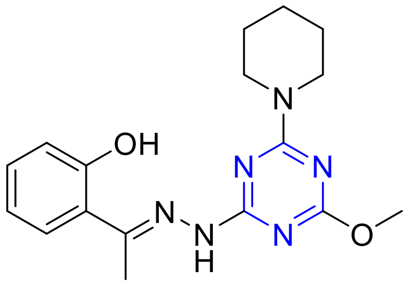
1. Synthesized 34 trisubstituted derivatives
2. Tested against cancer cell lines
   - ✓ Lung carcinoma (A549),
   - ✓ Hepatocellular carcinoma (HepG2),
   - ✓ Adenocarcinoma (MCF-7),
   - ✓ Human breast cancer (MCF, MDA-MB-231),
   - ✓ Human colorectal carcinoma (LoVo, HCT-116),
   - ✓ Human leukemia (K562)

A. El-Faham, S.M. Soliman, H.A. Ghabbour, Y.A. Elnakady, T.A. Mohaya, M.R.H. Siddiqui, F. Albericio, *J. Mol. Struct.*, **2016**, 1125, 121.
M. Farooq, A. Sharma, Z. Almarhoon, A. Al-Dhfyan, A. El-Faham, N.A. Taha, M.A.M. Wadaan, B.G. de la Torre, F. Albericio, *Bioorg. Chem.*, **2019**, 87, 457.
H. Al Rasheed, K. Dahlous, A. Sharma, E. Sholkamy, A. El-Faham, B.G. de la Torre, F. Albericio, *ACS Omega*, **2020**, 5, 15805.

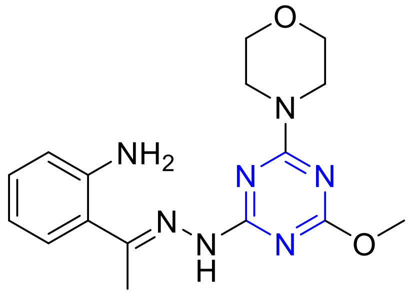
Our Journey with s-Triazine – Antiproliferative/Anticancer Agents



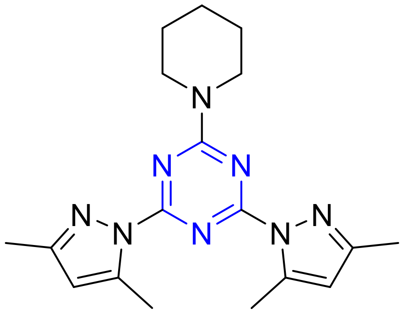
A549 = 19.9 (±2.1)
HepG2 = 15.5 (±1.7)



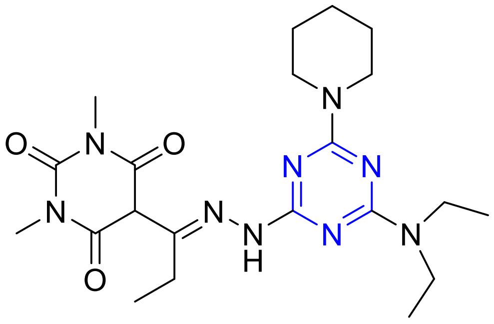
A549 = 5.6 (±1.1)
HepG2 = 6.5 (±1.7)



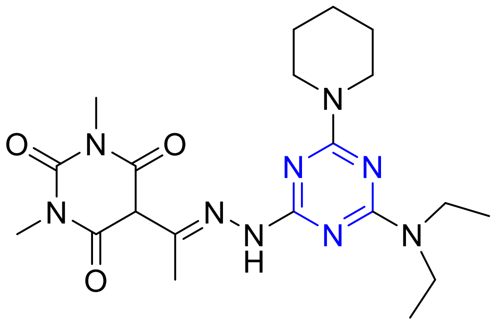
A549 = 9.3 (±1.6)
HepG2 = 1.5 (±0.25)



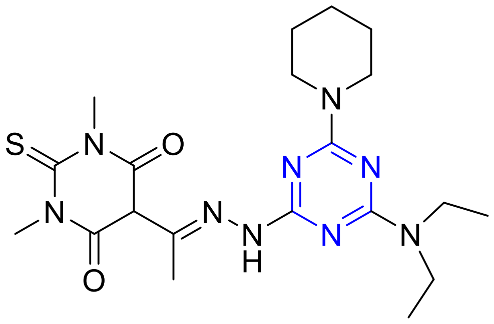
MCF = 7.5
MDA-MB-231 = 14
HepG2 = 17.5
LoVo = 6.1
K562 = 9.8



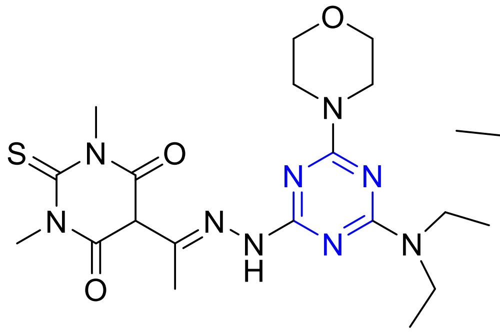
A549 = 5.6 (±1.1)
HepG2 = 6.5 (±1.7)
HCT-116 = 3.9 (±0.4)
MCF-7 = 16.1 (±1.5)



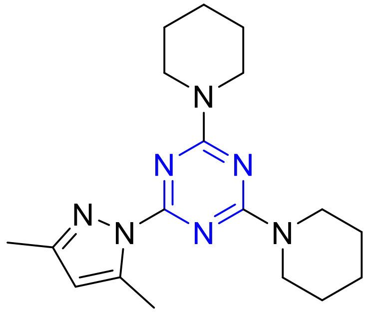
A549 = 9.4 (±7.2)
HepG2 = 15.2 (±1.8)
HCT-116 = 13.1 (±1.2)
MCF-7 = 19.1 (±0.7)



A549 = 1.6 (±0.6)
HepG2 = 3.8 (±0.3)
HCT-116 = 1.9 (±0.4)
MCF-7 = 1.2 (±0.5)



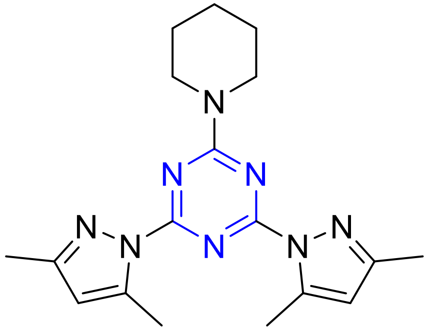
A549 = 9.7 (±3.3)
HepG2 = 11.0 (±0.2)
HCT-116 = 7.9 (±0.3)
MCF-7 = 15.8 (±0.7)



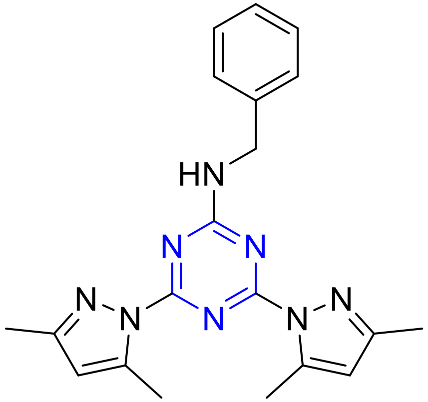
MCF = 5.0
MDA-MB-231 = 15
HepG2 = 21.2
LoVo = 8.4
K562 = 5.9

A. El-Faham, S.M. Soliman, H.A. Ghabbour, Y.A. Elnakady, T.A. Mohaya, M.R.H. Siddiqui, F. Albericio, *J. Mol. Struct*., **2016**, 1125, 121.
M. Farooq, A. Sharma, Z. Almarhoon, A. Al-Dhfyan, A. El-Faham, N.A. Taha, M.A.M. Wadaan, B.G. de la Torre, F. Albericio, *Bioorg. Chem*., **2019**, 87, 457.
H. Al Rasheed, K. Dahlous, A. Sharma, E. Sholkamy, A. El-Faham, B.G. de la Torre, F. Albericio, *ACS Omega*, **2020**, 5, 15805.

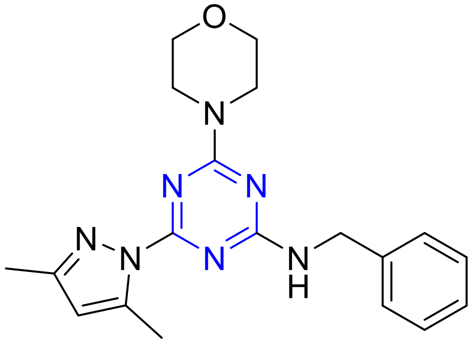
Our Journey with s-Triazine – Antimicrobial Agents



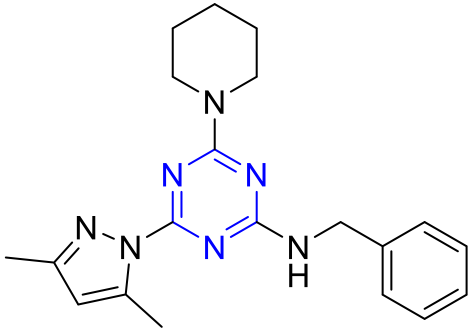
P. aeruginosa = 13
M. luteus = 17
MRSA = 11



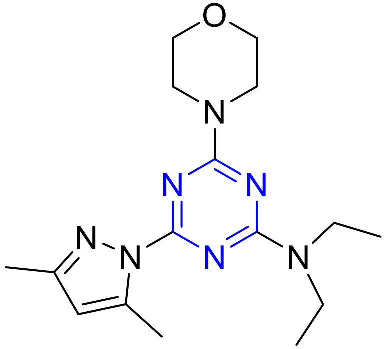
P. aeruginosa = 13
M. luteus = 15
MRSA = 7



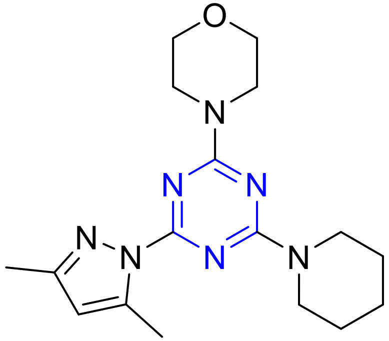
P. aeruginosa = 17
M. luteus = 9
MRSA = 8



P. aeruginosa = 19
M. luteus = 22
MRSA = 11



P. aeruginosa = 9
M. luteus = 17
MRSA = 8
C. albicans = 8



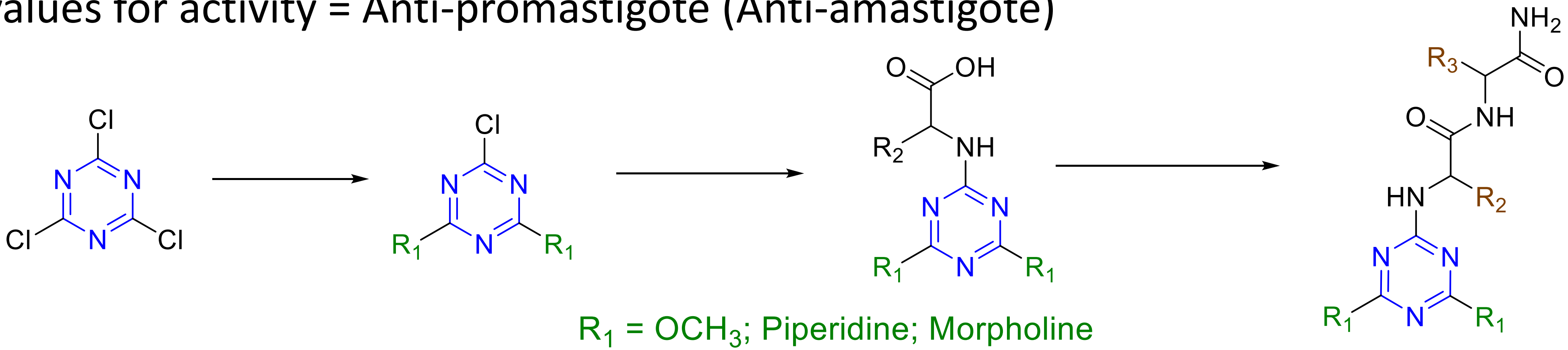
C. albicans = 9

A. Sharma, H. Ghabbour, S.T. Khan, B.G. de la Torre, F. Albericio, A. El-Faham, *J. Mol. Struct*., **2017**, 1145, 244.

Our Journey with s-Triazine – Antileishmanial Agents

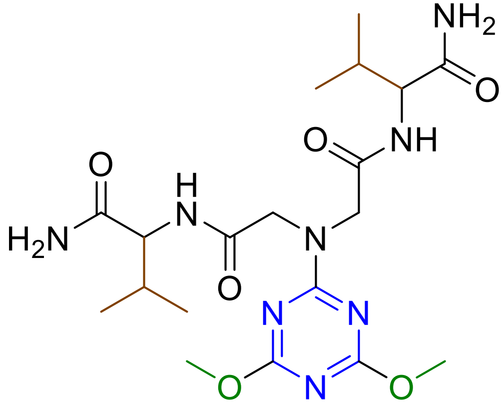
Synthesized total of 20 derivatives

IC_{50} values for activity = Anti-promastigote (Anti-amastigote)

