UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

2,4,6-Trichloro-1,3,5-triazine as a Triorthogonal Chemoselective Linker

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Chemistry, School of Chemistry and Physics of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, South Africa. The research was financially supported by NRF.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

As the candidate's supervisor, I have approved this thesis for submission.

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DECLARATION: PLAGIARISM

I, Sheyi Ebenezer Rotimi, declare that:

(i) The research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) This thesis has not been submitted in full or in part for any degree or examination to any other university;

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(vi) This thesis is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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ABSTRACT

Trisubstituted-1,3,5-triazine derivatives are usually obtained by the sequential aromatic nucleophilic substitution of TCT chlorine atoms. The third substitution is usually achieved at elevated temperatures which tend to prevent their application in the biological context. In this project, an easy synthetic route for the synthesis of a trisubstituted-1,3,5-triazine was reported. This was achieved at an ambient temperature compatible with the biological system. The nucleophiles used for this study are azide, phenol, isopentyl amine and 3-methyl-butane-1-thiol. All synthesized compounds were obtained in good yields, and characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

Furthermore, the concept of orthogonal chemoselectivity which describe an occurring reaction that proceeds with discrimination between reactive sites was demonstrated for the first time. The introduction of the azide as one of the nucleophiles into TCT opens the possibility of the incorporation of other substituents at low temperature.

ACKNOWLEDGEMENTS

I thank God almighty who enabled me to carry out this research work successfully in sound health and sound mind.

Appreciation they say is nothing, but at the same time is something because it spurs the mind, warm the soul, and transform lassitude into excitement. It is in this light that I submit my heartiest gratitude to my respected supervisors: Professor Fernando Albericio and Professor Beatriz G. de la Torre for offering to supervise me and for their sincere guidance and help for completing this project despite my excesses.

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Nobody has been more important to me in the course of this research than the members of my family. I would like to specially thank my mother; whose

love and prayers are always with me in my career. She remains my ultimate role model till eternity. Most importantly, I wish to thank my loving and supportive brother, Dr. Mathew Rotimi whose unending motivations and inspiration gave birth to this achievement.

LIST OF ABBREVIATIONS

ACN: Acetonitrile

ADC: Antibody drug conjugate

DCM: Dichloromethane

DFT: Density Functional Theory

DST: Disubstituted-1,3,5-triazine

DIEA: N, N-Diisopropylethylamine

Dts: Dithiassuccinoyl

EtOAc: Ethyl acetate

HPLC: High-Performance Liquid Chromatography

HRMS: High Resolution Mass Spectroscopy

MST: Monosubstituted-1,3,5-triazine

TA: s-Triazine

TCT: 2,4,6-Trichloro-1,3,5-triazine

TFA: Trifluoroacetic acid

TLC: Thin layer chromatography

TMS: Trimethyl silane

TST: Trisubstituted-1,3,5-triazine

^{1}H NMR: Proton Nuclear Magnetic Resonance

^{13}C NMR: Carbon Nuclear Magnetic Resonance

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CHAPTER 1

Introduction

Background of the study

Triazines are class of nitrogen-containing heterocycles comprised of three carbon atoms and three nitrogen atoms. They exist in three isomeric forms depending on the positions of the nitrogen atoms (Figure 1): 1,2,3-triazine, 1,2,4-triazine, and 1,3,5-triazine, which is also called s-triazine.[1]

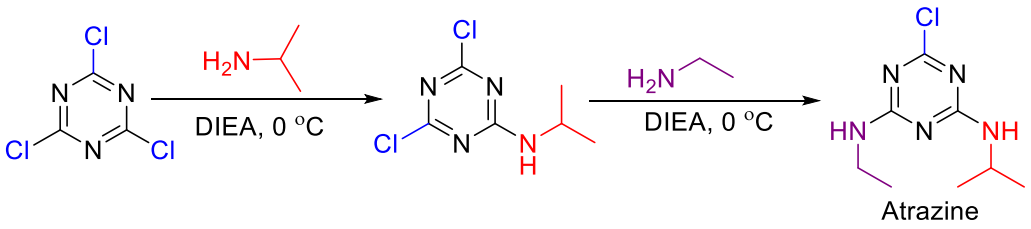
Figure 1: Isomers of triazine

In organic chemistry, the 1,3,5-triazine is considered an important moiety[2] due to its presence in several organic compounds because of its unique aromatic properties and its ability to participate in some distinctive chemical communications like hydrogen bonding, electrostatic and charge-transfer attraction, coordination, nucleophilic substitution, and π-π stacking.[3-5]

s-Triazine is thermally stable but easily decomposes to form hydrogen cyanide when heated above 600 °C. s-Triazine ring is fairly resistant to electrophilic substitution but highly susceptible to nucleophilic attack by readily undergoing ring cleavage.[6]

s-Triazine and its derivatives possess wide range of applications in medicine (as antibacterial, antiviral, anti-fungal, and anticancer agents),[7, 8] textile, rubber and plastics industries[9] and are also used as pesticides,[10] bearing in mind that TCT is the key reagent for obtaining most of these derivatives. The important aspect of TCT that makes it very suitable for the preparation of these derivatives is the reactivity of its chlorine atoms toward nucleophiles. For instance, atrazine, a useful herbicide used for the production of grains (like corn, millet, sorghum) and cotton is derived by the sequential reaction of TCT with

isopropylamine and ethylamine in the presence of DIEA (Scheme 1). Reports however revealed that it use poses some threats to the ecosystem which has led to its ban in many countries.[11]



Scheme 1: Synthesis of ~~Atrazine~~

Similarly, altretamine (Figure 2), a cytotoxic antineoplastic agent has been very effective in the treatment of ovarian cancer.[12]

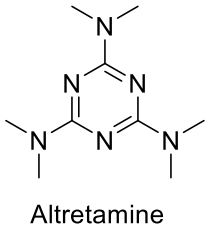


Figure 2: Altretamine

Also, ~~Enasidenib~~, a drug used to treat relapsed or refractory acute myeloid leukemia in people is built on triazine (Figure 3).[13]

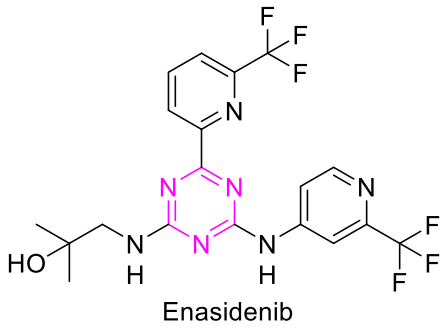
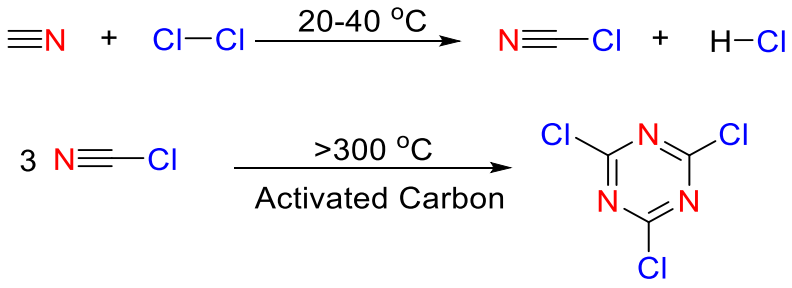


Figure 3: Structure of ~~Enasidenib~~

2,4,6-Trichloro-1,3,5-triazine (TCT)

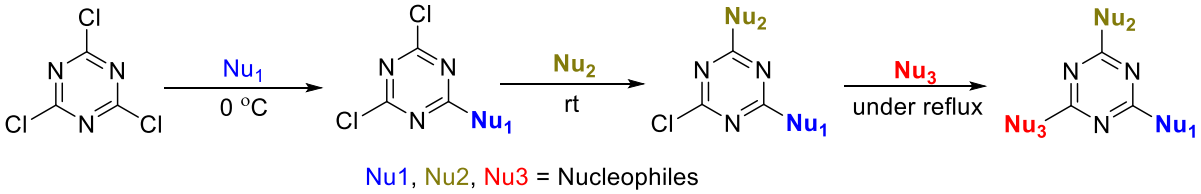
2,4,6-Trichloro-1,3,5-triazine (TCT), the best precursor for s-triazine derivatives is prepared industrially in two steps (Scheme 2). First is the chlorination of hydrogen cyanide to

give cyanogen chloride followed by the trimerization of the cyanogen chloride at an elevated temperature over a carbon catalyst.[3, 6]



Scheme 2: Industrial preparation of 2,4,6-trichloro-1,3,5-triazine

A unique characteristic of TCT is ~~its~~ ability to readily undergo nucleophilic aromatic substitution (S_NAr) reaction by the successive replacement/substitution of its triorthogonal chlorine atoms in a base dependent thermal reaction (Scheme 3).[14] The substitution of the first chlorine atom is usually accompanied with a release of energy and because this substitution is exothermic, the reaction is carried out at 0 °C.[14] The substitution of the second chlorine atom is usually achieved at room temperature, and the third substitution is performed at a high temperature (heating at ≥ 65 °C).[14, 15]



Scheme 3: Differential reactivity of 2,4,6-trichloro-1,3,5-triazine[15]

The synthesis and evaluation of TCT derivatives remain a focal objective of a broad number of research groups because of its wide range of applications. This work focus on exploring TCT[9] as a linker. In this context, we define a linker as a molecule which is able to bind/link other molecules. We intend to achieve this by taking advantage of the ability of TCT to readily undergo nucleophilic substitution reaction as it was discussed above.

Further, we have explored in this work a new chemical concept, the orthogonal chemoselectivity.

Orthogonality

The term orthogonality applied to protection was first introduced in chemistry by Merrifield and Barany in 1977,[16] and later demonstrated by Barany and Albericio in 1985 in a triorthogonal protection scheme.[17] Orthogonal protection system refer to 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.

Demonstrating the removal of a three-dimensional protection scheme (Scheme 4), Barany and Albericio prepared a leucine-enkephalin peptide (H-Tyr-Gly-Gly-Phe-Leu-OH) using different set of protecting groups.[17] In this context, the preferred way to prepare the peptide was to grow up the peptide with dithiassuccinoyl (Dts) amino acids (Figure 4),[17,18] having side-chain functions protected with the *tert*-butyl (tBu) group, on a *o*-nitrobenzyl based resin. The removal of the Dts group is carried out by thiolysis, the tBu is removed by acidolysis, and the peptide is cleaved from the resin by ~~photolysis(Scheme 4).~~

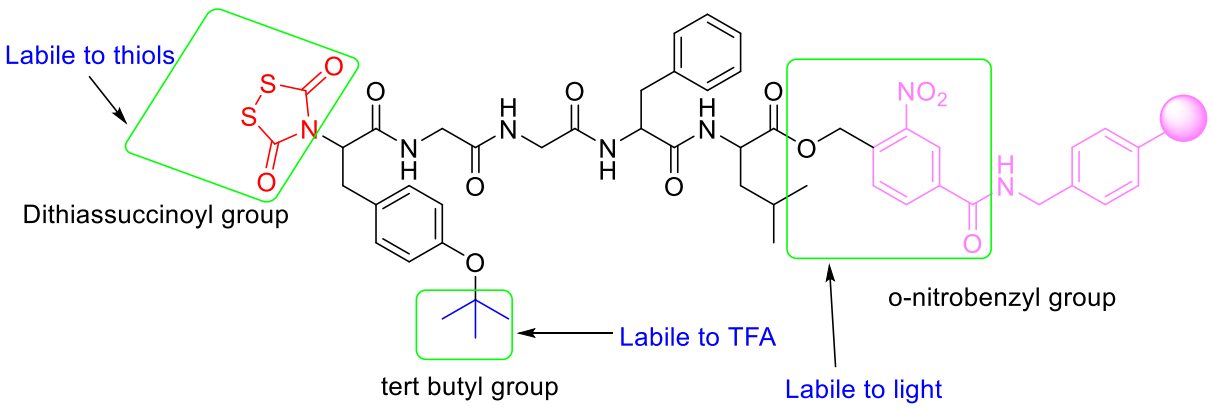
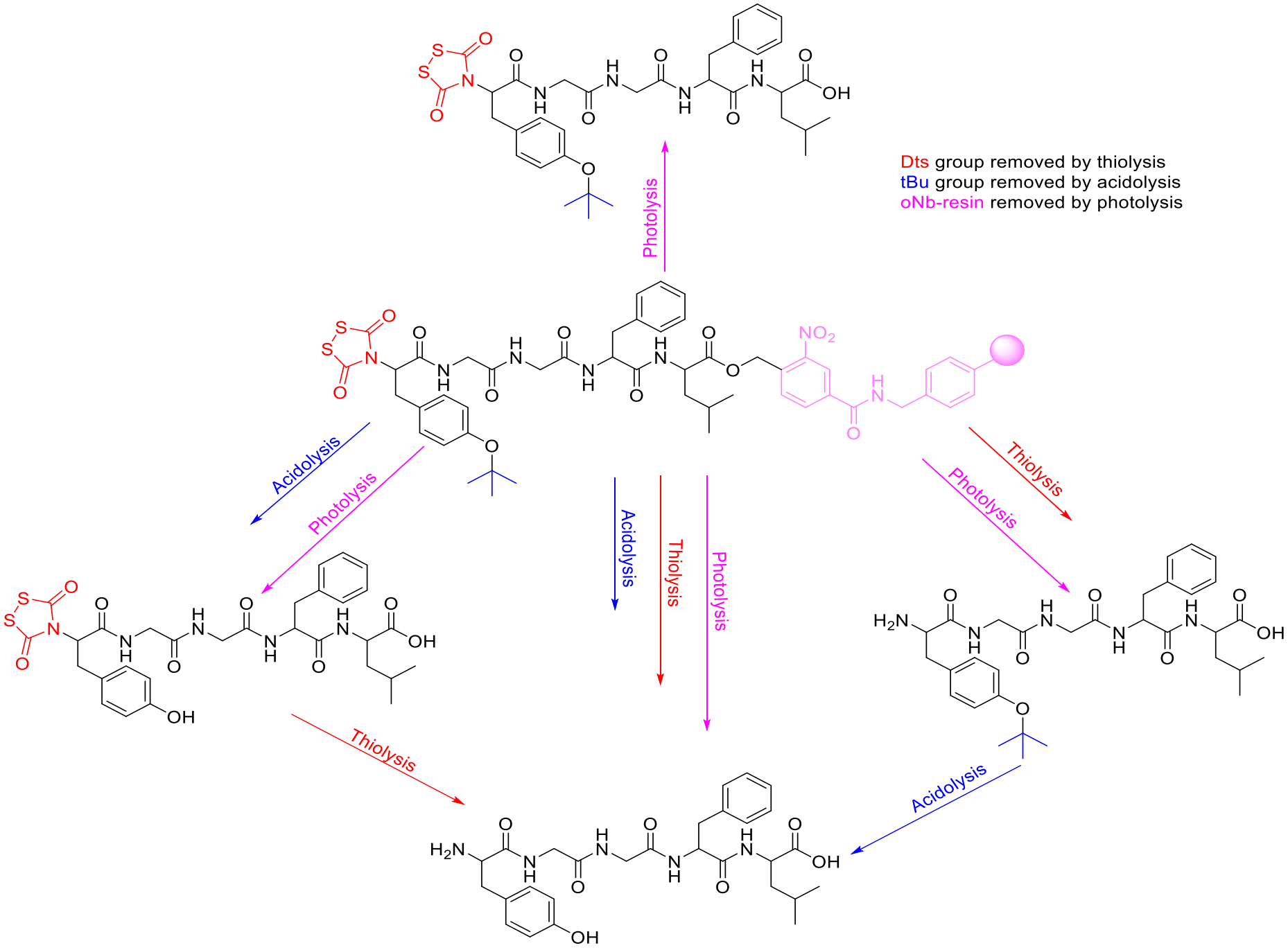