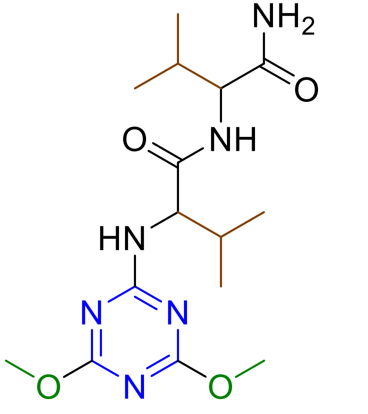


R_1 = OCH_3; Piperidine; Morpholine

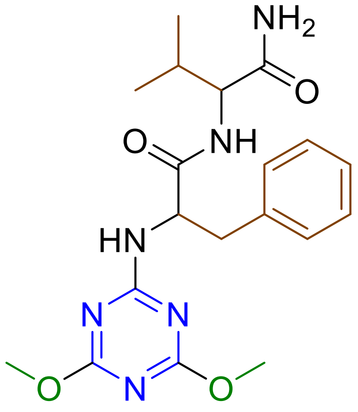
R_2/R_3 = Side chain of amino acid



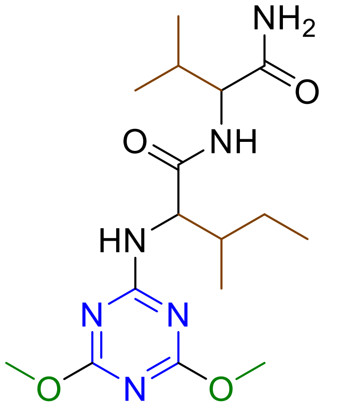
1.4±0.04 (0.02±0.02)



4.7±0.11 (0.47±0.08)



2.3±0.06 (0.37±0.06)



5.0±0.08 (0.55±0.08)

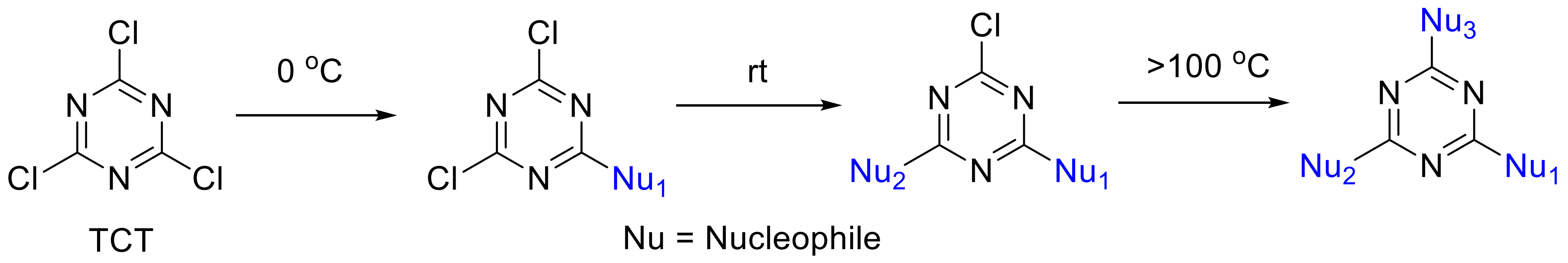
S.N. Khattab, H.H. Khalil, A.A. Bekhit, M.M.A. El-Rahman, B.G. de la Torre, A. El-Faham, F. Albericio, *ChemMedChem*, **2018**, 13, 725.

Conclusion: s-Triazine in Medicinal Chemistry

- ✓ **s-Triazine** as a **privileged structure**.
- ✓ **Amine-bearing s-triazine motif** is the "**Master Key**" as it is integral part of several commercial drugs.
- ✓ Piperidine is better compared to morpholine in enhancing the antiproliferative/anticancer activity.
- ✓ Thiobarbituric analogue with piperidine possess better anticancer activity.
- ✓ One pyrazole is better than two in addition to benzylamine and piperidine for antimicrobial activity.
- ✓ Dimethoxy substituent is better than piperidine and morpholine for antileishmanial activity.
- ✓ Hydrophobic amino acids with amide at C terminal enhances antileishmanial activity.

S.N. Khattab, H.H. Khalil, A.A. Bekhit, M.M. El-Rahman, A. El-Faham, F. Albericio, *Molecules*, **2015**, 20, 15976.
A. El-Faham, S.M. Soliman, H.A. Ghabbour, Y.A. Elnakady, T.A. Mohaya, M.R.H. Siddiqui, F. Albericio, *J. Mol. Struct.*, **2016**, 1125, 121.
M. Farooq, A. Sharma, Z. Almarhoon, A. Al-Dhfyan, A. El-Faham, N.A. Taha, M.A.M. Wadaan, B.G. de la Torre, F. Albericio, *Bioorg. Chem.*, **2019**, 87, 457.
H. Al Rasheed, K. Dahlous, A. Sharma, E. Sholkamy, A. El-Faham, B.G. de la Torre, F. Albericio, *ACS Omega*, **2020**, 5, 15805.
A. Sharma, H. Ghabbour, S.T. Khan, B.G. de la Torre, F. Albericio, A. El-Faham, *J. Mol. Struct.*, **2017**, 1145, 244.
S.N. Khattab, H.H. Khalil, A.A. Bekhit, M.M.A. El-Rahman, B.G. de la Torre, A. El-Faham, F. Albericio, *ChemMedChem*, **2018**, 13, 725.

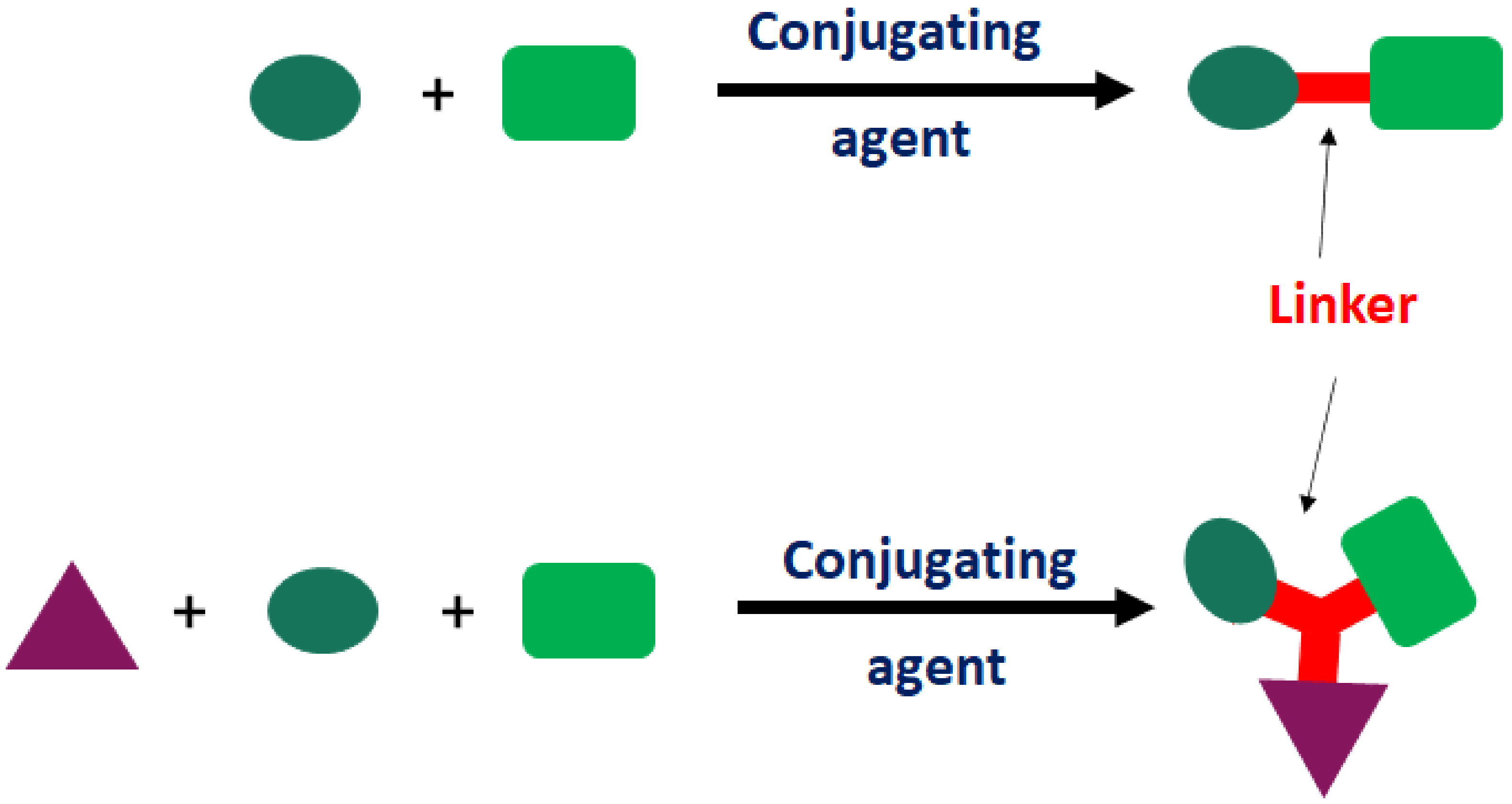
s-Triazine – As a Linker in Bioconjugation



- ✓ Taking advantage of the three position, and sequential addition of nucleophiles:
- ➢ s-Triazine can be used in **bioconjugation** as **linker**!!!
- ➢ Exploring **orthogonal chemoselectivity** in TCT.

Bioconjugation

- Refers to attachment of one molecule to another (covalent bond) of which one is of biological origin.
- Usually between **2 molecules** or **3 molecules**.



Orthogonality

The idea of orthogonality was first introduced in 1977 by Barany and Merrifield

Demonstrated by Barany and Albericio in 1985 as applied to protecting groups

J. Am. Chem. Soc. **1985**, *107*, 4936–4942

A Three-Dimensional Orthogonal Protection Scheme for Solid-Phase Peptide Synthesis under Mild Conditions[1,2]

George Barany*[3a] and Fernando Albericio[3b]

Contribution from the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received December 20, 1984*

protecting groups including the anchoring linkage are removed by the same chemical mechanism (acidolysis), so that chemical selectivity must be attained by modulation of kinetic parameters.

With Prof. R. B. Merrifield, we have defined[5b,8,9] an *orthogonal* system as a set of completely independent classes of protecting groups, such that each class of groups can be removed in any order and in the presence of all other classes. An orthogonal protection scheme offers the prospect for use of deblocking reagents that are substantially *milder* than those used in schemes based on graduated lability to the same type of reagent, because in the orthogonal case, selectivity can be attained on the basis of differences in chemistry rather than in reaction rates.