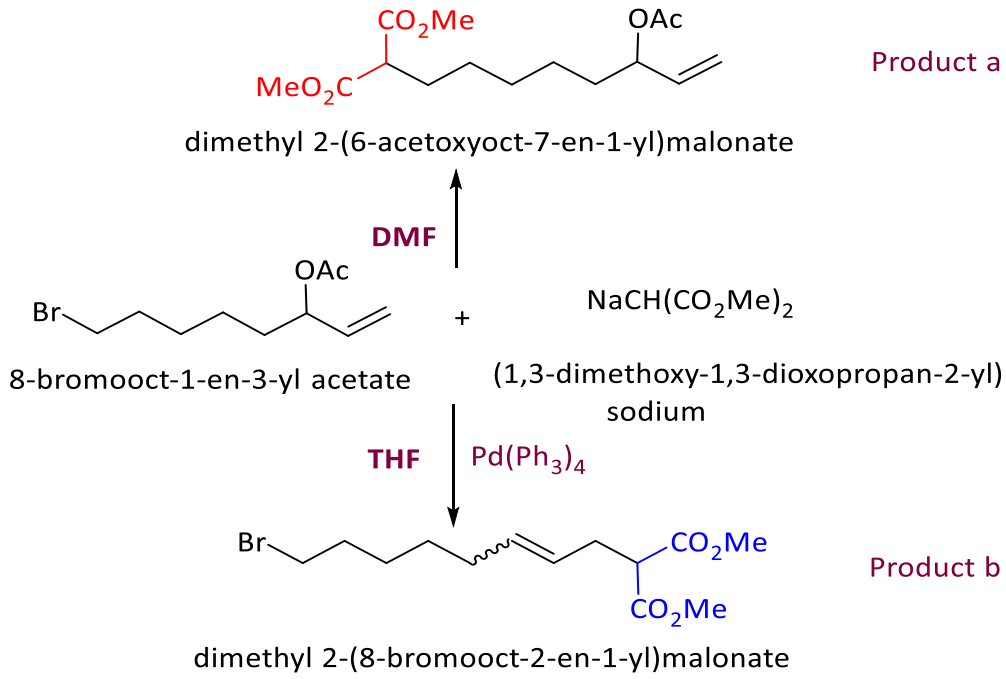
Figure 4: Leu-Enk [Dts-Tyr(t-Bu)-Gly-Gly-Phe-Leu-ONb-resin]



Scheme 4: The concept of orthogonal protection scheme showing different possible mechanism for the removal of orthogonal protecting groups present

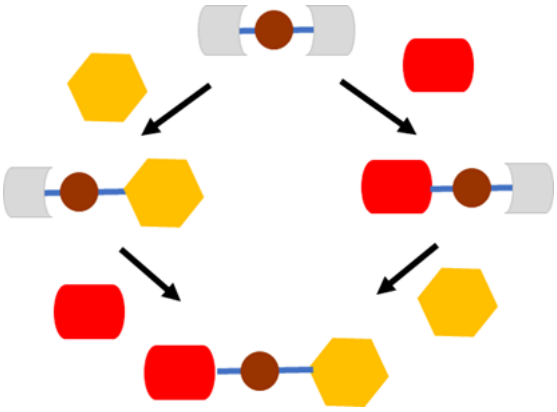
Chemoselectivity

In 1983, Trost introduced the concept of chemoselectivity which is described as the ability of a chemical reagent or functional group to discriminate among reactive sites.[19] He demonstrated the concept of chemoselectivity in a reaction of 8-bromooct-1-en-3-yl acetate with a sodium salt of dimethyl malonate in 2 different solvents (DMF and THF).[20] He observed an exclusive displacement of bromide by sodium salt with the reaction in DMF to form dimethyl 2-(6-acetoxyoct-7-en-1-yl)malonate. However, addition of a palladium(O) catalyst in tetrahydrofuran (THF) activates an allylic acetate as a result of prior coordination with the olefin. This lead to allylic acetate reordering to give dimethyl 2-(8-bromooct-2-en-1-yl)malonate.[20, 21] This differential displacement of bromide and allylic acetate in two different chemical mechanisms (Scheme 5) describes the chemoselectivity of the two reactive sites.

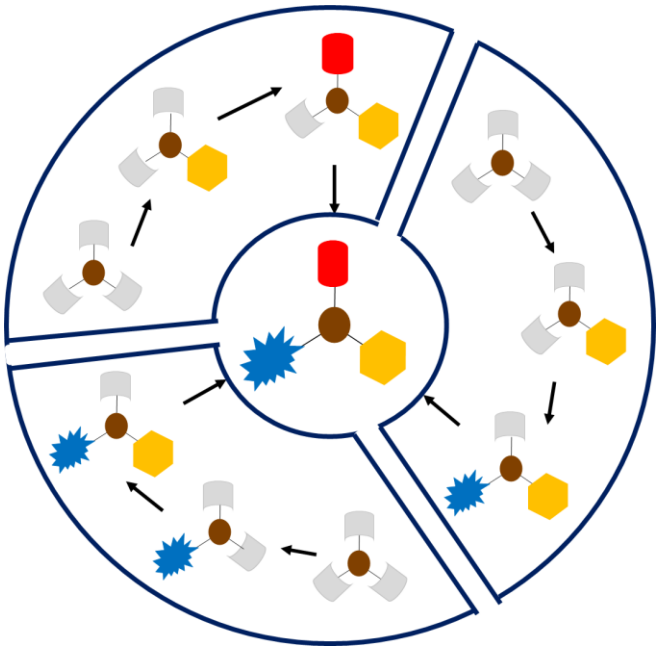


Scheme 5: The Concept of Chemoselectivity as described by Trost[21]

Joining these two concepts, our group has proposed orthogonal chemoselectivity as occurring reaction that proceeds with the discrimination between reactive sites which take place in any order (Figure 5).



Bi-orthogonal chemoselectivity



Tri-orthogonal chemoselectivity

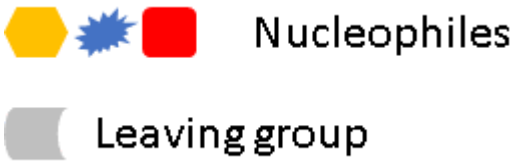
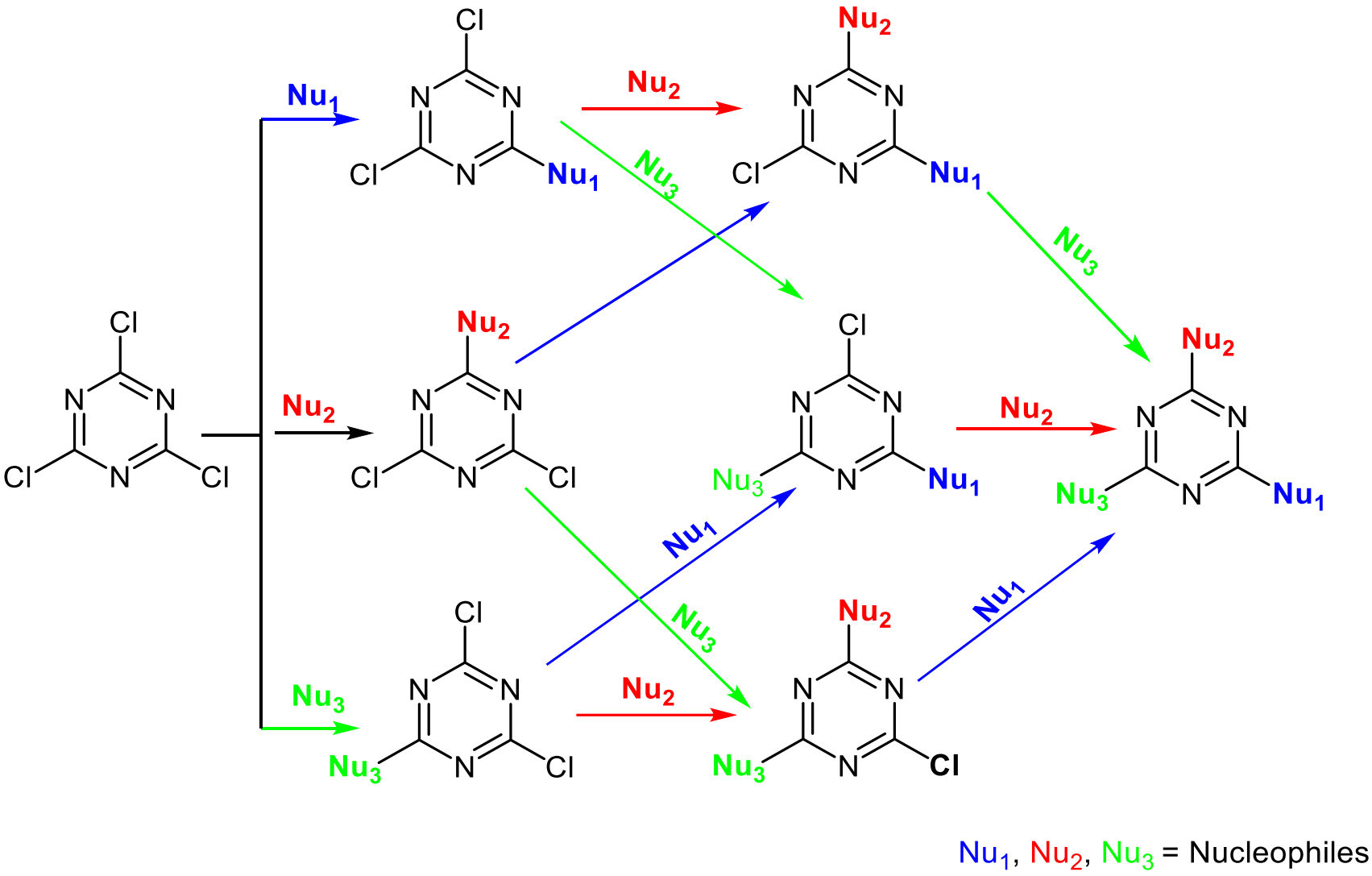


Figure 5: A sequential scheme of triorthogonal chemoselectivity

Objectives of the Research

As stated earlier, the substitution of TCT chlorine atoms occur sequentially with the substitution of the first chlorine at 0 °C, second chlorine at room temperature and the third chlorine at high temperature (reflux condition in most cases), the goal of this research is to achieve this trisubstitution at an ambient temperature compatible with the biological context

(for instance, a temperature less than 40 °C). Hence, this research is designed to establish the best order and condition to incorporate different nucleophiles into TCT at a temperature compatible with in a biological context, and to also explore the possibility of establishing the concept of orthogonal chemoselectivity using the proposed scheme below (Scheme 6).



Scheme 6: Different possible routes for the synthesis of a trisubstituted TCT using 3 different ~~nucleophile~~

Chapter 2 of this work discusses the incorporation of three different nucleophiles into TCT core under an ambient temperature condition. Here, phenol, isopentyl amine and 3-methyl-butane-1-thiol nucleophiles are sequentially incorporated into TCT. A series of reactions were carried out following the above scheme to explore the best order of incorporation. The compounds synthesized here were characterized by ^{1}H NMR and ^{13}C NMR

In **chapter 3** of this work, TCT was explored as a triorthogonal chemoselective linker. Here, a series of 43 reactions were performed to proof this concept using tridentate s-triazine as a model. Amine nucleophile was replaced with azide (which has a high electron withdrawing ability). The introduction of azide here is to check the possibility of incorporating other nucleophiles.

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CHAPTER 2

Phenol as a Modulator in the Chemical Reactivity of TCT: Rules of the Game II

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Phenol as a Modulator in the Chemical Reactivity of TCT: Rules of the Game II

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Abstract

TCT is a privileged core that has the capacity to undergo sequential nucleophilic substitution reactions. Three nucleophiles, namely ~~phenol, thiol and amine~~, were studied and the preferential order of incorporation on TCT was found to be first phenol, second thiol and third amine. The introduction of phenol was achieved at -20 ºC. The incorporation of this nucleophile in TCT helped to replace the third "Cl" at 35 ºC, which is compatible with a biological context. The atomic charges on "Cl" calculated by theoretical approaches were consistent with the experimental findings.

1. Introduction

s-Triazine (~~TCT~~) is one of the most privileged core units, finding applications ranging from industrial usage, such as melamine resins[1, 2] and energetics[3], to pharmaceutical molecules[4, 5]. The major advantage associated with TCT is the reactivity of the "Cl" atoms, which are easily controlled by temperature. This feature thus allows the incorporation of nucleophiles to prepare mono-, di- and tri-substituted triazines (**Figure 1**)[6-9].

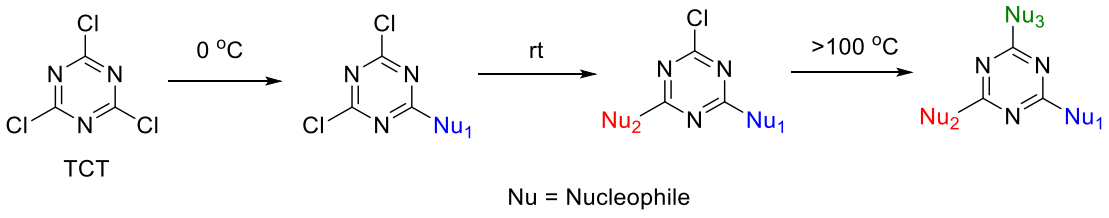


Figure 1: Nucleophilic substitution reaction for TCT

Orthogonality and chemoselectivity are two common terminologies in the field of synthetic chemistry. Orthogonality was introduced in 1977 by Barany and Merrifield to explain the context of protecting group[10], while chemoselectivity was coined by Trost in 1983 to differentiate between reactive sites[11]. In 1985, Barany and Albericio introduced and demonstrated triorthogonality to explain the removal of different protecting groups in any sequential order[12]. We recently reported the concept of "orthogonal chemoselectivity", which is defined as the discrimination between reactive sites in any order, and demonstrated the concept preserving TCT as the core unit[13]. Triorthogonality has been demonstrated using azide, thiol and phenol as nucleophiles. The concourse of alcohol (due to poor reactivity and deactivation of the aromatic ring as a result of its electron-donating behavior) and amine (as once it is substituted, only one further amine can be incorporated) was not present in the previous study as these compounds restrict the triorthogonality principle[14]. However, TCT carrying amines as substituents is a key player in the biological arena[9]. Furthermore, phenol can mimic the side chain of Tyr and a priori should be electron-withdrawing, a property that can enhance reactivity. Given these considerations, here we examined the preferential order of incorporation of phenol, thiol and amine. We demonstrate the influence of phenol on the TCT core for undergoing further nucleophilic substitution and unveil the preferential order of incorporation of three distinct nucleophiles onto this core.

2. Result and Discussion

As a model, we used phenol, thiol and amine as nucleophiles (**Figure 1**). ~~Phenol~~ shows considerably high reactivity due to the stabilization of phenoxide ion by resonance.