Chemoselectivity

Trost (1983) introduced the concept of chemoselectivity

Selectivity: A Key to Synthetic Efficiency

Barry M. Trost

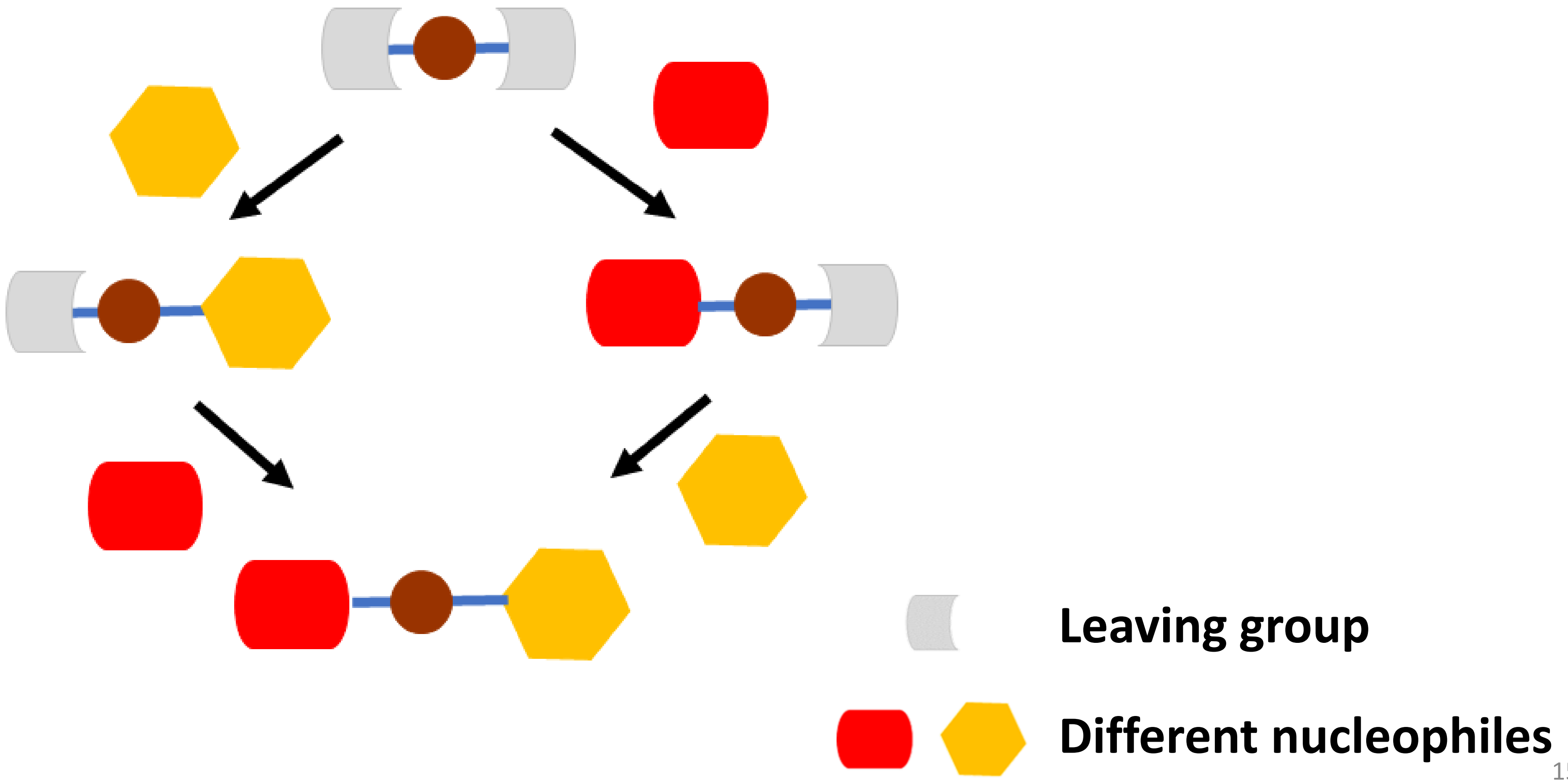
The demand for ready access to complex organic molecules has increased markedly. Increased sophistication of the bulk chemical industry has created the need for more elaborate inexpensive raw materials. The isolation and identification of complex organic molecules that play important roles in living systems, but whose availability from natural sources is precluded because of very low pouring of new tools—namely, reactions and reagents.

In searching for such tools, selectivity becomes the prime motivator. Three general classes of selectivity can be recognized. Complex organic molecules normally have more than one reactive site or functional group. The ability to discriminate among the reactive sites is referred to as chemoselectivity. For ex-

Orthogonal chemoselectivity

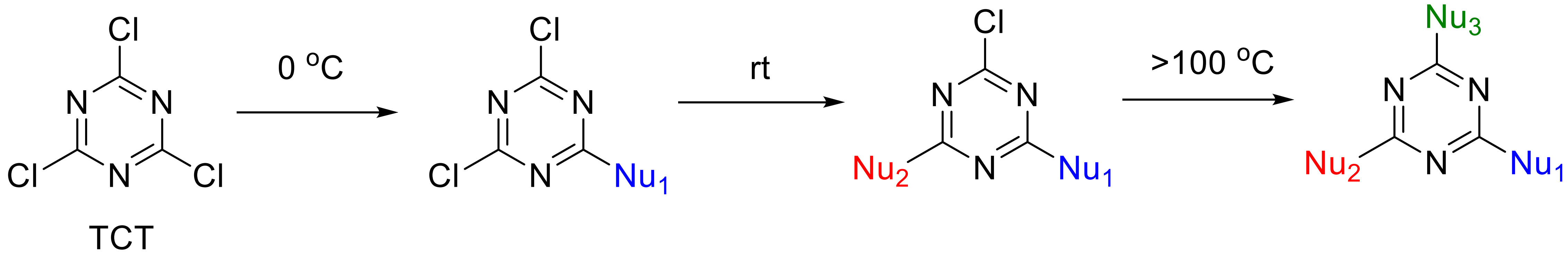
Fusion of these concepts as **“discrimination between reactive sites in any order”**

Bi-orthogonal chemoselectivity



TCT Reactivity

First to demonstrate the orthogonal chemoselectivity* concept onto TCT !!!



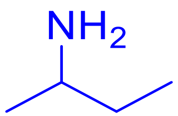
Nu = Nucleophile

Major problem in bioconjugation!!!
High temperature

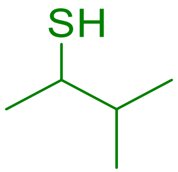
*Discrimination between reactive sites in any order

Orthogonal chemoselectivity onto TCT

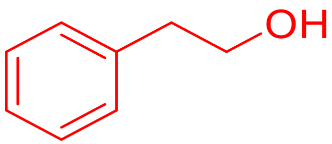
To prove concept: Following nucleophiles were chosen



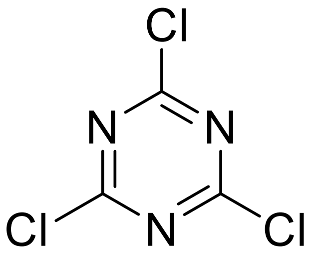
amine



thiol



alcohol



TCT

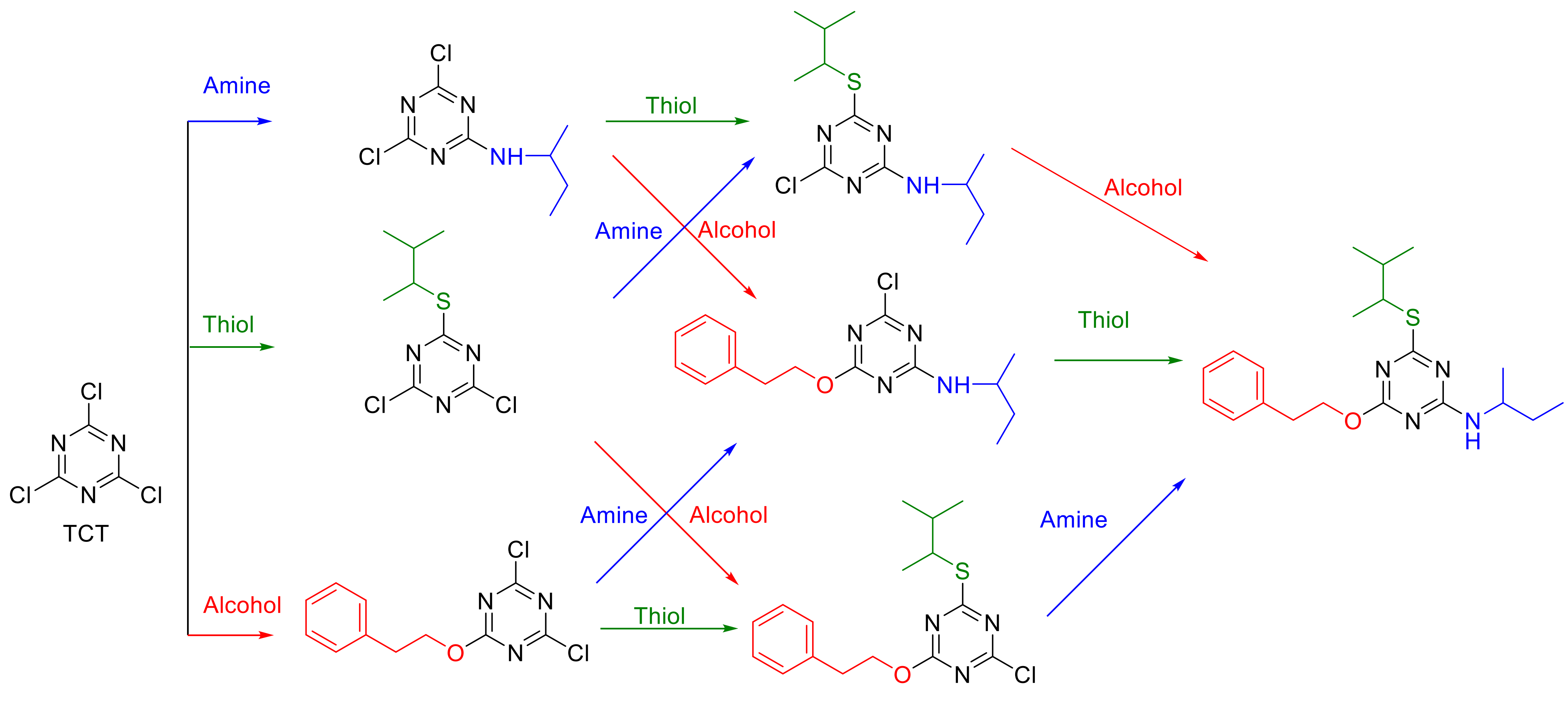
Objectives

1. To study the preferential order of incorporation

2. To demonstrate orthogonal chemoselectivity

A. Sharma, A. El-Faham, B.G. de la Torre, F. Albericio, *Front. Chem.*, **2018**, 6, 512.

Orthogonal chemoselectivity: Scheme

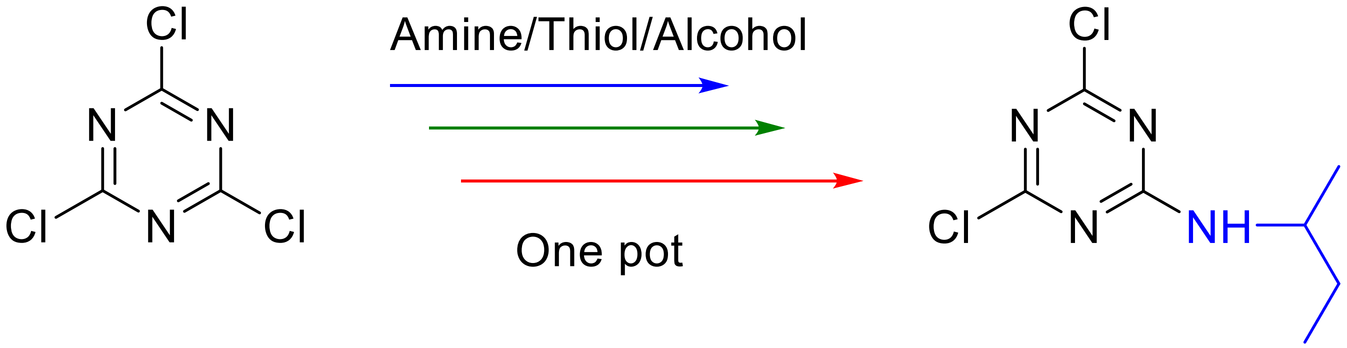


Orthogonal chemoselectivity: Results

1. Preferential order of incorporation: **alcohol, thiol and amine**

2. Final incorporation at higher temperature (90 °C).

3. Competitive test, only **amine is the champion**.

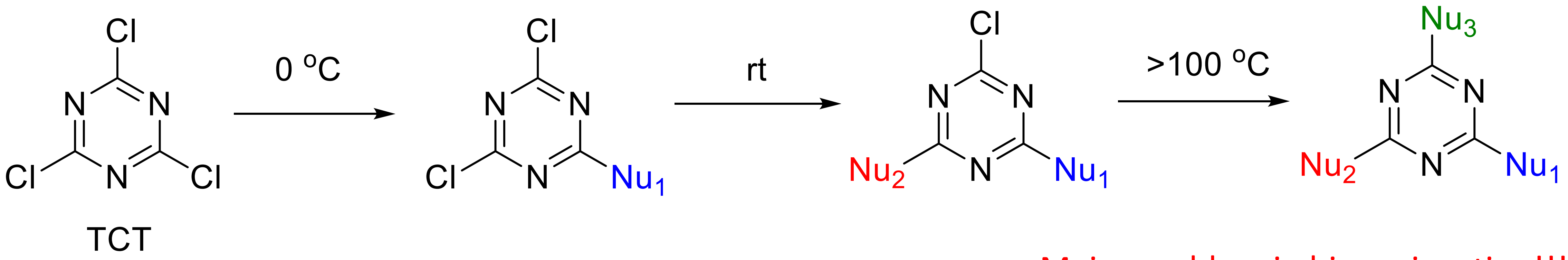


4. Once amine is incorporated, only another amine can be introduced.

A. Sharma, A. El-Faham, B.G. de la Torre, F. Albericio, *Front. Chem.*, **2018**, 6, 512.

Orthogonal chemoselectivity onto TCT

First to demonstrate the orthogonal chemoselectivity concept onto TCT !!!



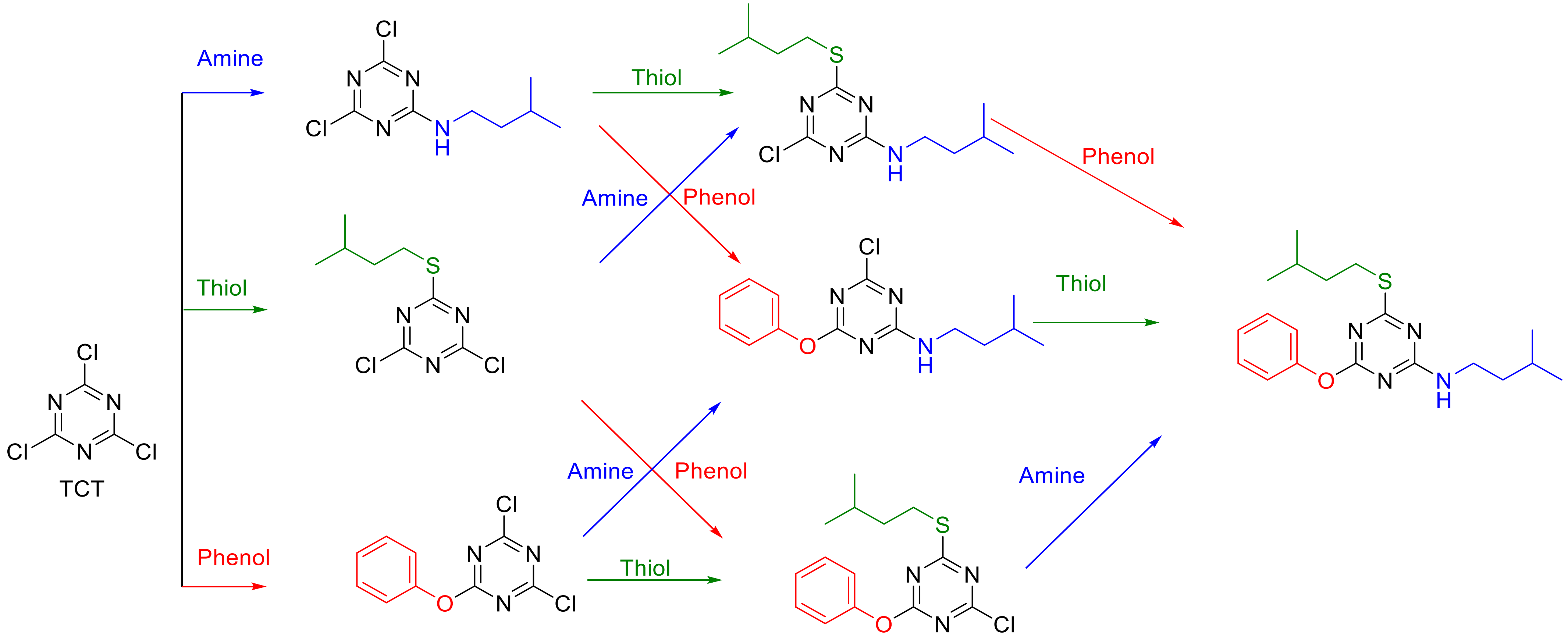
Nu = Nucleophile

Major problem in bioconjugation!!!
High temperature

Objective

Alcohol was replaced by phenol.

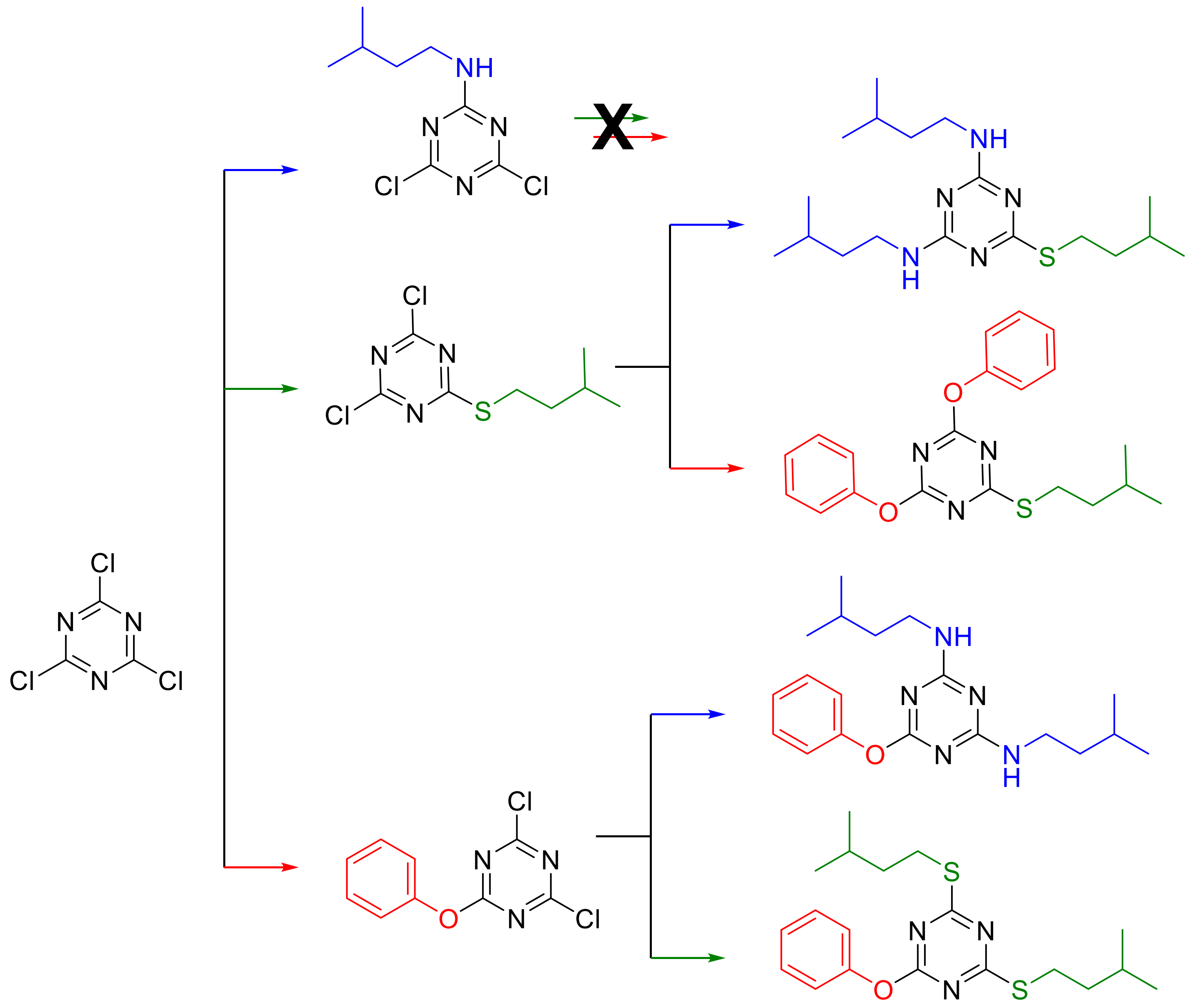
Reactivity of TCT with Phenol as one nucleophile



1. The preferential order of incorporation: **phenol, thiol and amine**
2. The final incorporation at ambient temperature (35 ^oC).

R. Sheyi, A. Sharma, A. El-Faham, B.G. de la Torre, F. Albericio, *Aust. J. Chem.*, **2020**, 73, 352.

Reactivity of TCT: 1+2 addition (For dendrons synthesis)



Results

1. No nucleophile undergoes reaction once amine is present onto TCT.

2. With thiol and phenol at 1st position onto TCT, 2 eq. of amine/thiol/phenol reacts at ambient temperature (35 °C).

R. Sheyi, A. Sharma, A. Kumar, A. El-Faham, B.G. de la Torre, F. Albericio, *Arkivoc*, **2020**. DOI: 10.24820/ark.5550190.p011.245

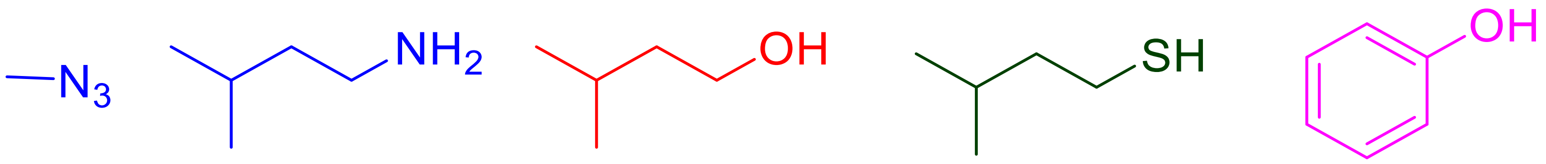
TCT Reactivity and further modulations

Phenol helps to lower the temperature (compatible for bioconjugation), but the preferential order of incorporation limits its usage to the 1st position.