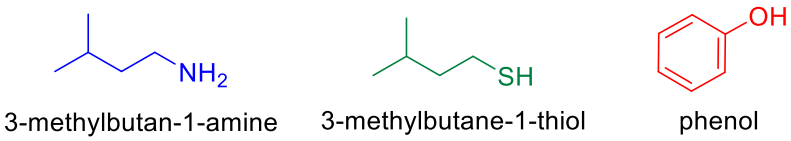
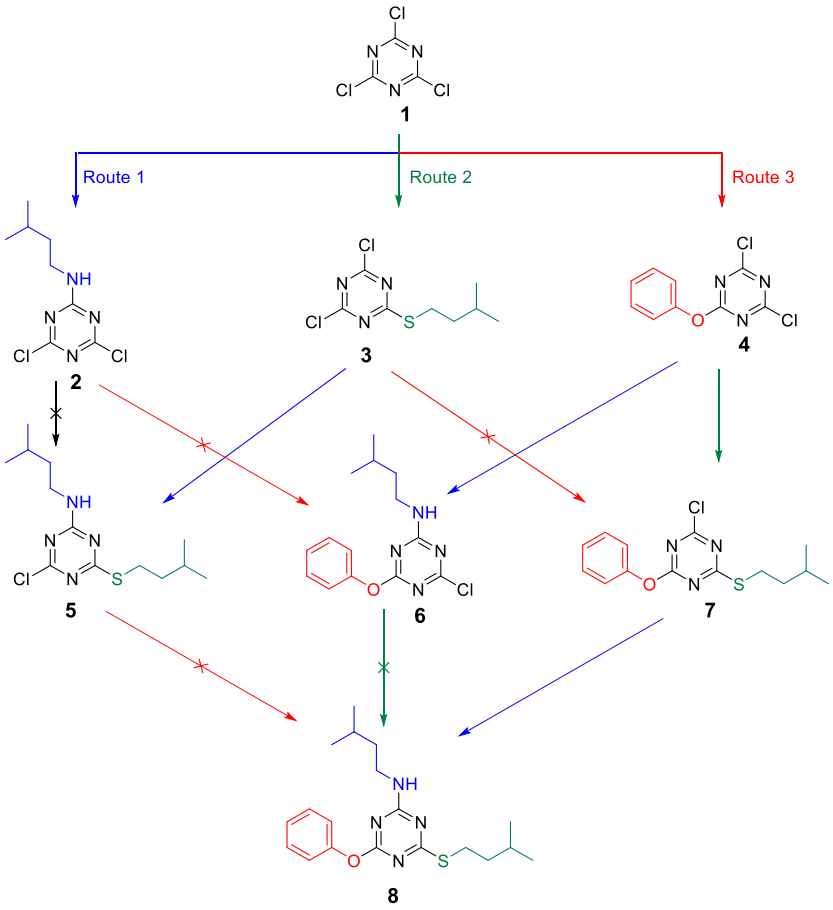


Figure 1: Nucleophiles chosen for the study

To study the order of nucleophile incorporation, several reactions were attempted using ethyl acetate as solvent and DIEA as base, as shown in **Scheme 1**. Three routes were tested for the synthesis of **8**, which contains ~~phenol, thiol and amine~~ as three substituents on the TCT core. In route 1, TCT was reacted with ~~amine~~ first to form amine derivative (**2**). This was followed by ~~thiol,~~ and then phenol or phenol, and finally thiol. In route 2, TCT was reacted with thiol first to form thiol derivative (**3**), then followed by amine and then phenol, or phenol and then amine. In route 3, phenol derivative (**4**) was formed first by reaction of TCT with

phenol, followed by amine and thiol, or thiol and amine to form **8**. The routes are shown in **Scheme 1**.



Scheme 1: A priori approaches to form compound **8**

Upon reaction with the above-mentioned nucleophiles at 0 ºC for 30 min, TCT formed di-chloro substituted triazine (DST). When amine and thiol were used, the reaction was completed in 30 min, as monitored by TLC, affording amine and thiol derivative (**2** and **3**) in high yields. However, in case of the synthesis of phenol derivative (**4**), two spots were formed on TLC. The reaction condition for **4** was further optimized by lowering the temperature to -20 ºC, which yielded **4** in high purity and good yield (as confirmed by HPLC). This result could be explained by the high nucleophilicity of phenol caused by greater stability of phenoxide ion compared to phenol alone. This result was the first indication supporting the hypothesis regarding the suitability of phenol to enhance reactivity. After the synthesis of DST, we addressed the preparation of mono-chloro substituted triazine (MST). The reactions were performed at rt using the same conditions as explained previously. The next possibility for amine derivative (**2**) was the reaction with thiol or phenol. Therefore, **2** was reacted with thiol and phenol at rt for 3 h using ethyl acetate as solvent. However, no product was observed. The reaction was then stirred at rt for overnight, but still only amine derivative (**2**) was there in the reaction mixture. This agrees well with our earlier findings where we reported, once amine is

incorporated, it is not possible to incorporate any other nucleophile except another amine owing to their high nucleophilicity[13]. Thus, route 1 was ruled out for the synthesis of **8**. In parallel, the thiol derivative (**3**) was left to react with amine and phenol at rt for 3 h using the reaction conditions mentioned before. These conditions led to the formation of thiol-amine derivative (**5**) and thiol-phenol derivative (**7**). Compound **5** was obtained in good yield and high purity, while poorer results were obtained for **7**. TLC monitoring showed a spot corresponding to product **3**, in addition to several other spots including starting material (**3**). It was concluded that these results could be due to the high reactivity of phenol. Therefore, another attempt was made to conduct the reaction at 0 ºC for 3 h. Under these conditions, only thiol derivative (**3**) was observed by TLC. Thus, thiol-phenol is not a suitable approach for the synthesis of **8**.

The reaction of thiol-amine derivative (**5**) with phenol was first carried out for the third position to form **8** at >60 ºC. Several attempts were made at a higher temperature, as TCT usually requires more elevated temperature for the replacement of the third "Cl". However, no product was observed. These results are consistent with our earlier work, in that no nucleophile can be incorporated after the amine has been introduced. Therefore, this route was ruled out for the synthesis of **8**.

Following route 3, compound **4** was reacted with amine and thiol separately for its incorporation at position 2 to form phenol-amine derivative (**6**) and phenol-thiol derivative (**7**). The reaction was attempted at rt for 3 h using same reaction condition as earlier. Compounds **6** and **7** were obtained in good yield and high purity. Replacement of the third "Cl" in **6** was attempted with thiol at 35 ºC. However, no product was observed. The reaction was also attempted at >60 ºC. As expected, no product formation was detected, since amine had already been incorporated onto the TCT core. However, in the case of **7**, the reaction was carried out at 35 ºC for 12 h with amine as nucleophile. TLC showed complete consumption of **7,** affording the formation of **8**.

~~Theoretical calculations~~

To understand the electronic effect on TCT and several intermediates (as shown in Scheme 1) for the synthesis of **8,** including the charges carried by "Cl" after each substituent, we performed a density functional theory (DFT) geometry optimization using the Gaussian09 program package and employing the B3LYP (Becke three parameters Lee–Yang–Parr exchange correlation functional) and the 6-311G++(d,p) basis set in gas phase[15]. Natural bond orbital (NBO) calculations were made on the optimized geometries to determine the atomic charges[16]. The charges carried by "Cl" in each case were compared (**Table 1**).

Table 1: Charges carried by "Cl" in the molecules

Entry	Charges carried by "Cl"		
	First "Cl"	Second "Cl"	Third "Cl"
1	0.088	0.088	0.088
2	-	0.049	0.049
3	-	0.062	0.062
4	-	0.069	0.069
5	-	-	0.025
6	-	-	0.030
7	-	-	0.044

The atomic charges carried by "Cl" in each molecule reveals important details about the chemical reactivity of each one upon reaction with nucleophiles[13, 14]. In the case of **1**, the charge carried by "Cl" was 0.088, which accounts for the high reactivity of TCT when treated with nucleophiles. Hence, the reaction was carried out at lower temperature (0 ºC or even lower). Upon the first substitution, the charges present on "Cl" decreased, lowering the reactivity of DST. In case of **2**, **3** and **4,** the charges decreased to 0.049, 0.062 and 0.069, respectively. Therefore, the conditions for further reactions required a longer time and higher temperature (rt in this case). ~~Of **2**~~, **3** and **4**, the former carried the least charge, whereas **4** carried the highest, thereby indicating the high reactivity of this compound. Based on these results, the incorporation of phenol as the first substituent emerges as the best approach to maintain the reactivity of triazine core.

To further determine the preferential order of substitution, we compared the charges carried by last "Cl" present on disubstituted TCT. The charge carried by "Cl" in the case of **5**, **6** and **7** was 0.025, 0.030 and 0.044, respectively (Table 1). Of all the derivatives, **7** showed the highest charge, thus confirming it as the best choice to afford **8**. In contrast, **5** and **6** might require elevated temperature or harsh conditions to afford **8**. Our findings therefore show that the ideal order to incorporate the nucleophiles is first phenol, second thiol and third amine.

3. Conclusion

Here we report on the preferential incorporation of nucleophiles (~~phenol, amine and thiol~~) onto TCT. The order was found to be first phenol, second thiol and third amine. Taking advantage of the high chemical reactivity of phenol, the third replacement with amine was done at 35 ºC, a temperature compatible with biological systems. However, the presence of amine on TCT blocks the incorporation of phenol, despite its high reactivity. A comparison of these results with our previous findings [13] reveals that both alcohol and phenol show the same trend

of incorporation. However, the presence of phenol facilitates the incorporation of the rest of the nucleophiles. This is significant in case of amine incorporation in the third position, which was carried out at 35 ºC instead of 75 ºC when the alcohol was present. Theoretical calculations were performed and compared with the experimental findings. NBO calculations helped to determine the atomic charges of "Cl" in each molecule. The charges found supported the experimental results, thus validating the findings. Replacement of the third "Cl" at 35 ºC extends the use of TCT beyond a triorthogonal linker in the biological context, thereby paving the way for nucleophilic reactions involving various peptides, antibodies, and drugs.

4. Experimental

Materials and Methods

2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride, TCT), 3-methylbutan-1-amine, 3-methylbutane-1-thiol, phenol, and diisopropylethylamine were purchased from Sigma-Aldrich (Sigma-Aldrich, Germany). The solvents used were of analytical and HPLC reagent grade. Magnetic resonance spectra (^{1}H and ^{13}C) were recorded on a Bruker 400 MHz instrument. Chemical shift values were reported in δ units (ppm) using TMS as internal standard. Follow-up of the reactions and checks of the purity of the compound was done by TLC on silica-gel-protected aluminum sheets 60 F254 (Merck), and the spots were detected by exposure to UV light at λ = 254 nm. Analytical HPLC was performed on an Agilent 1100 system using a Phenomenax C_{18} column (3 µm, 4.6 × 50 mm). Data were processed using Chemstation software. Buffer A: 0.1% TFA in H_2O; buffer B: 0.1% TFA in CH_3CN were used in HPLC. LCMS was performed on Shimadzu 2020 UFLC using a YMC- Triart C_{18} (5 µm, 4.6 × 150 mm) column and data processing was carried out using Lab Solution software. Buffer A: 0.1% formic acid in H_2O; and buffer B: 0.1% formic acid in CH_3CN were used.

Synthesis of 4,6 dichloro 2-substituted s-triazine (DST)

TCT (50 mg, 0.27 mmol) was dissolved in EtOAc (1 mL) and cooled to 0 ºC for 5 min. Nucleophile (0.27 mmol) was then added to the above stirring solution, followed by addition of DIEA (47 µL, 0.27 mmol). The reaction was stirred at 0 ºC (-20 ºC in the case of phenol) for 30 min. The progress of the reaction was monitored by TLC (EtOAc/hexane as mobile phase) until no starting material was observed. The solution was then concentrated to dryness, and the residue was dissolved in EtOAc and washed several times with water to remove DIEA salts. The organic layer was collected, dried over $MgSO_4$, filtered and concentrated to afford pure product, which was used for the next step without further purification.

4,6-dichloro-N-isopentyl-1,3,5-triazin-2-amine (**2**)

Off-white semi-solid; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 7.6 min; ^{1}H NMR (400 MHz, CDCl3): 0.90 (d, *J* = 6.8 Hz, -CH_3), 1.40 (t, *J* = 6.8 Hz, -CH_2), 1.60 (m, ~~*J* = 6.8 Hz,~~ -CH), 3.4 (q, *J* = 6.0 Hz, -CH_2), 5.7 (~~m,~~ -NH); ^{13}C NMR (100 MHz, CDCl3): ~~21.2, 26.5, 28.3, 36.2, 169.0, 182.0, 185.6~~

2,4-dichloro-6-(isopentylthio)-1,3,5-triazine (**3**)

Yellowish oil; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 10.5 min; ^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (d, *J* = 6.4 Hz, -CH_3), 1.55 (m, *J* = 6.4 Hz, -CH_2), 1.65 (m, ~~*J* = 6.4 Hz,~~ -CH), 3.1 (t, *J* = ~~6.4~~ Hz, -CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): ~~21.2, 26.5, 28.3, 36.2, 169.0, 182.0, 185.6~~

2,4-dichloro-6-phenoxy-1,3,5-triazine (**4**)

Off-white solid; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 6.7 min; ^{1}H NMR (400 MHz, $CDCl_3$): ~~7.10 (d, *J* = 1.6 Hz, -CH), 7.30 (t, *J* = 1.6 Hz, -CH), 7.40 (t, *J* = 2.0 Hz, -CH);~~ ^{13}C NMR (100 MHz, $CDCl_3$): ~~114.2, 120.0, 125.7, 128.7, 150.7, 170.0, 172.0~~

Synthesis of 2-chloro-4,6-disubstituted s-triazine (MST)

Nucleophile (0.27 mmol) was added to DST (0.27 mmol) in EtOAc (1 mL), followed by addition of DIEA (47 µL, 0.27 mmol). The reaction was stirred at rt for 3 h. The progress of the reaction was monitored by TLC (ethyl acetate/hexane as mobile phase) until no starting material was observed. The solution was concentrated to dryness and the residue was dissolved in EtOAc and washed several times with water to remove DIEA salts. The organic layer was collected, dried over $MgSO_4$, filtered and concentrated to afford pure product, which was used for the next step without further purification.