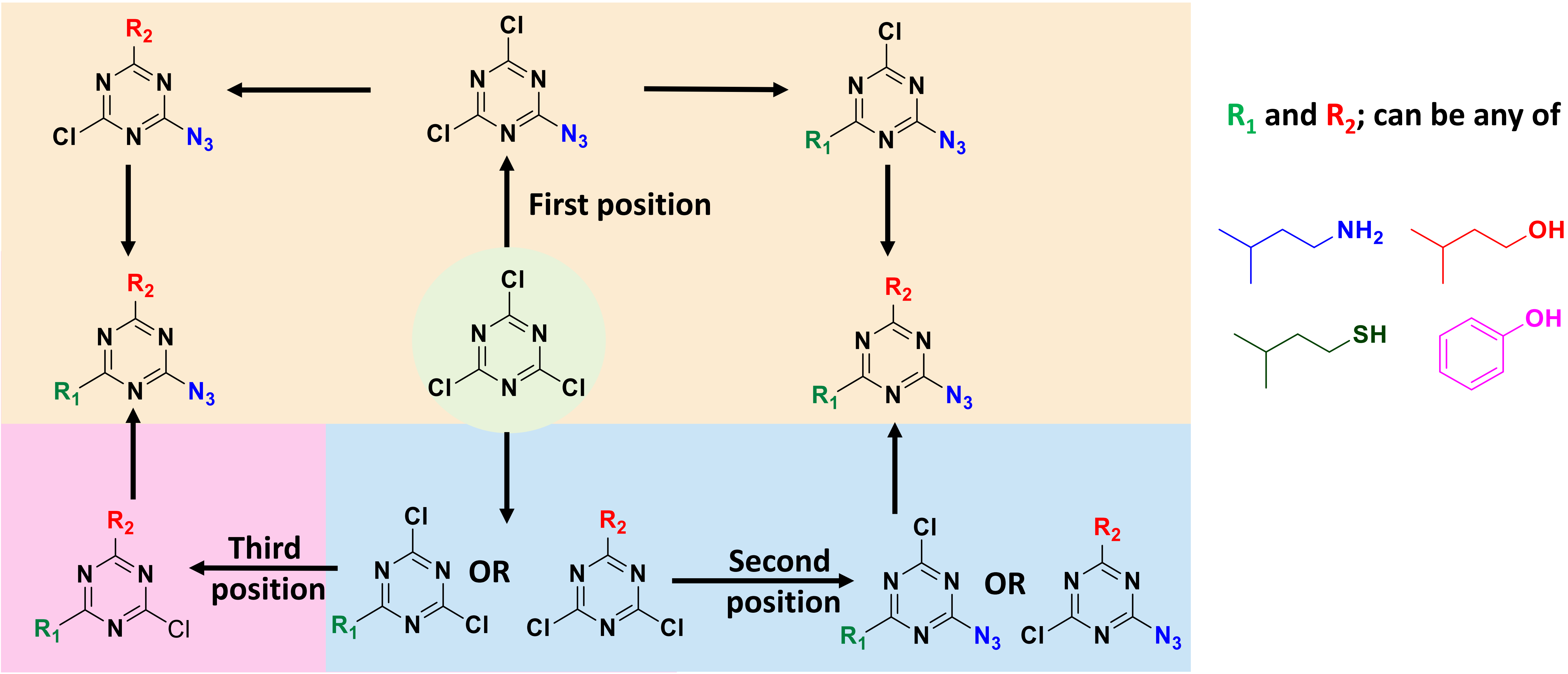
Azide (N_3) as one of the nucleophiles onto TCT as:

✓ *Azide is key due is electron withdrawing which enhances reactivity.*

Following nucleophiles were chosen



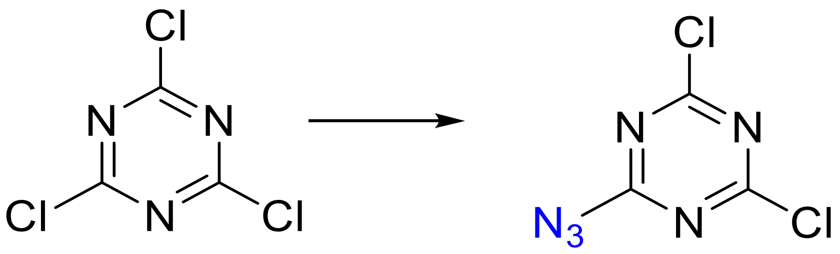
Tri-Orthogonal chemoselectivity onto TCT: Proposed Scheme



A. Sharma, R. Sheyi, A. Kumar, A. El-Faham, B.G. de la Torre, F. Albericio, *Org. Lett.*, **2019**, 21, 7888.

A. Sharma, A. Kumar, A. El-Faham, B.G. de la Torre, F. Albericio, *Bioorg. Chem.*, **2020**, 104, 104334.

TCT: Azide in first position



1. No proper procedure in literature. 

2. Requires inexpensive starting material (TCT), sodium azide and water/acetone as solvent.

3. Reaction temperature controlled well. Its important to avoid di-substitution!!!

4. Reaction optimized well and scaled up to 5 g with around 95% yield. 

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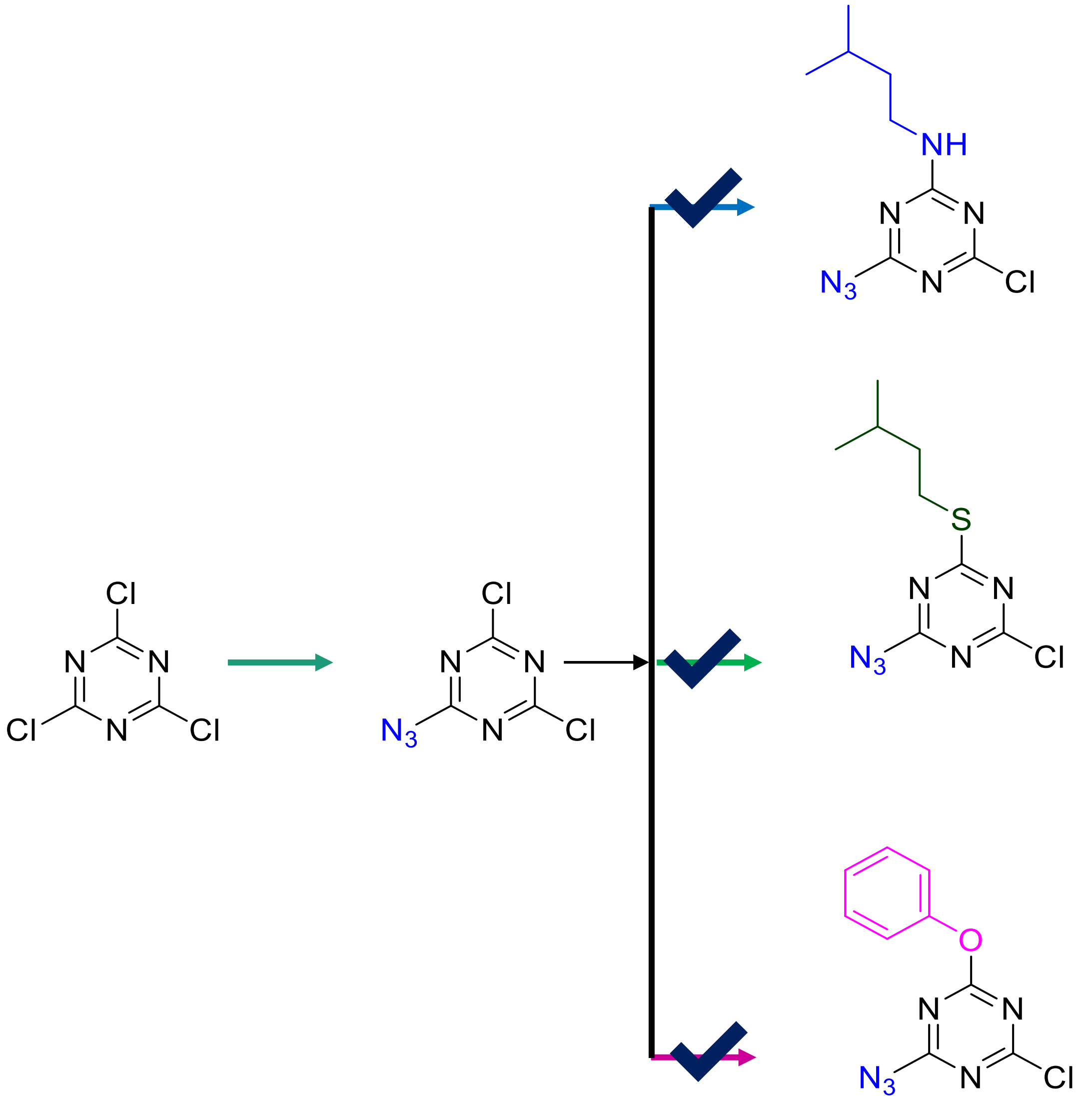
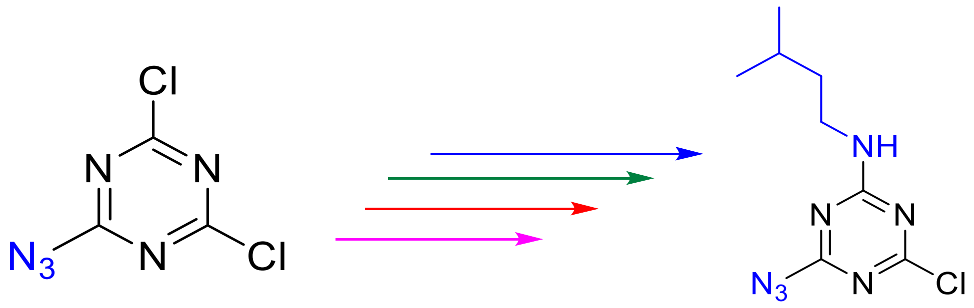
TCT: Azide in first position

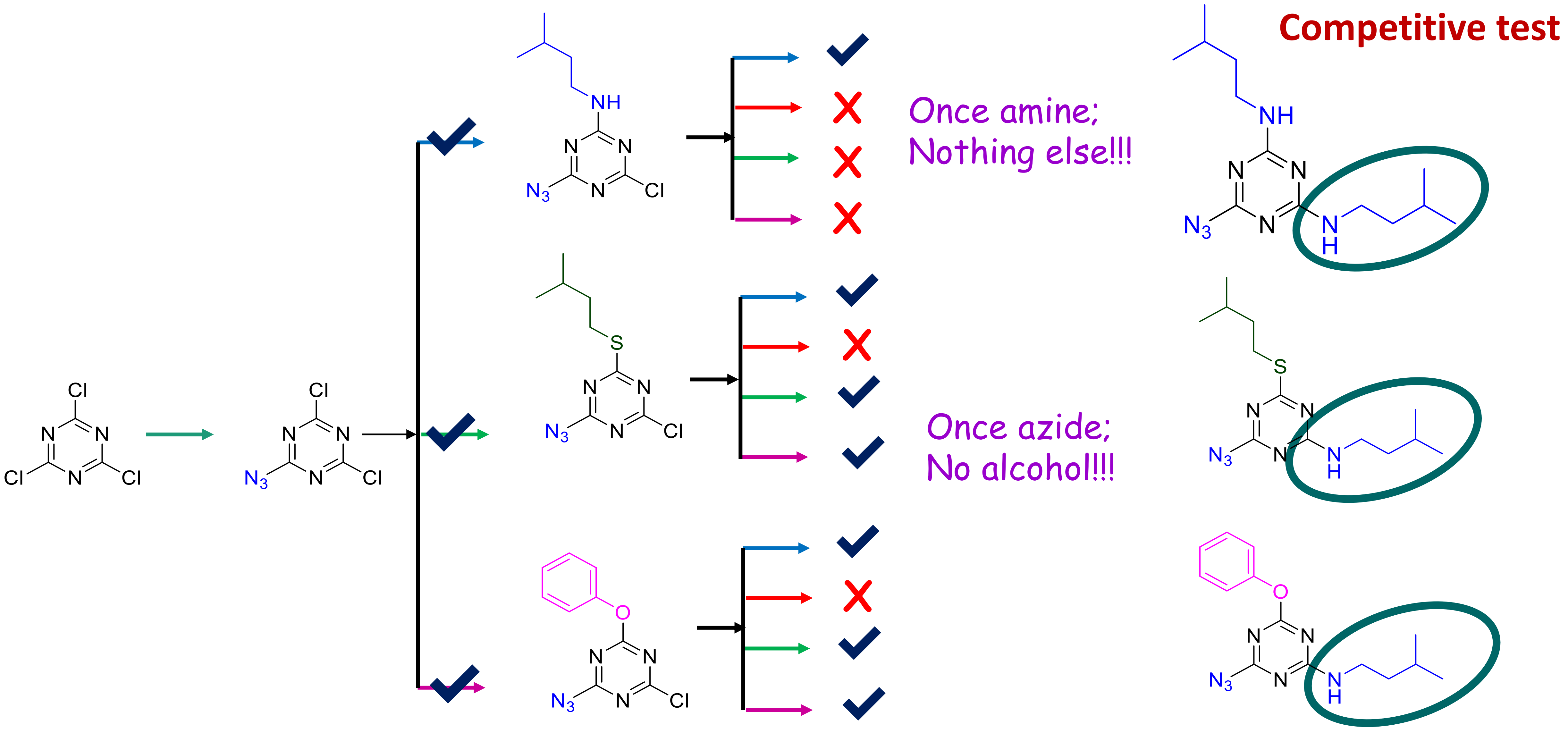


Competitive test*



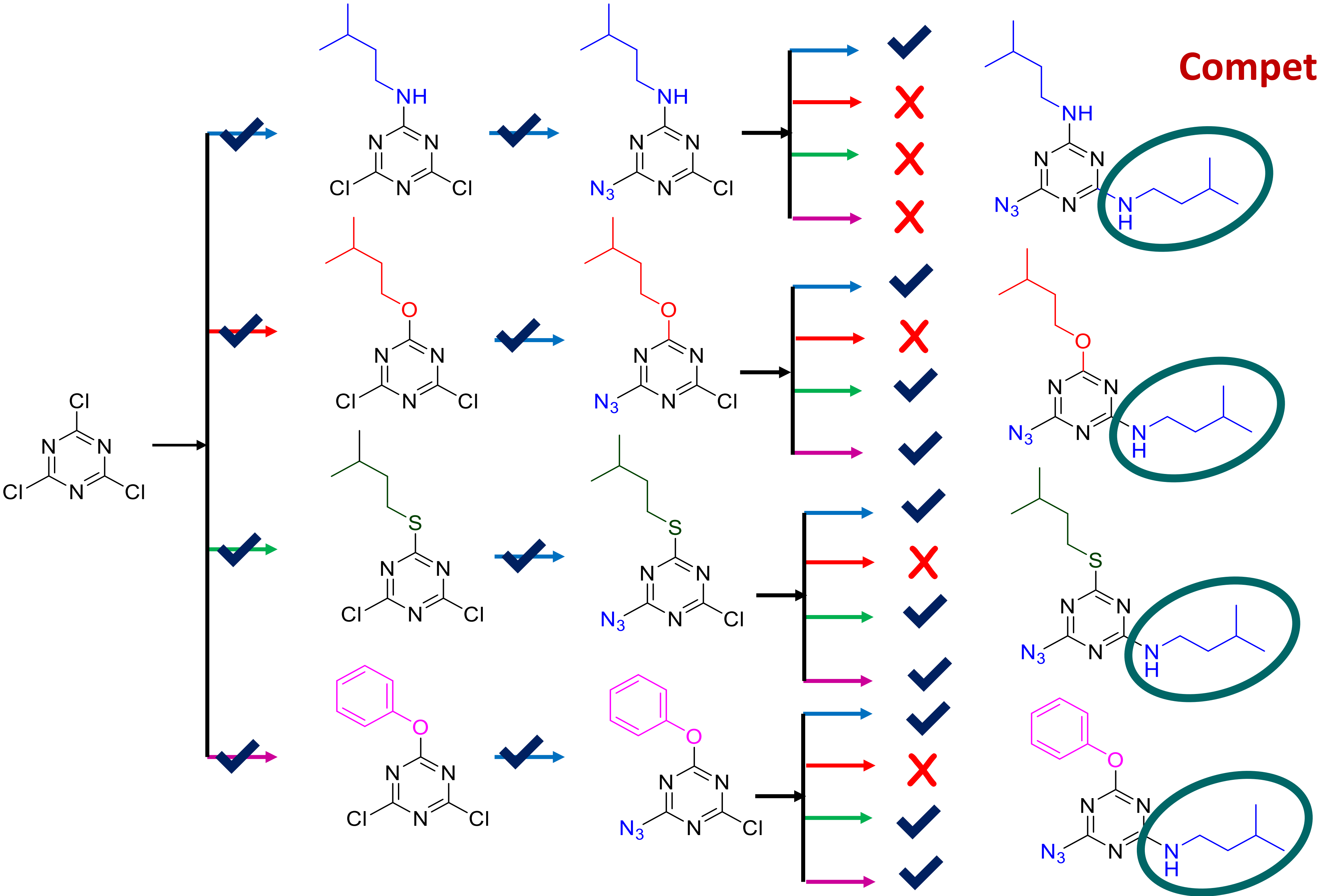
*Reactant with amine/thiol/alcohol/phenol in one pot in presence of TEA as base