4-chloro-N-isopentyl-6-(isopentylthio)-1,3,5-triazin-2-amine (**5**)

Creamy oil; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 10.7 min; ^{1}H NMR (400 MHz, $CDCl_3$): 0.86 (m, *J* = 6.6 Hz, -CH_3), 1.51 (m, *J* = 7.1 Hz, -CH_2), 1.62 (m, *J* = 6.6 Hz, -CH), 3.20 (q, *J* = 6.1 Hz, -CH_2), 5.28 (t, *J* = 5.4, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): 21.3 24.7, 26.5, 27.1, 37.3, 38.1, 161.7, 178.2, 179.2

4-chloro-N-isopentyl-6-phenoxy-1,3,5-triazin-2-amine (**6**)

Off-white solid; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 9.1 min; ^{1}H NMR (400 MHz, $CDCl_3$): 0.80 (m, *J* = 6.6 Hz, -CH_3), 1.45 (m, *J* = 6.6 Hz, -CH_2),

1.55 (m, *J* = 6.6 Hz, -CH), 3.27 (q, *J* = 6.2 Hz, -CH_2), 7.21 (m, *J* = 7.6, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): 21.1, 26.4, 27.7, 36.9, 124.9, 128.4, 150.7, 170.0, 172.6, 185.6

2-chloro-4-(isopentylthio)-6-phenoxy-1,3,5-triazine (**7**)

Yellowish semi-solid; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 12.4 min; ^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (m, *J* = 6.4 Hz, -CH_3), 1.50 (m, *J* = 6.4 Hz, -CH_2), 1.60 (m, *J* = 7.0 Hz, -CH), 3.0 (t, *J* = 7.6 Hz, -CH_2), 7.3 (m, *J* = 7.4, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): ~~21.1, 26.4, 27.7, 36.9, 124.9, 128.4, 150.7, 170.0, 172.6, 185.6~~

Synthesis of N-isopentyl-4-(isopentylthio)-6-phenoxy-1,3,5-triazin-2-amine (**8**)

Isopentyl amine (0.27 mmol) was added to a stirring solution of MST (0.27 mmol) in EtOAc (1 mL), followed by addition of DIEA (0.27 mmol). The reaction mixture was heated to 35°C for 8 h. The progress of the reaction was monitored by TLC (ethyl acetate/hexane as mobile phase) until the complete consumption of starting material. Solvent was removed under vacuum, and the residue was dissolved in EtOAc (5 mL). The organic layer was washed several times with water to remove DIEA salts. The organic layer was collected, dried over $MgSO_4$, filtered and concentrated to afford pure product.

Brownish semi-solid; HPLC [30-95 % of CH_3CN (0.1% TFA/ H_2O (0.1 %TFA) over 15 min] t_R = 13.3 min; ^{1}H NMR (400 MHz, $CDCl_3$): 0.85 (m, *J* = 6.8 Hz, -CH_3), 1.55 (m, *J* = 7.6 Hz, -CH_2), 3.37 (t, *J* = 7.6 Hz, -CH_2), 5.30 (t, *J* = 7.6 Hz, -NH), 7.30 (m, -ArH); ^{13}C NMR (100 MHz, $CDCl_3$): 21.3 24.7, 26.5, 27.2, 37.3, 38.3, 120.9, ~~120.9,~~ 124.4, 128.2, 151.1, 164.6, 168.6, 182.6.

Theoretical calculations

The models were drawn using GaussView05[17], and all quantum chemical calculations were performed using Gaussian09 with B3LYP functional and 6-311G++(d,p) basis set. No solvent corrections were made with these calculations. Vibration analysis showed that the optimized structure indeed represented a minimum on the potential energy surface (no negative eigenvalues). NBO calculations were performed on the optimized geometries to determine atomic charges.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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I. Chromatogram and spectra for derivatives

Figure 1. HPLC of **2**

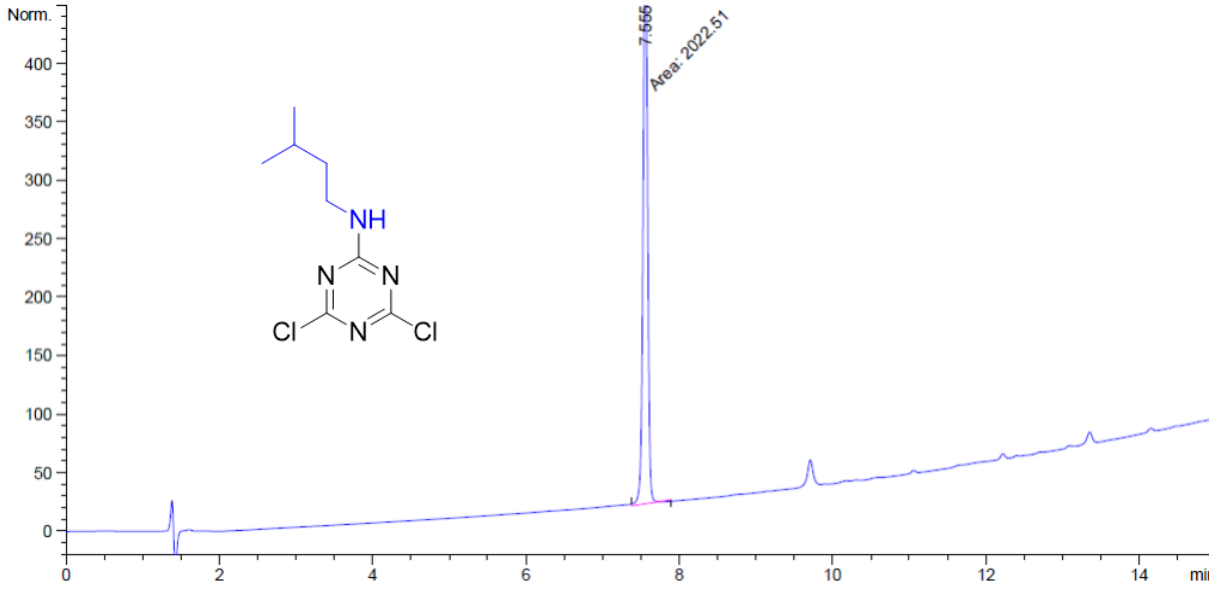


Figure 2. ^{1}H NMR of **2**

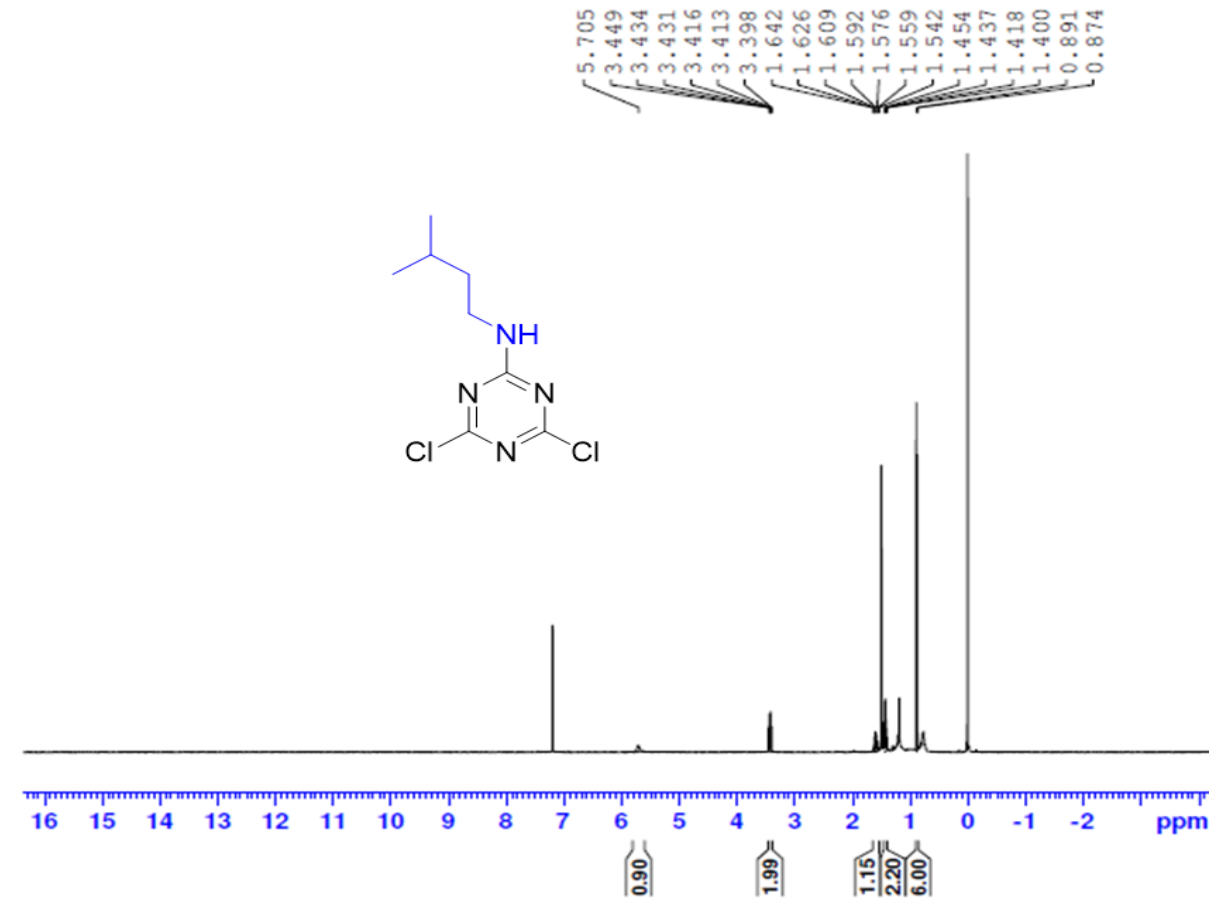


Figure 3. ^{13}C NMR of **2**

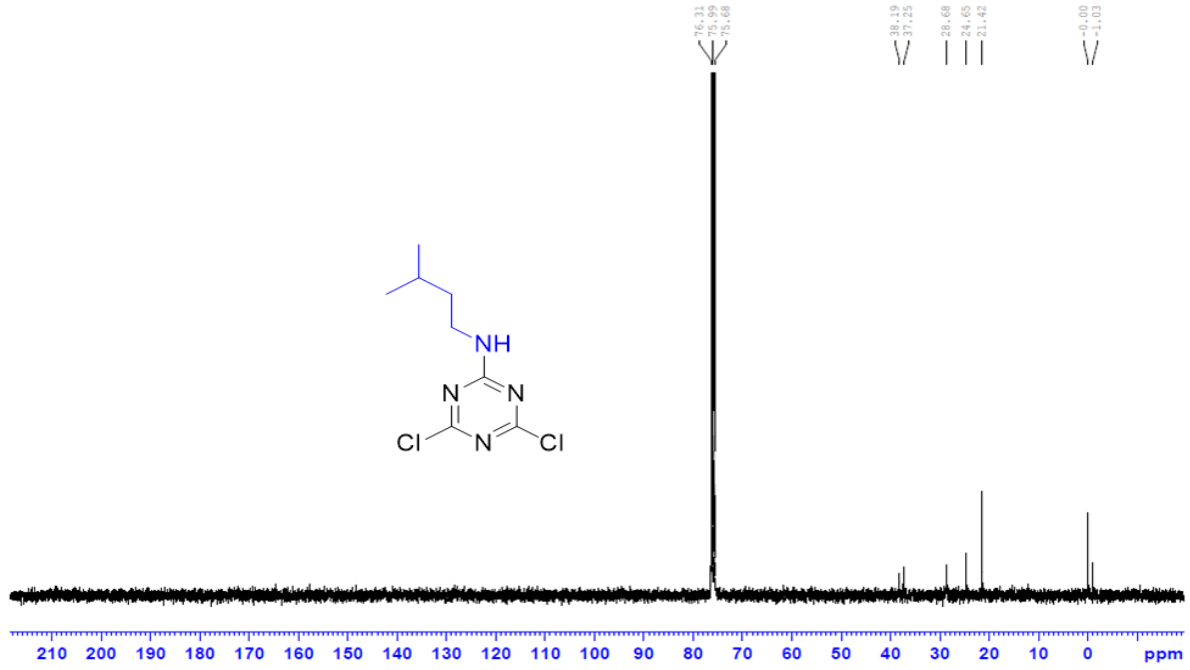


Figure 4. HPLC of **3**

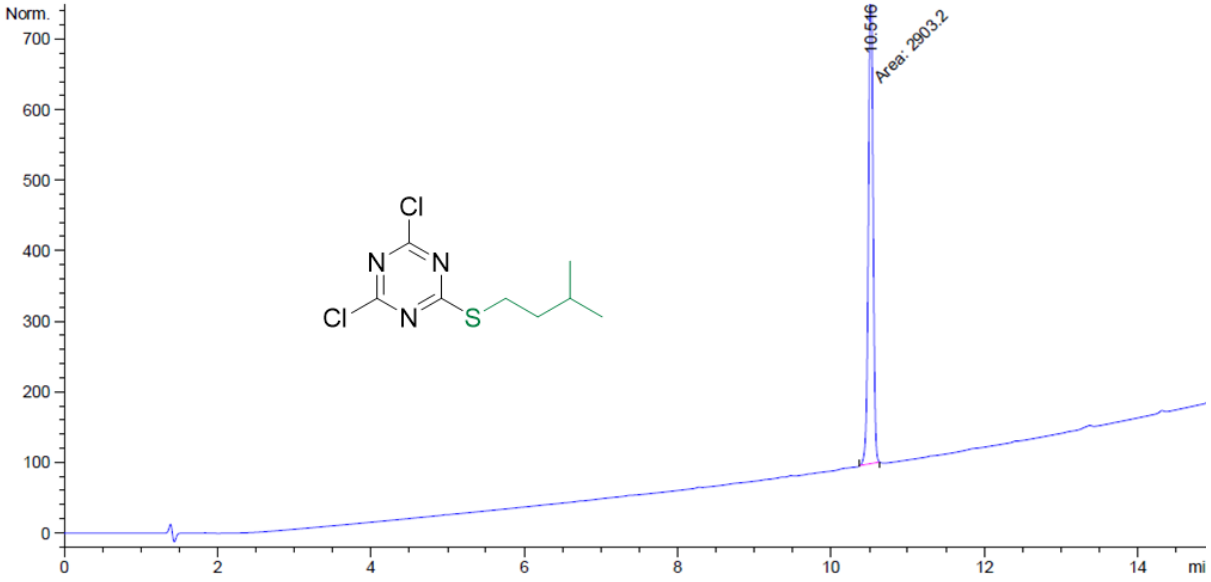


Figure 5. ^{1}H NMR of **3**

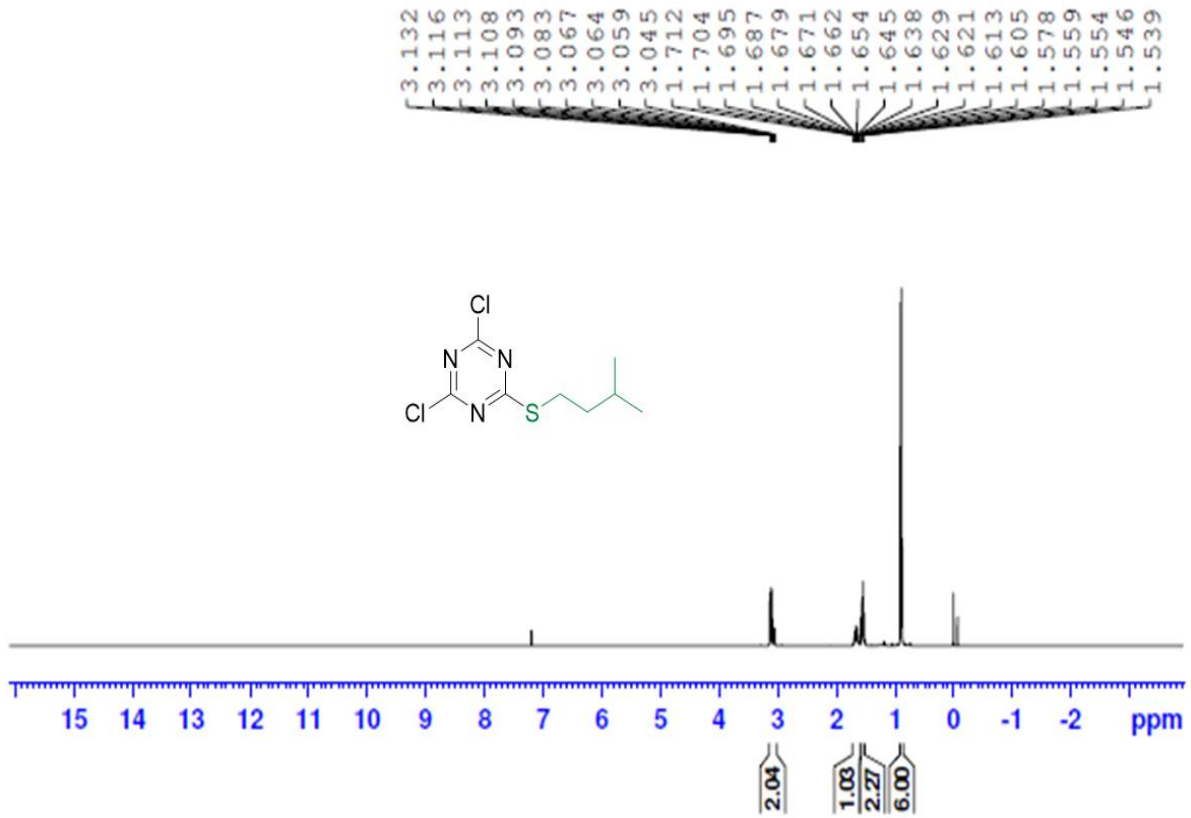


Figure 6. ^{13}C NMR of **3**

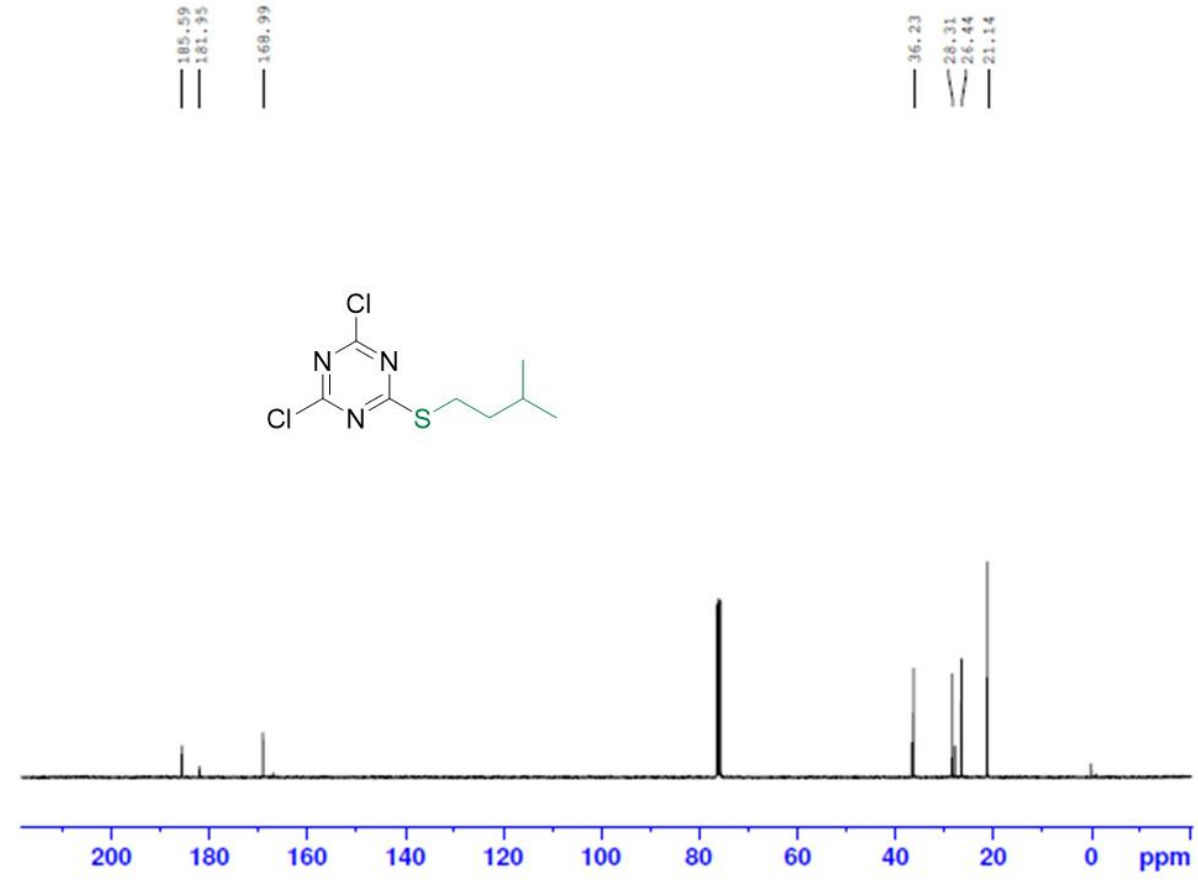


Figure 7. HPLC of **4**

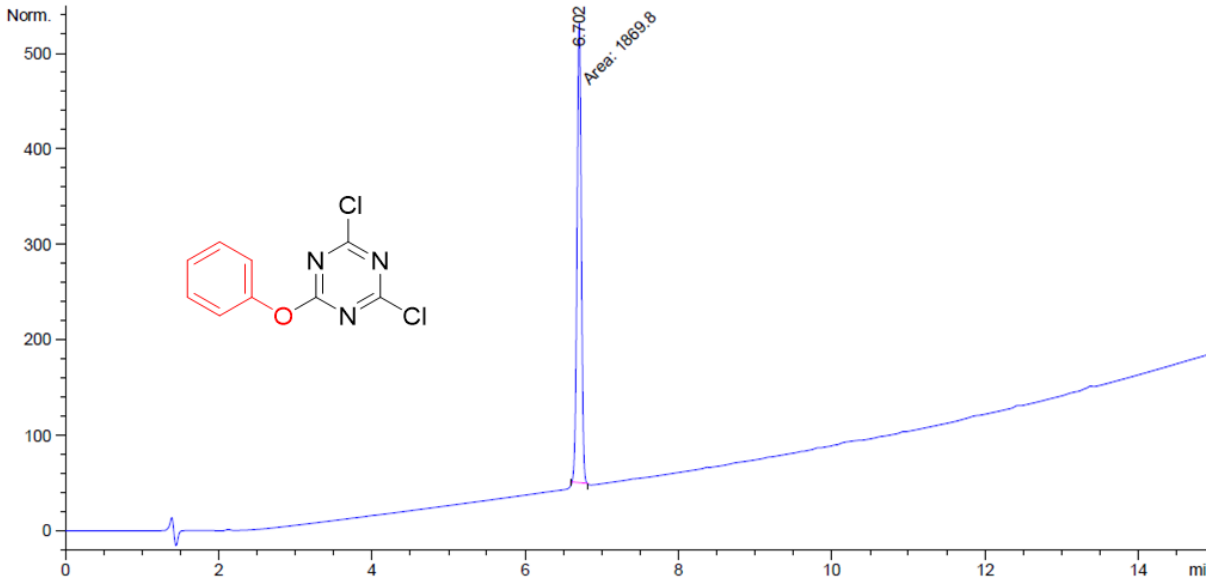