TCT: Azide in first position

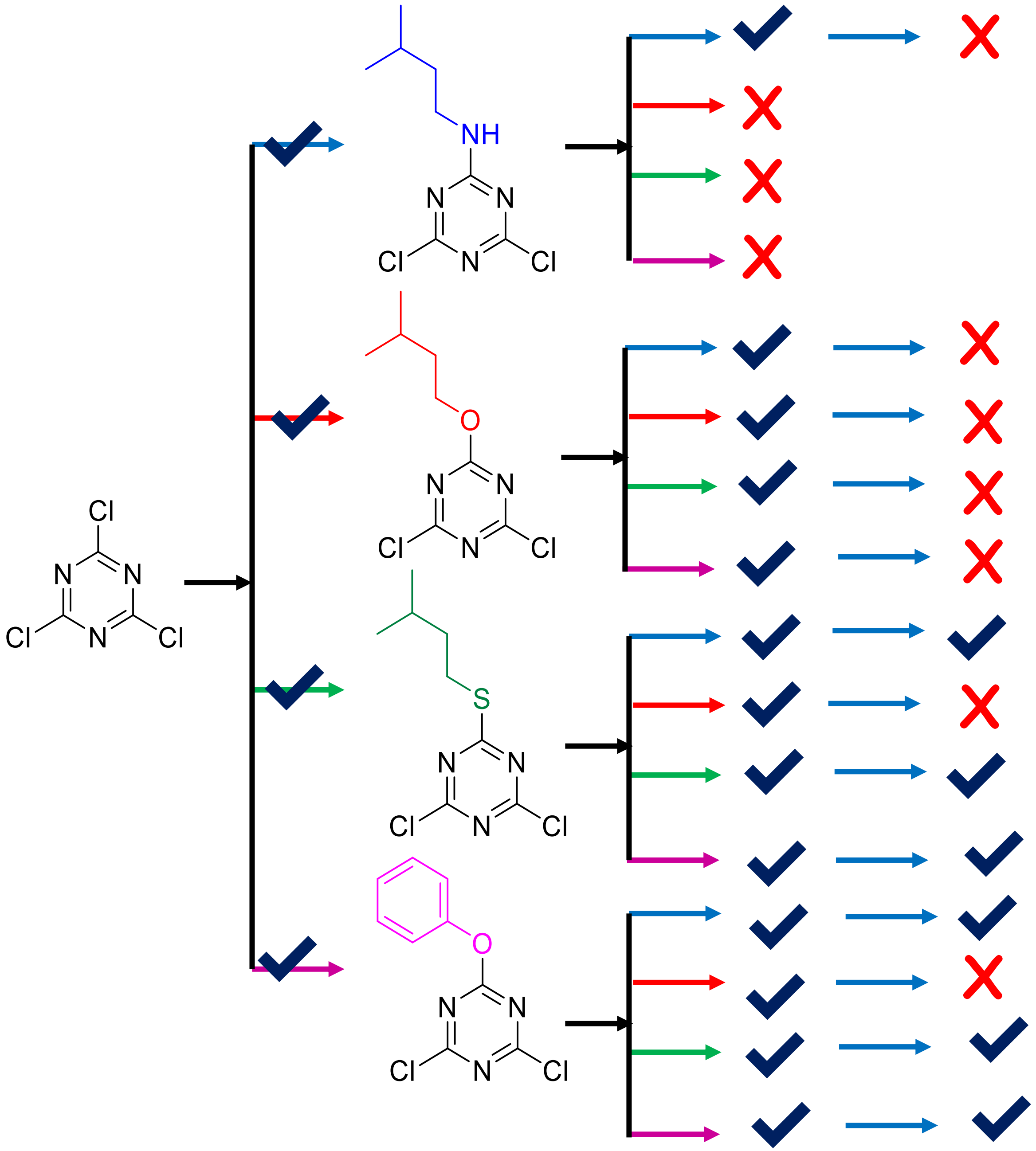


TCT: Azide in second position

Competitive test

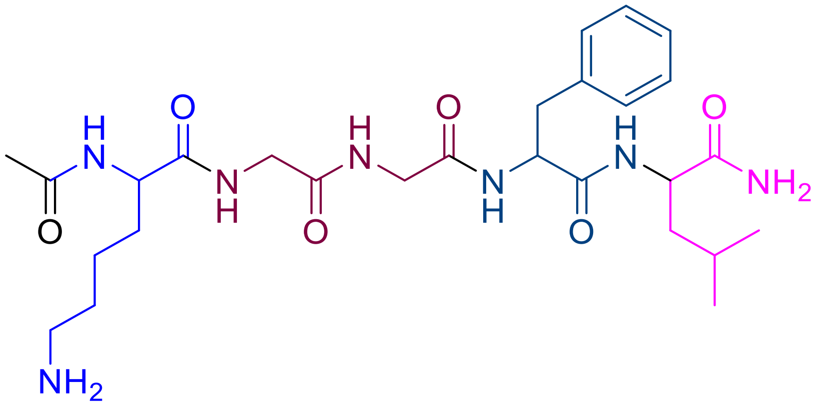


TCT: Azide in third position

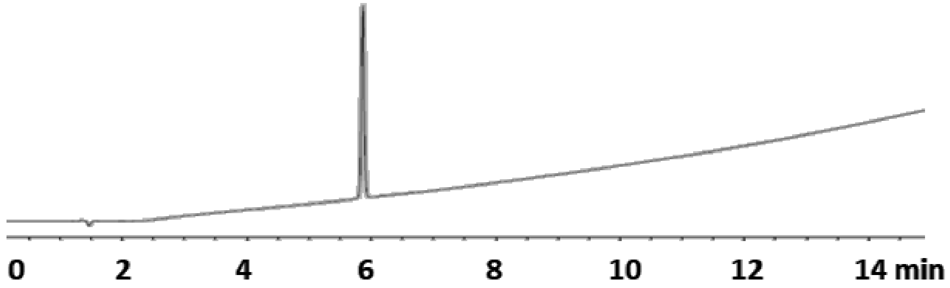


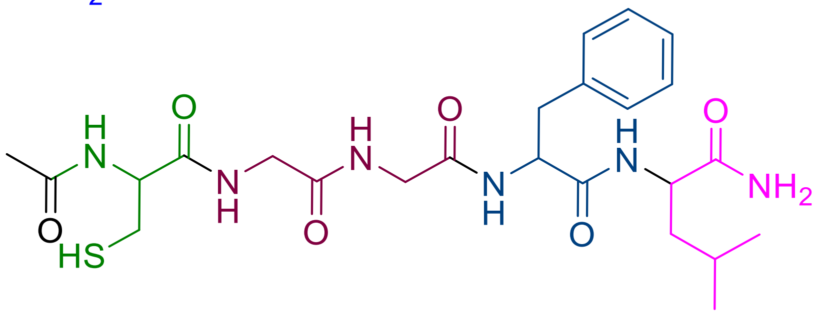
Application using model peptides

SPPS for following peptides bearing Lys, Cys and Tyr amino acids

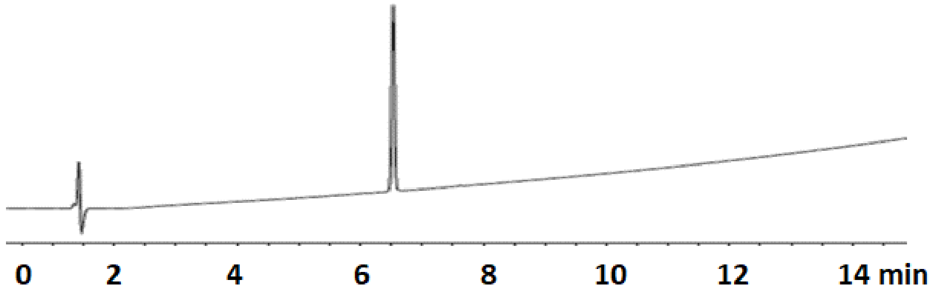


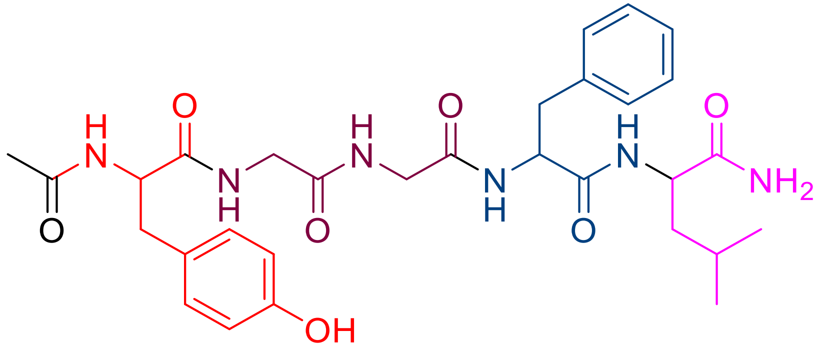
≡ **Ac—K-G-G-F-L—NH_2**
Lys-peptide



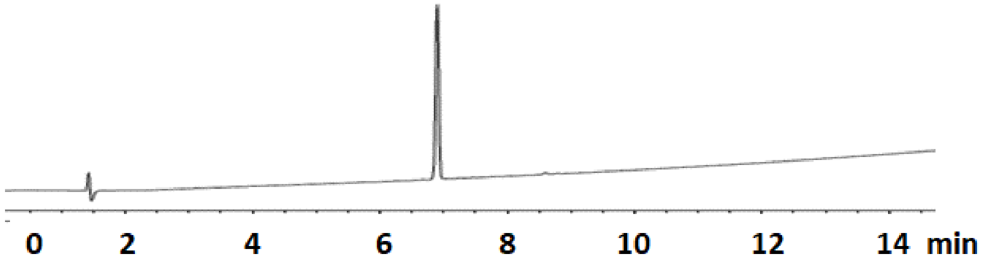


≡ **Ac—C-G-G-F-L—NH_2**
Cys-peptide



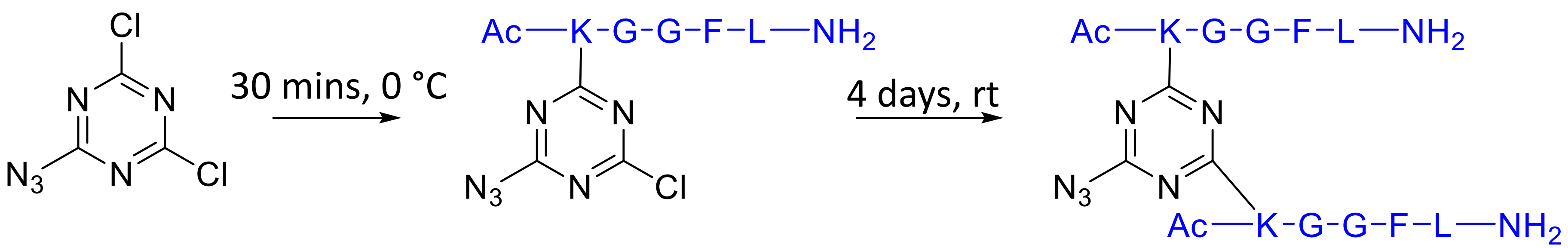


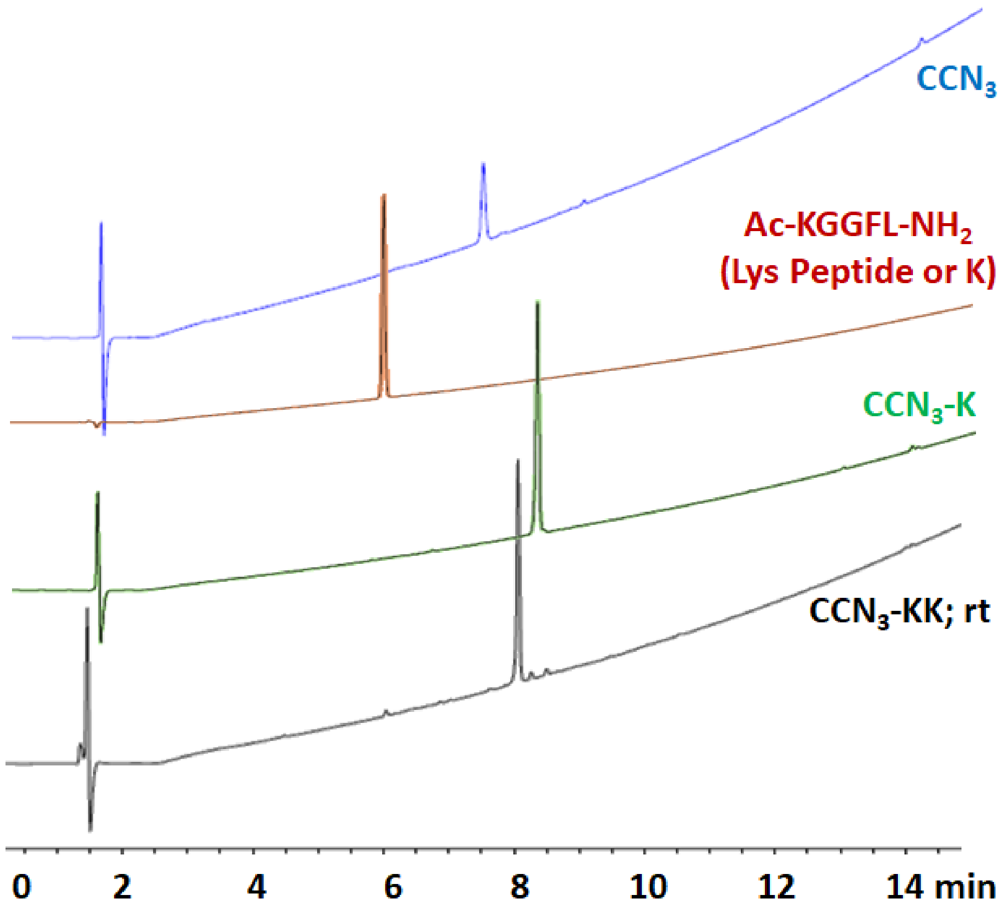
≡ **Ac—Y-G-G-F-L—NH_2**
Tyr-peptide



A. Sharma, A. Kumar, A. El-Faham, B.G. de la Torre, F. Albericio, *Bioorg. Chem.*, **2020**, 104, 104334.

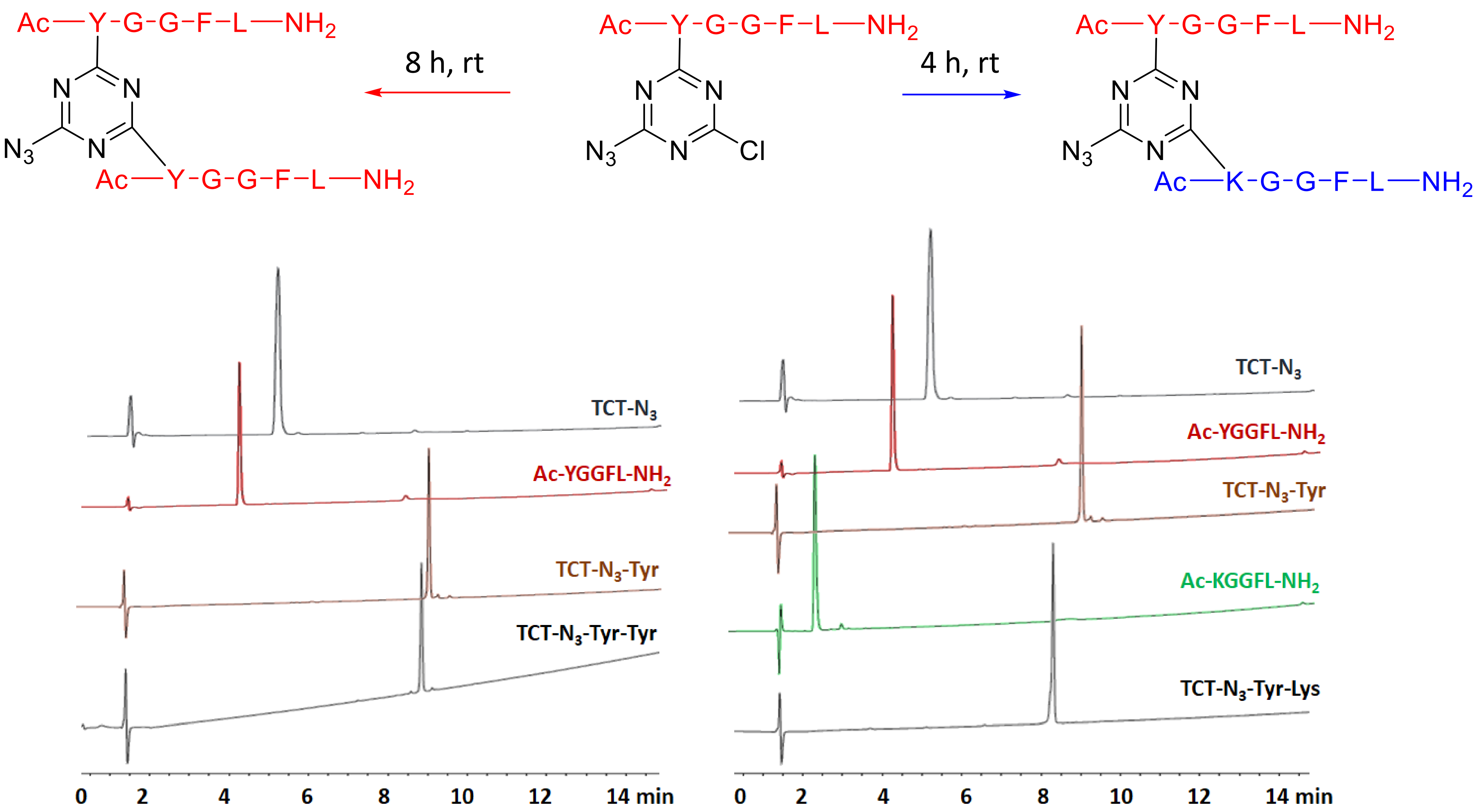
Reaction of CCN_3 with Ac-KGGFL-NH_2





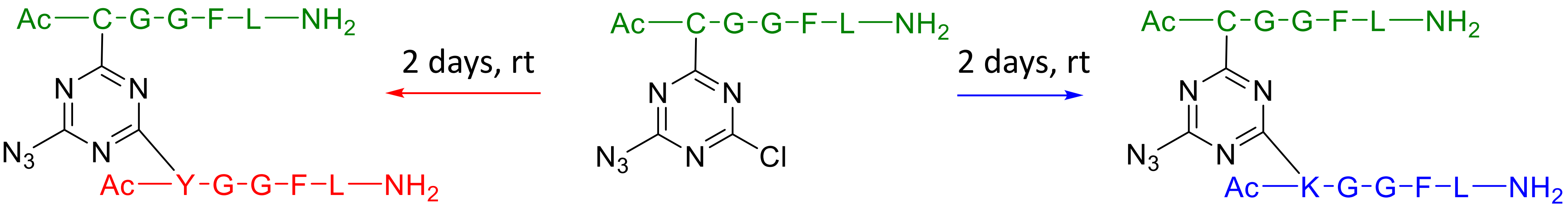
A. Sharma, A. Kumar, A. El-Faham, B.G. de la Torre, F. Albericio, *Bioorg. Chem.*, **2020**, 104, 104334.

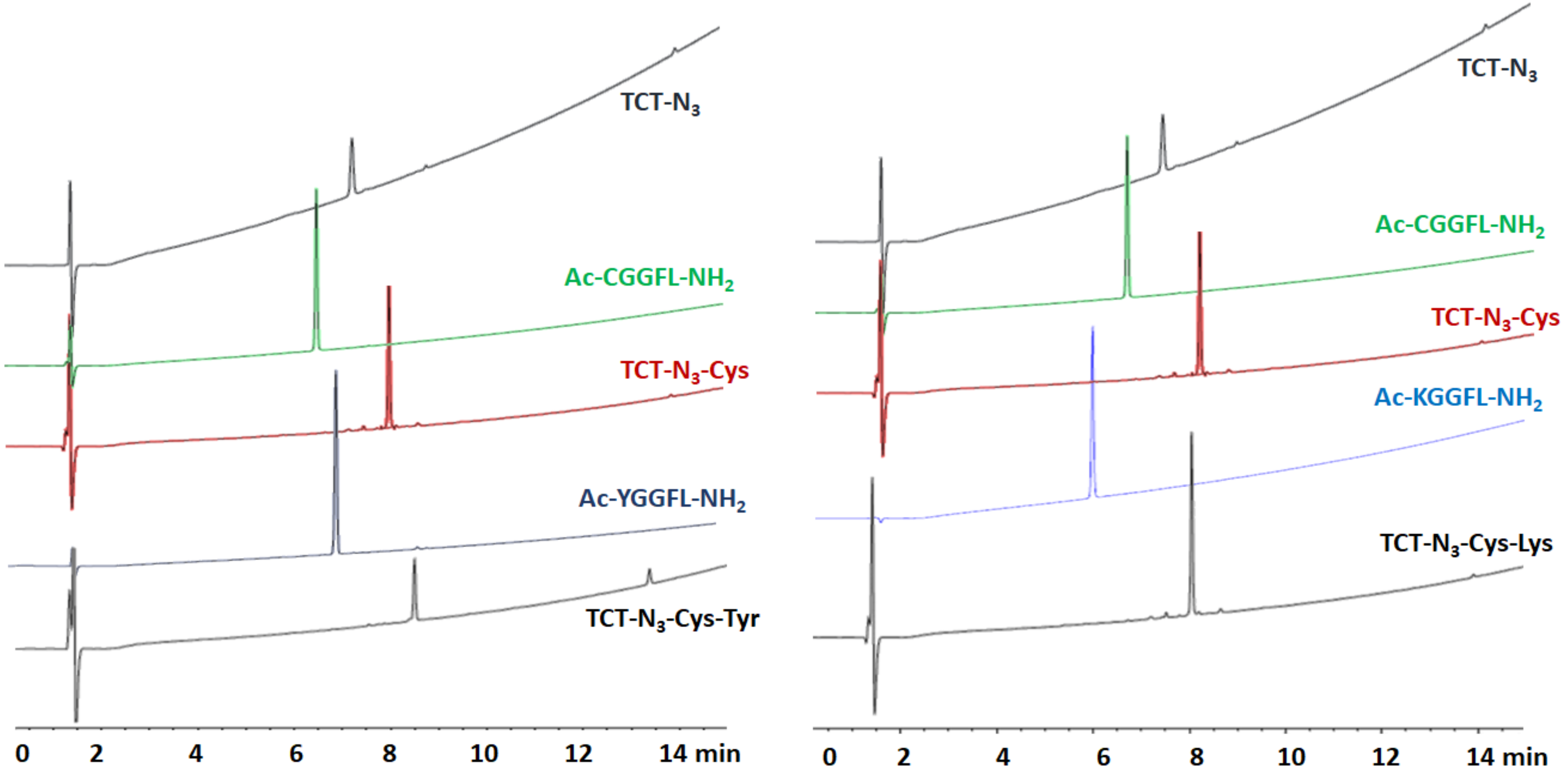
Reaction of CCN_3 with Ac-YGGFL-NH_2 and Ac-KGGFL-NH_2



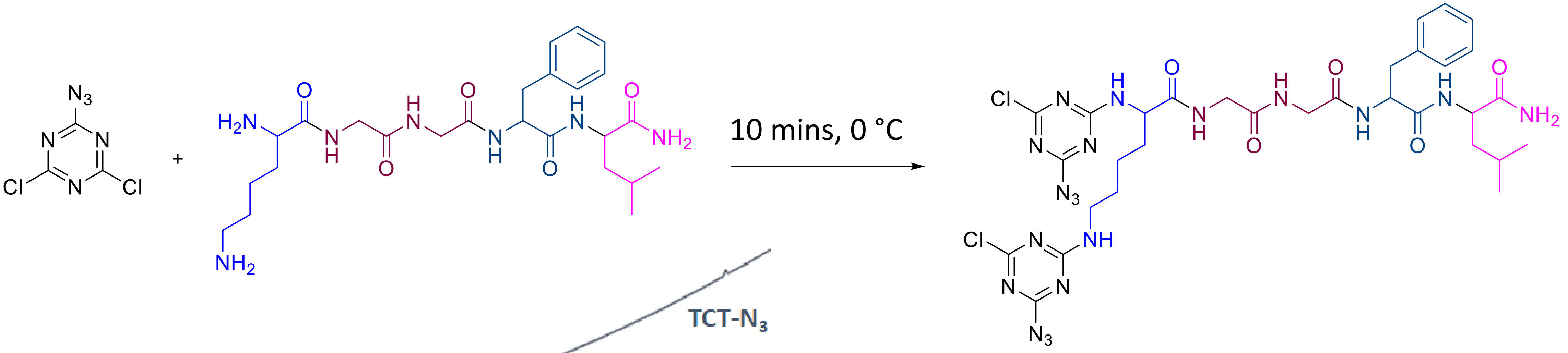
A. Sharma, A. Kumar, A. El-Faham, B.G. de la Torre, F. Albericio, *Bioorg. Chem.*, **2020**, 104, 104334.

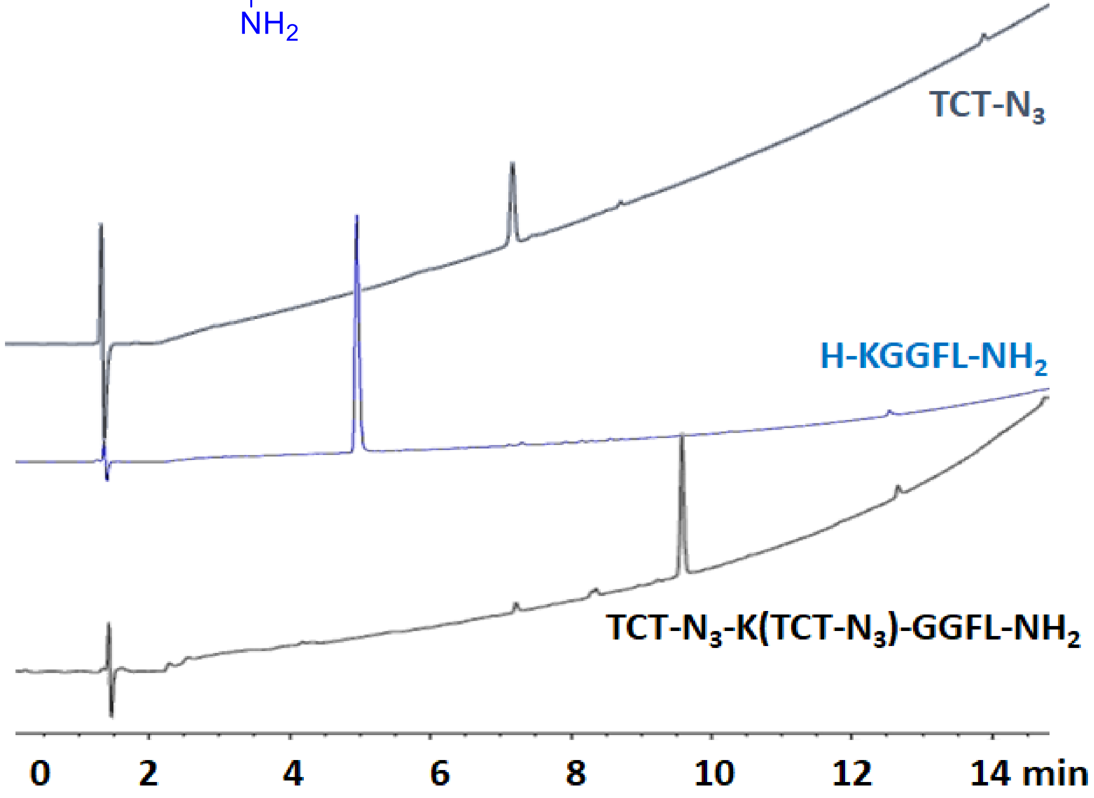
Reaction of TCT-N_3-Ac-CGGFL-NH_2 with Ac-YGGFL-NH_2 and Ac-KGGFL-NH_2





Reaction of TCT-N_3 with α and ε NH_2 of Lys in H-KGGFL-NH_2





1. The reaction is 5 mins at 0 °C, both NH_2 react.

2. Product confirmed by LCMS

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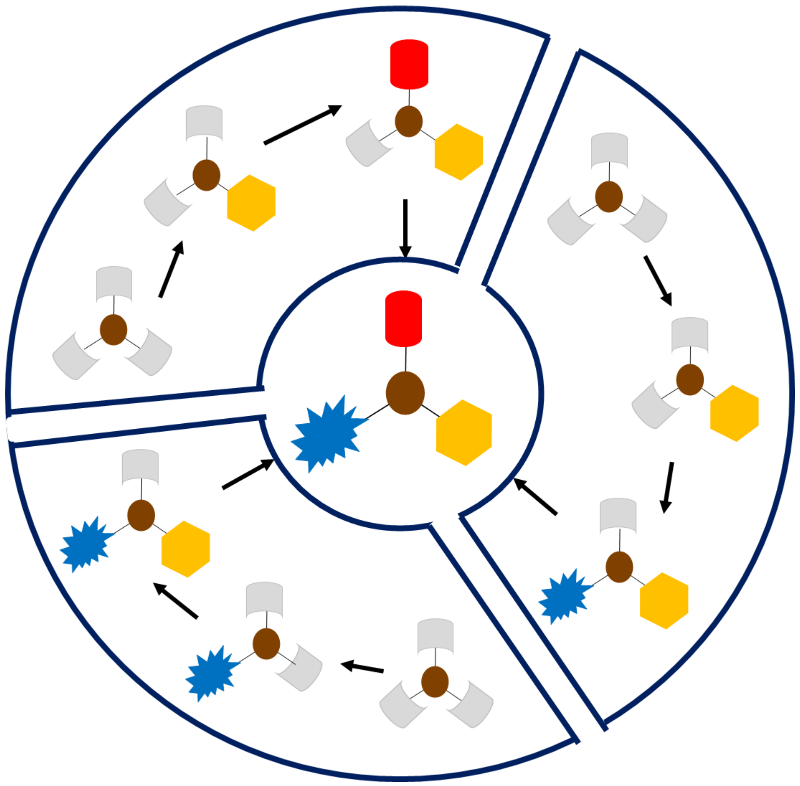
Conclusion: Tri-orthogonal chemoselectivity explained



1. N_3, Phenol and Thiol can be substituted in any order.
2. Other combination by 3 different strategies.
3. N_3 can be introduced in any position but once N_3 is introduced, OH cannot be incorporated even with higher temperature.
4. N_3 placement in either position 1 or 2 facilitates the introduction of the remaining nucleophiles at room temperature.
5. Competitive tests has been done in all cases and amine always wins the race.

Amine was best for incorporation in the last position.

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Asante
Gràcies
Medaase
Eshe
Dankie
Gracias
Thank you
ধন্যবাদ
Obrigado
நன்றி
धन्यवाद
شكرا جزيلا
ధన్యవాదాలు
Na gode
Merci
Ngiyabonga
Mpusia