Figure 8. ^{1}H NMR of **4**

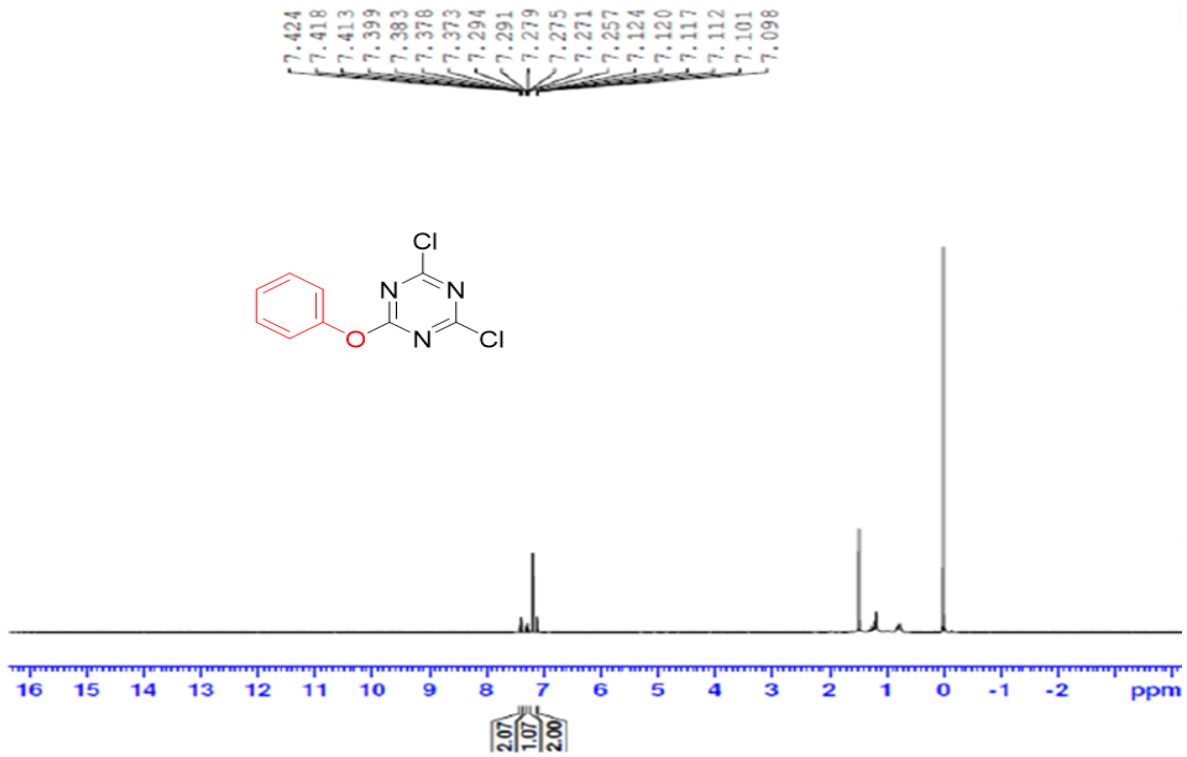


Figure 9. ^{13}C NMR of **4**

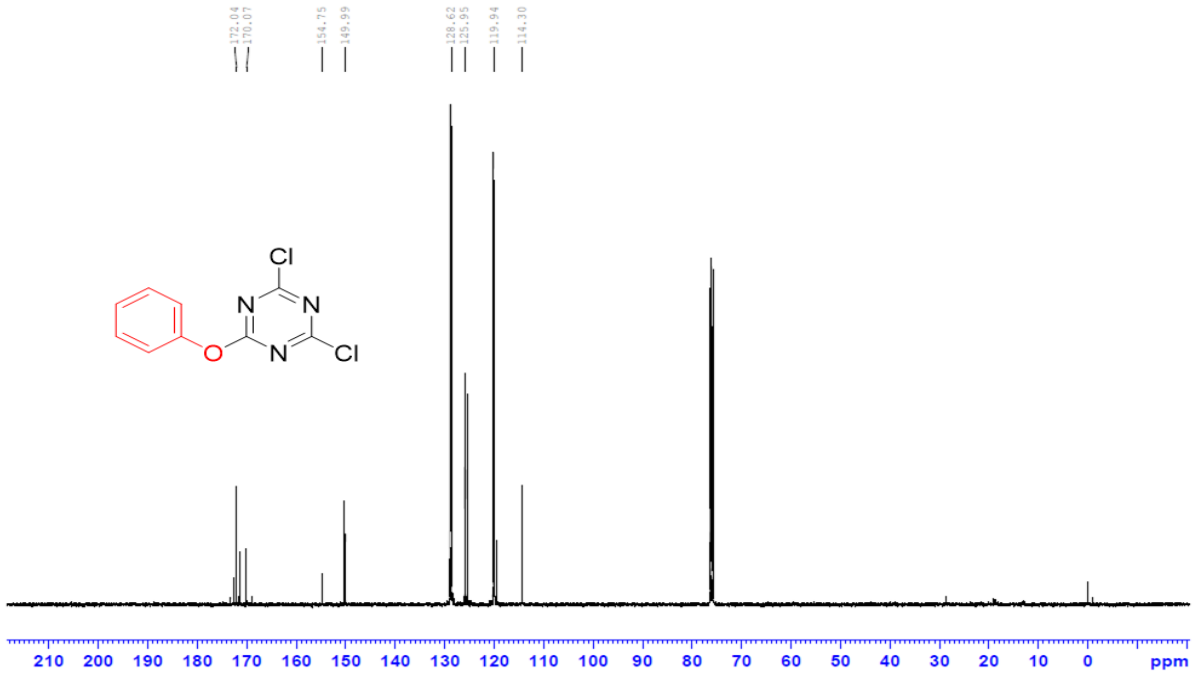


Figure 10. HPLC of **5**

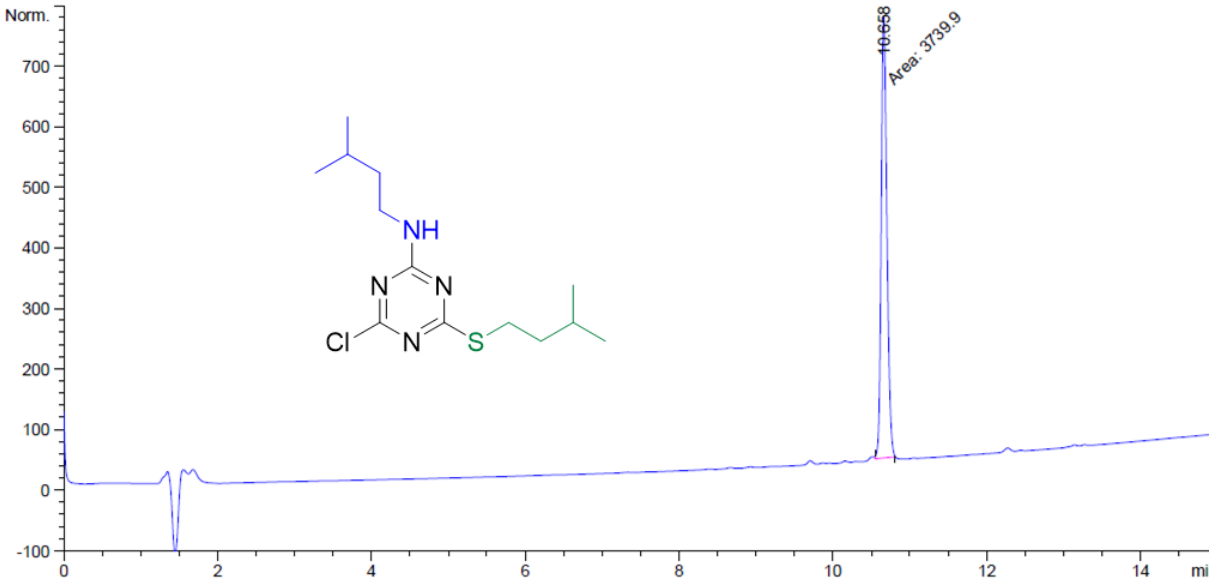


Figure 11. ^{1}H NMR of **5**

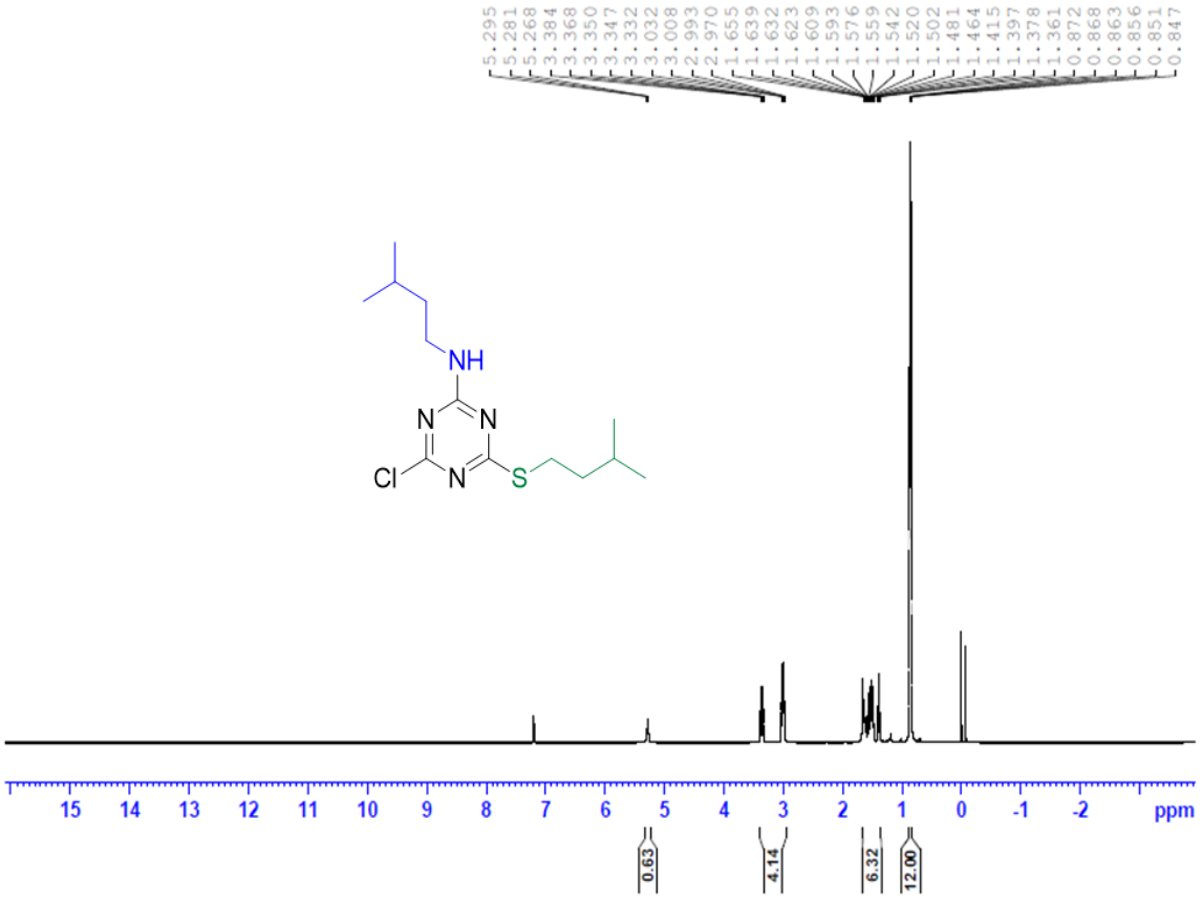


Figure 12. ^{13}C NMR of **5**

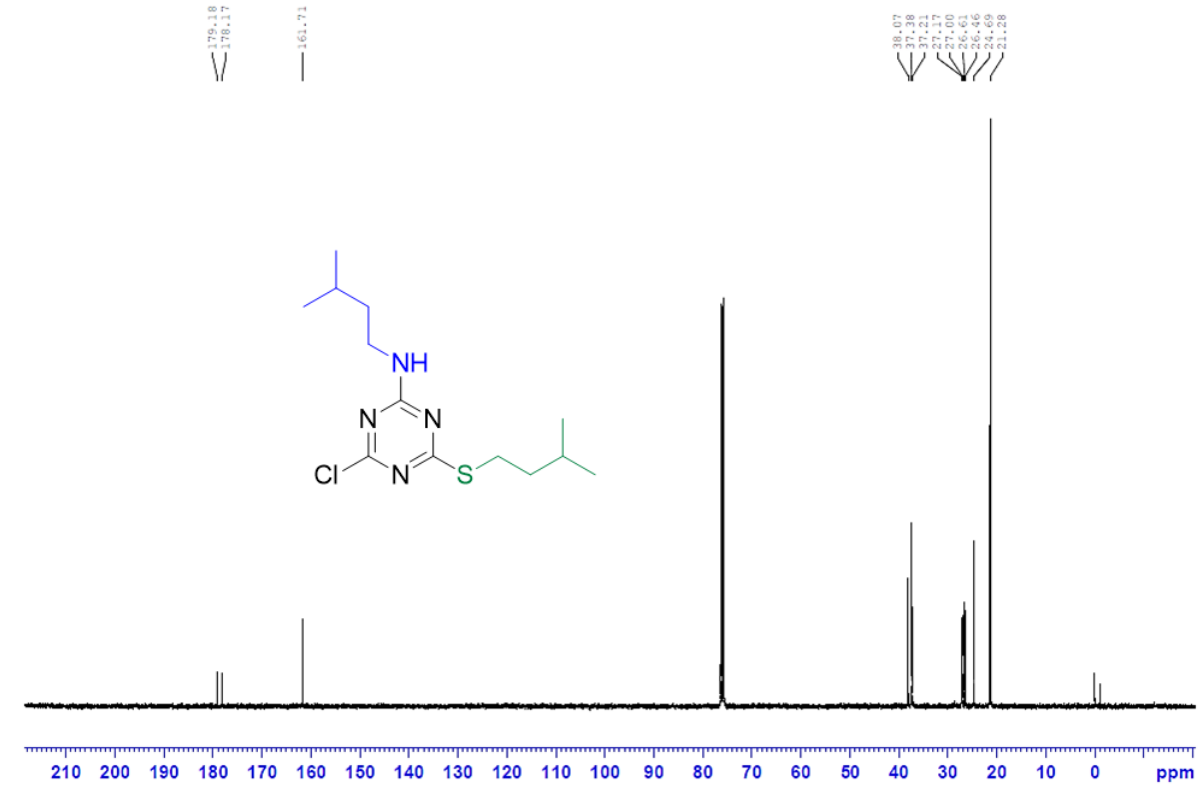


Figure 13. HPLC of **6**

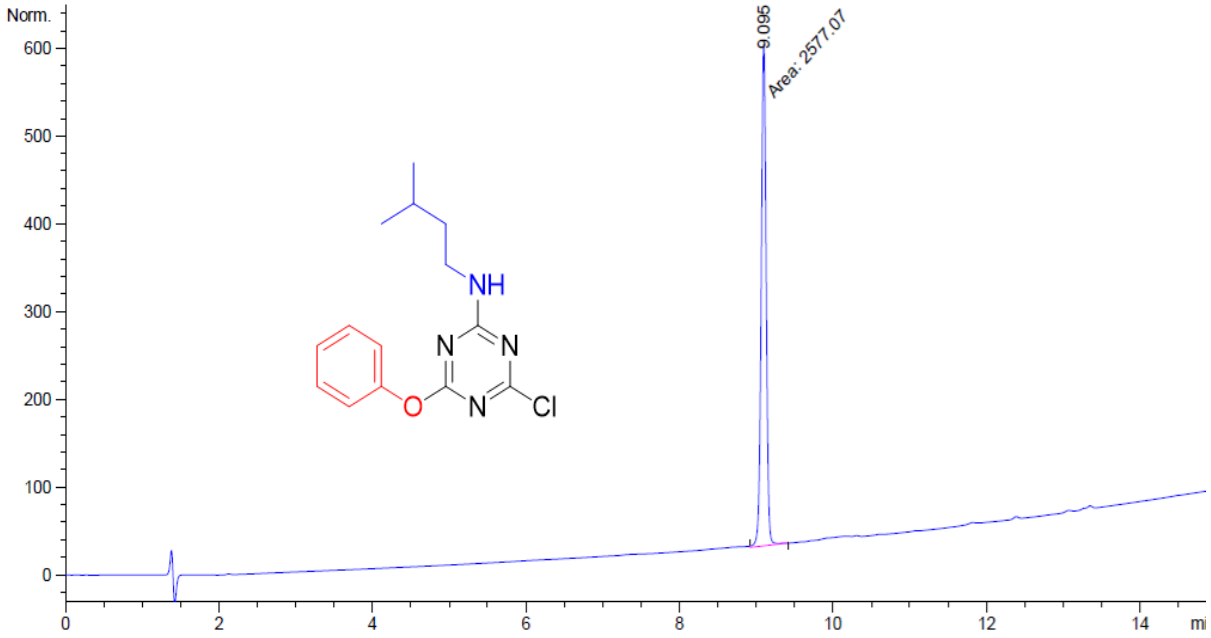


Figure 14. ^{1}H NMR of **6**

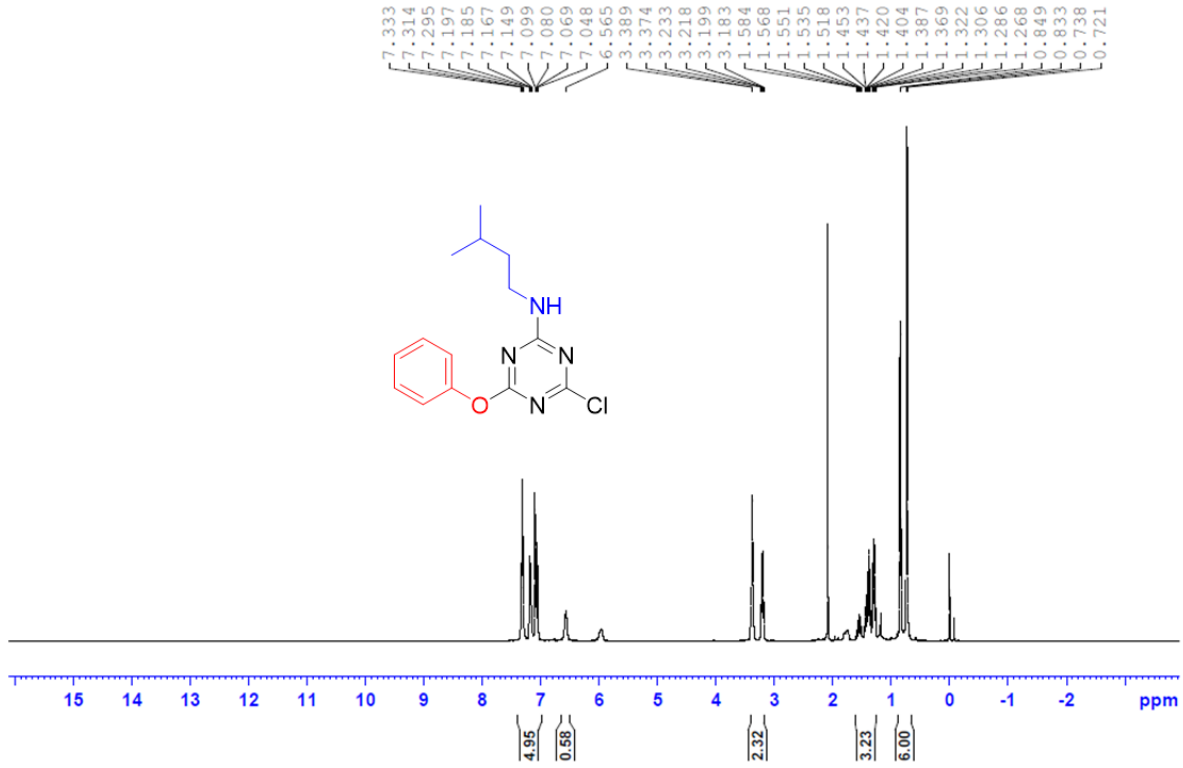


Figure 15. ^{13}C NMR of **6**

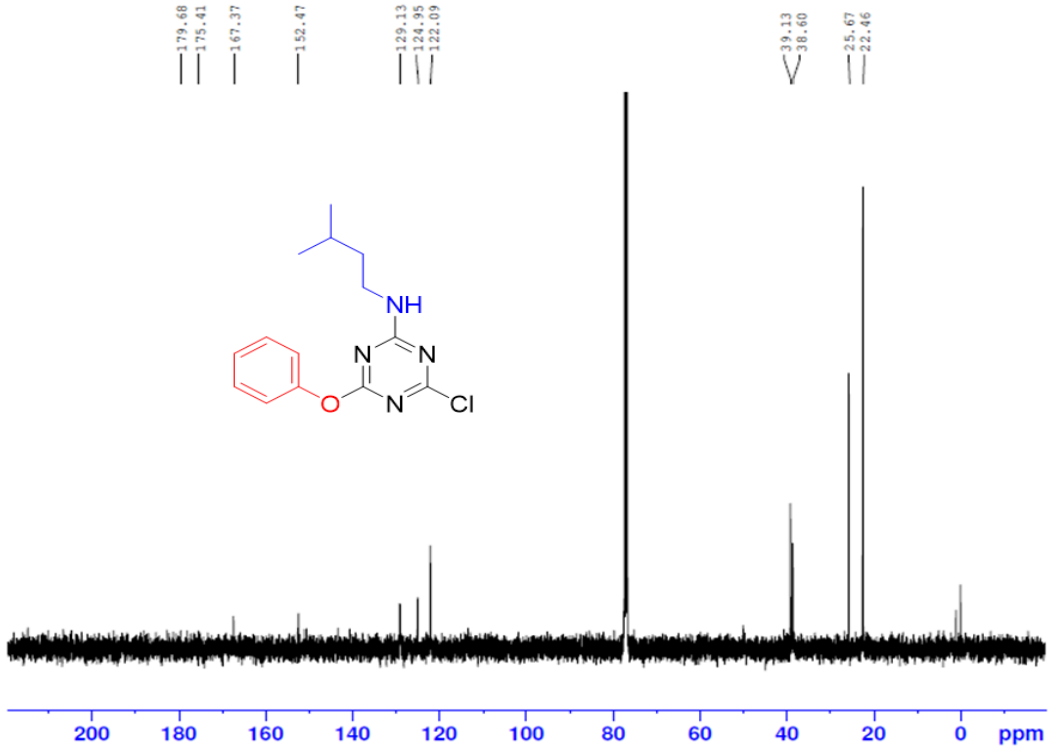


Figure 16. HPLC of **7**

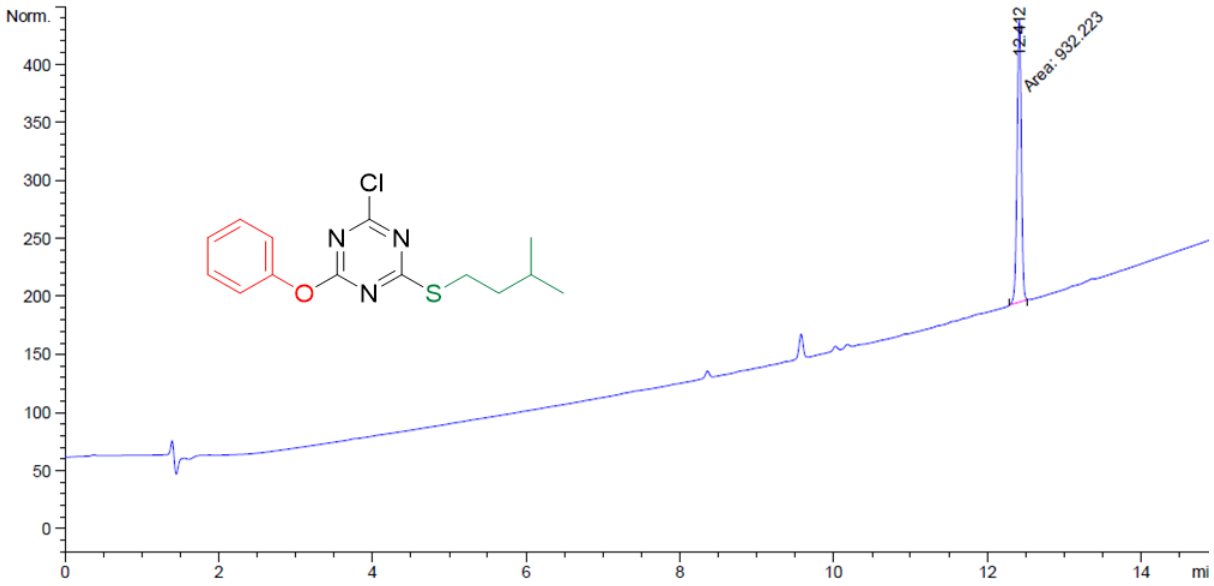


Figure 17. ^{1}H NMR of **7**

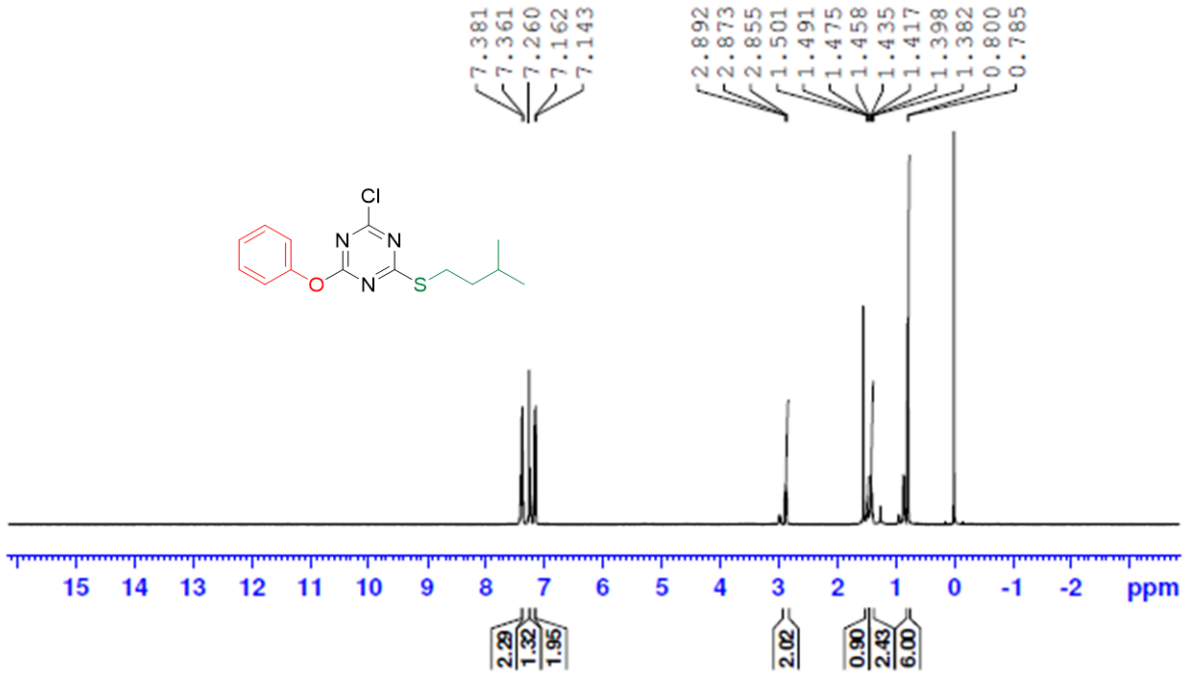


Figure 18. ^{13}C NMR of **7**

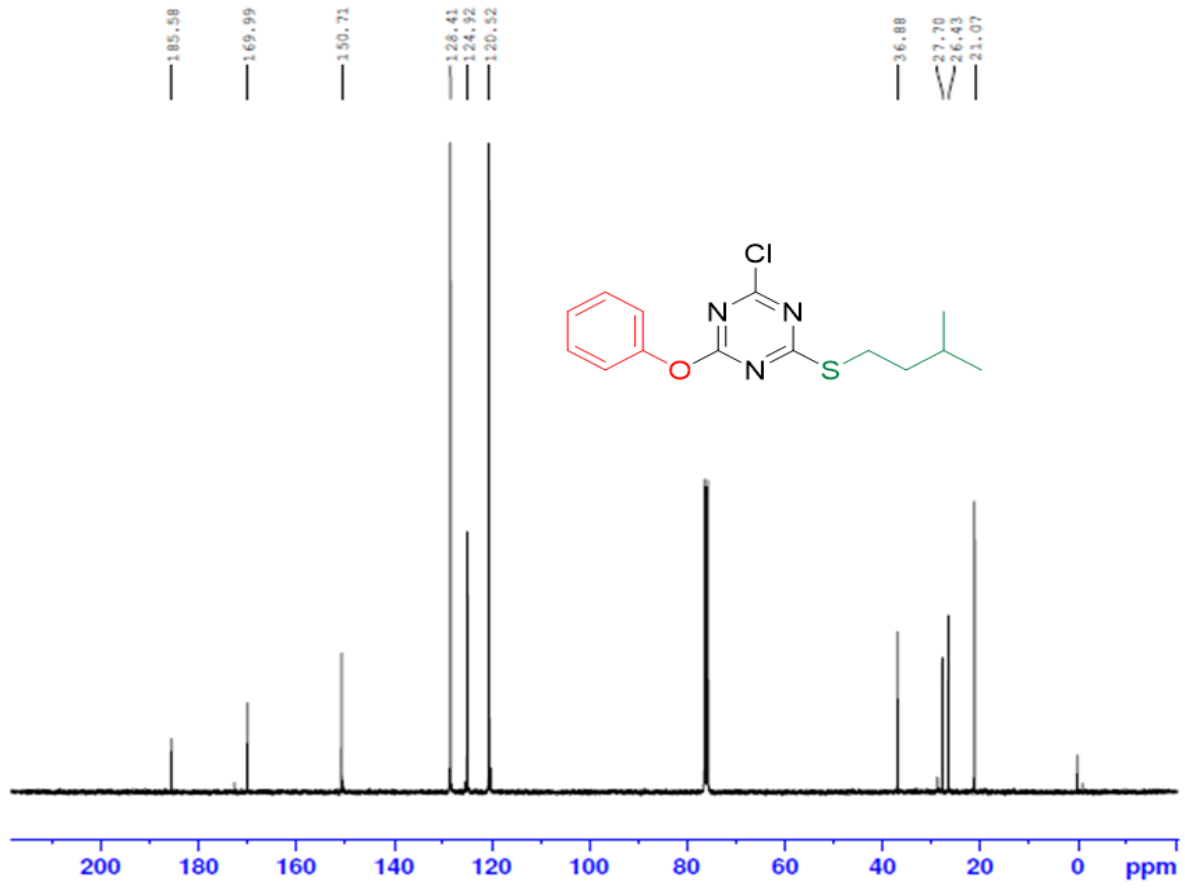


Figure 19. HPLC of **8**

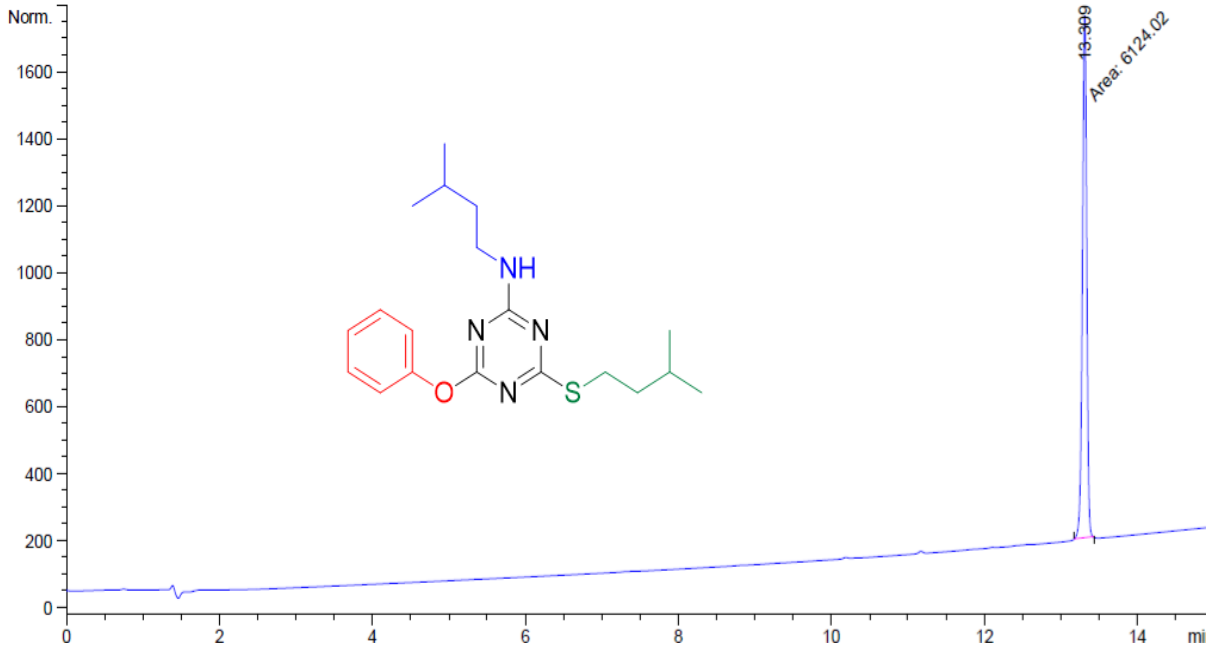


Figure 20. ^{1}H NMR of **8**

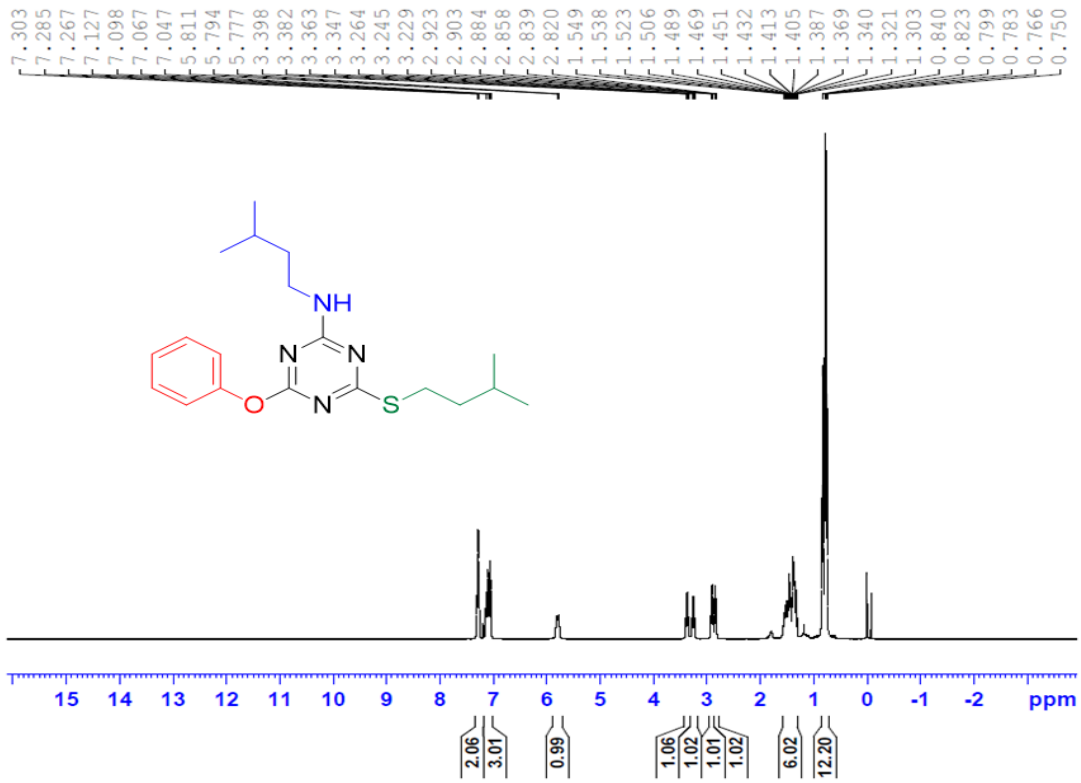
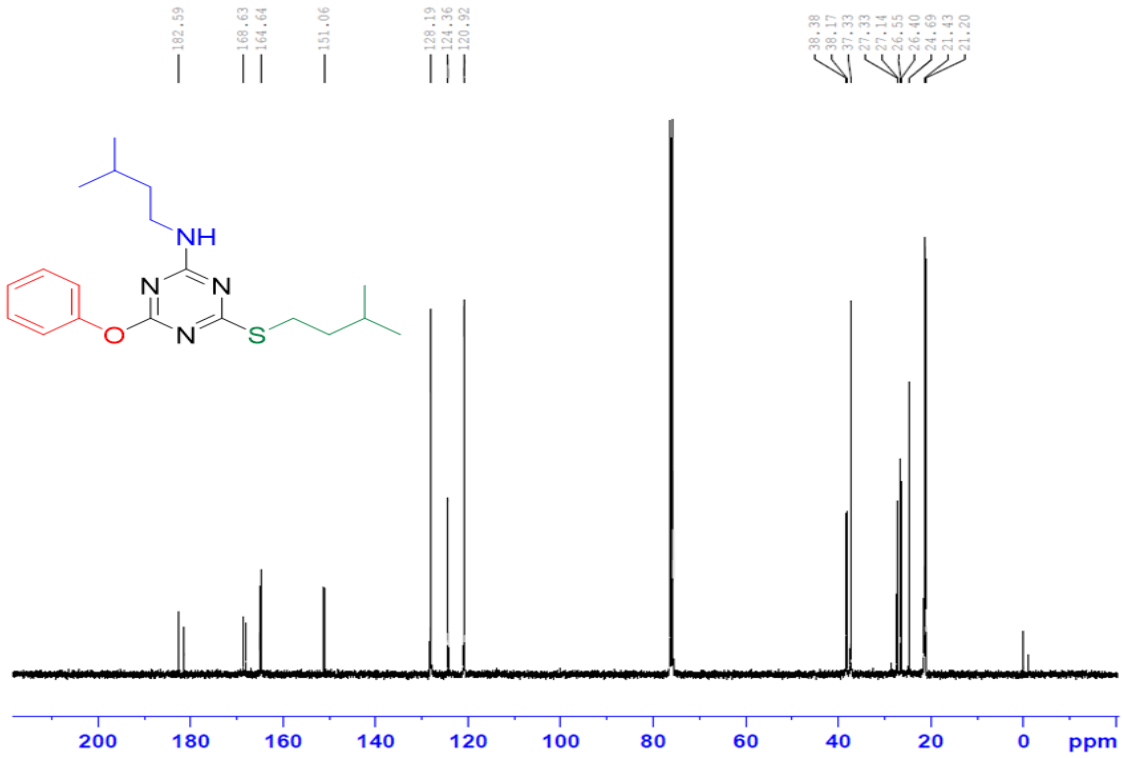


Figure 21. ^{13}C NMR of **8**



II. Cartesian co-ordinates for the NBO calculations

1. Optimized Co-ordinates of 1 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	1.27012700	-0.16535400	-0.00003900
C	-0.77814500	-1.01726200	0.00001100
C	-0.49180600	1.18253700	0.00006400
Cl	-1.15664700	2.78037700	-0.00007600
Cl	-1.82980500	-2.39171400	0.00000200
Cl	2.98629300	-0.38858700	0.00005600
N	0.83176700	1.08718400	0.00000400
N	-1.35730100	0.17665000	0.00001300
N	0.52577000	-1.26395100	-0.00000500

1 6 1.0 7 1.5 9 1.5
2 5 1.0 8 1.5 9 1.5
3 4 1.0 7 1.5 8 1.5
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2. Optimized Co-ordinates of 2 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	2.66253600	0.67681700	-0.10054300
C	1.55298100	-1.22955200	0.02799200
C	0.49210300	0.69350500	0.44250400
Cl	4.15296000	1.53064400	-0.40855800
N	1.62210300	1.40919600	0.21584900
N	2.72150600	-0.64929700	-0.21747300
N	0.41654900	-0.65219100	0.35251600
N	-0.60456900	1.38714800	0.78393000
H	-0.48731600	2.38997700	0.78757300
C	-1.93189700	0.81660700	1.00500700
H	-1.79045600	-0.20551000	1.35170800
H	-2.40048300	1.38316300	1.81547600
C	-2.81374900	0.85659900	-0.25007000
H	-2.33489800	0.26354900	-1.03803200
H	-2.85411400	1.88984200	-0.61511400
C	-4.25038500	0.34898000	-0.02478500
H	-4.67906000	0.91833200	0.81206800
C	-5.11121500	0.62566800	-1.26543800

H	-4.72670400	0.08315300	-2.13577700
H	-6.14517900	0.30682300	-1.10667200
H	-5.12350800	1.69078500	-1.51493100
C	-4.29468600	-1.14193500	0.34258000
H	-3.84664800	-1.75042700	-0.45021200
H	-3.76018900	-1.36036300	1.27052500
H	-5.32723600	-1.47689500	0.47543900
Cl	1.52660100	-2.97044100	-0.10081200

1 4 1.0 5 2.0 6 1.5
2 6 1.5 7 2.0 26 1.0
3 5 1.5 7 1.5 8 1.5
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3. Optimized Co-ordinates of 3 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	-1.91990500	1.27675100	0.07190000
C	-2.97313400	-0.66708200	-0.04279100
C	-0.74073500	-0.61868000	0.01124400
Cl	-1.97019300	3.01533600	0.14998700
N	-0.72002200	0.72099600	0.07470400
N	-3.09057400	0.65868700	0.01617900
N	-1.85779100	-1.36597400	-0.05004600
C	2.02020100	-0.19421200	0.10300300
H	1.85240700	0.48546900	-0.73059000
H	1.86111800	0.35504000	1.03189800
C	3.40976600	-0.83366800	0.06228700

H	3.54972700	-1.35496300	-0.89241000
H	3.47968200	-1.59413900	0.84790000
C	4.55807900	0.17784500	0.25801600
H	4.37919400	0.70296200	1.20594800
C	5.89606600	-0.56461600	0.37853700
H	6.12402600	-1.11237700	-0.54225500
H	6.71731000	0.13410400	0.56036000
H	5.88137000	-1.28550300	1.20105600
C	4.61796000	1.22626900	-0.86237100
H	4.75778600	0.74620300	-1.83732900
H	3.71038800	1.83287000	-0.91414800
H	5.45731300	1.90973000	-0.70712200
Cl	-4.46195800	-1.56252000	-0.11789300
S	0.75282200	-1.52680400	0.00414400

1 4 1.0 5 2.0 6 1.5
2 6 1.5 7 2.0 24 1.0
3 5 1.5 7 1.5 25 1.0
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11 12 1.0 13 1.0 14 1.0
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16 17 1.0 18 1.0 19 1.0
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4. Optimized Co-ordinates of 4 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	1.36540900	1.24195700	-0.00006300
C	0.29787000	-0.70897500	0.00001000
C	2.51750700	-0.64707200	0.00000700
Cl	1.32084900	2.97943100	-0.00013000
Cl	4.04882200	-1.46814100	0.00007700
O	-0.80288700	-1.45620800	0.00001200

C	-2.07228900	-0.84310300	-0.00000400
C	-2.69628200	-0.59714700	1.21391100
C	-2.69651200	-0.59760100	-1.21387100
C	-3.98599100	-0.06931000	1.20737400
H	-2.18155200	-0.81746200	2.14102600
C	-3.98622700	-0.06975600	-1.20725600
H	-2.18193300	-0.81830400	-2.14097700
C	-4.63059000	0.19608900	0.00006400
H	-4.48637800	0.13060500	2.14763200
H	-4.48679700	0.12979800	-2.14749600
H	-5.63427000	0.60464100	0.00008100
N	2.56716700	0.68339600	-0.00005300
N	0.19772400	0.62142500	-0.00005300
N	1.43971700	-1.40511300	0.00003500

1 4 1.0 18 1.5 19 2.0
2 6 1.5 19 1.5 20 1.5
3 5 1.0 18 1.5 20 2.0
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6 7 1.0
7 8 1.5 9 1.5
8 10 1.5 11 1.0
9 12 1.5 13 1.0
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14 17 1.0
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5. Optimized Co-ordinates of 5 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	0.12956000	1.51771100	-0.21629500
C	1.40189900	-0.31490200	-0.27524300
C	-0.84313200	-0.48337900	-0.17361600
Cl	-0.00751100	3.26831000	-0.20641800
N	-1.01239600	0.85348600	-0.16340000
N	1.35249400	1.03884200	-0.27822500
N	0.32100200	-1.11772100	-0.22642600
C	-3.64299500	-0.31713500	-0.06105900
H	-3.47895600	0.33947600	0.79129100
H	-3.60192900	0.28585600	-0.96894700
C	-4.96406300	-1.08271600	0.03996600

H	-4.98977400	-1.65459400	0.97571000
H	-5.01896000	-1.81411800	-0.77418600
C	-6.21130800	-0.17810200	-0.03242800
H	-6.14654500	0.40185900	-0.96297300
C	-7.48129500	-1.03749700	-0.10330100
H	-7.59595000	-1.64097300	0.80380400
H	-8.37387500	-0.41295400	-0.20068100
H	-7.45517300	-1.72051400	-0.95741600
C	-6.29073200	0.81137700	1.13899900
H	-6.32059900	0.27848300	2.09607500
H	-5.43872900	1.49507900	1.16227400
H	-7.19664000	1.42046000	1.07092700
S	-2.25856300	-1.52938800	-0.11382900
C	3.88935200	-0.22670500	-0.44273100
H	4.10461800	-0.02431700	-1.50036700
H	3.79566200	0.73776100	0.05425400
C	5.01067300	-1.06909200	0.16822300
H	4.98652500	-2.06986900	-0.28269100
H	4.81671500	-1.20422700	1.23944100
C	6.42040700	-0.48117100	-0.02851500
H	6.57060900	-0.32975800	-1.10620800
C	7.48340900	-1.47681500	0.45612600
H	7.39948800	-2.43676400	-0.06195800
H	8.49183100	-1.09055900	0.28351300
H	7.38038100	-1.66642900	1.53009200
C	6.59080900	0.87631200	0.66921700
H	5.90261700	1.63164900	0.28217600
H	6.41537400	0.78396000	1.74676900
H	7.60623200	1.25790300	0.53059600
N	2.60691200	-0.91249200	-0.31637800
H	2.58019300	-1.92059700	-0.36618800

1 4 1.0 5 2.0 6 2.0
2 6 1.5 7 1.5 41 1.5
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37 38 1.0 39 1.0 40 1.0
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41 42 1.0
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6. Optimized Co-ordinates of 6 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	-0.89307200	2.08325500	0.06335700
C	-0.91586100	-0.12435000	-0.13597000
C	1.02979900	0.97956600	-0.20728400
Cl	-1.76708000	3.58730400	0.27507900
O	-1.54787500	-1.30761100	-0.18612800
C	-2.94557900	-1.35966400	-0.06932500
C	-3.50931700	-1.55560500	1.18366800
C	-3.72055400	-1.31741700	-1.22001900
C	-4.89177400	-1.69831600	1.28535300
H	-2.87266100	-1.59442900	2.05904800
C	-5.10200500	-1.46107500	-1.10732200
H	-3.24543900	-1.17374500	-2.18270500
C	-5.68934400	-1.64973600	0.14293300
H	-5.34389900	-1.84912800	2.25887600
H	-5.71790100	-1.42648100	-1.99860200
H	-6.76415000	-1.76132700	0.22630600
N	0.40885700	2.17951200	-0.04276300
N	-1.63903600	0.98589000	0.03033500
N	0.40075500	-0.20413800	-0.26554700
N	2.37039200	1.01799000	-0.30899400
H	2.77118900	1.94469300	-0.30612800
C	3.23535000	-0.13353200	-0.54478500
H	2.72852500	-1.00795500	-0.13980700
H	3.35170100	-0.29921800	-1.62412600
C	4.60495100	0.07853700	0.10360800

H	4.47749000	0.17534100	1.18873600
H	5.01799400	1.03295400	-0.24839500
C	5.62535300	-1.03442000	-0.19774800
H	5.70382600	-1.12802600	-1.28955400
C	7.01010400	-0.64508500	0.33854300
H	6.98999600	-0.53376700	1.42811500
H	7.75415700	-1.40937800	0.09752600
H	7.35360900	0.30231600	-0.08729000
C	5.19094800	-2.39597000	0.36465300
H	5.06186400	-2.34415800	1.45142200
H	4.24946900	-2.74427800	-0.06718200
H	5.94681100	-3.15858500	0.15724800

1 4 1.0 17 2.0 18 1.5
2 5 1.5 18 1.5 19 1.5
3 17 1.5 19 1.5 20 1.5
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5 6 1.0
6 7 1.5 8 1.5
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30 31 1.0 32 1.0 33 1.0
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34 35 1.0 36 1.0 37 1.0
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7. Optimized Co-ordinates of 7 for NBO calculations

b3lyp/6-311++g(d,p) pop=(nbo,savenbo) geom=connectivity

Title Card Required

0 1

C	-0.49754600	1.23455600	0.09259100
C	-1.55426500	-0.71513600	-0.01616200
C	0.68063800	-0.65534300	0.02712000
Cl	-0.55342900	2.98001200	0.17000100
N	0.70482200	0.68878600	0.09091400
N	-1.66439000	0.61769100	0.04248800
N	-0.42163200	-1.40641400	-0.02796400
C	3.44552400	-0.21068400	0.10560200
H	3.26004200	0.47846900	-0.71617700
H	3.29882300	0.32694300	1.04331900
C	4.83951100	-0.83789000	0.03470900
H	4.97110200	-1.33796600	-0.93255500
H	4.92498200	-1.61490700	0.80257300
C	5.98357800	0.17650500	0.23823400
H	5.81588400	0.67612700	1.20191700
C	7.32901500	-0.55794700	0.31867600
H	7.54712200	-1.07840700	-0.62021400
H	8.14792500	0.14180900	0.50763600
H	7.33183000	-1.30148300	1.12098500
C	6.01835200	1.25391200	-0.85541400
H	6.14449800	0.79979400	-1.84464600
H	5.10582100	1.85479200	-0.87635100
H	6.85598700	1.93889900	-0.69672600
S	2.18796000	-1.55224000	0.01109600
O	-2.66415300	-1.45875900	-0.07135300
C	-3.92741100	-0.84220000	-0.05622200
C	-4.53965700	-0.58146800	1.16157600
C	-4.56763300	-0.60610400	-1.26421700
C	-5.82811300	-0.05133800	1.16488100
H	-4.01449400	-0.79131800	2.08527800
C	-5.85659300	-0.07627600	-1.24886900
H	-4.06284200	-0.83658700	-2.19435800
C	-6.48701700	0.20293600	-0.03735900
H	-6.31697400	0.15979500	2.10888900
H	-6.36746900	0.11475700	-2.18547500
H	-7.48997600	0.61341100	-0.02976100

1 4 1.0 5 2.0 6 2.0
2 6 1.5 7 1.5 25 1.5
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CHAPTER 3

Investigating Triorthogonal Chemoselectivity. Effect of AzideSubstitution on the Triazine Core

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Investigating Triorthogonal Chemoselectivity. Effect of Azide Substitution on the Triazine Core

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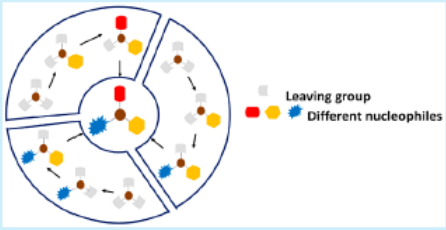
Supporting Information

ABSTRACT: An example of triorthogonal chemoselectivity is reported here for the first time. In this regard, a series of 43 reactions were performed using tridentate *s*-triazine as a model. In all of the possible cases, the three substitutions were carried out using different nucleophiles at a temperature compatible with biological systems.



In the chemical arena, the concepts of *orthogonality* and *chemoselectivity* are often linked. Applied to a protection scheme, *orthogonality* was first introduced by Barany and Merrifield in 1977[1] and then demonstrated in a *triorthogonal* mode by Barany and Albericio in 1985.[2] "Orthogonal protecting groups" are those that can be successfully removed in any order by different chemical mechanisms and in the presence of other protecting groups. At the same time, Trost defines *chemoselectivity* as the ability to discriminate between reactive sites.[3] The terms *orthogonality* and *chemoselectivity* have commonly been used interchangeably in the literature, and the former has even been considered a subclass of the latter.[4] However, these two concepts are in fact complementary and can be merged, thus defining *orthogonal chemoselectivity*.

In this regard, we have recently introduced *orthogonal chemoselectivity* as "discrimination between reactive sites in any order".[5] However, using the cyanuric chloride or *s*-triazine (TA) core, which has three reactive sites, the concept was proved using only two substituents (Figure 1, top).

Herein and using the same TA core, we describe for the first time an example of *triorthogonal chemoselectivity*, where three distinct substituents are sequentially introduced into the TA core in any order and, in some cases, at a temperature compatible with biological systems (Figure 1, down).

TA is inexpensive and has been widely exploited by synthetic chemists. In this regard, it has three readily tunable and independent ring positions, a feature that facilitates sequential nucleophilic substitutions reactions. However, the reactivity of TA is reduced upon substitution due to gain of π-orbital electron donation of the incorporated nucleophile (alcohol, amine, thiol) and thus requires elevated temperature for further reactions. While the first two substitutions can be performed under conditions compatible with biological systems [0 °C for the first and room temperature (rt) for the second], the third usually requires $T > 90$ °C, thereby precluding full application of this approach in such systems.[6−10] In addition to its ability to undergo sequential nucleophilic substitution reaction, the TA core also offers several interaction capacities, such as coordination, hydrogen bonding, charge-transfer attractions, and electrostatic and $\pi-\pi$ stacking. Thus, TA is a suitable candidate for several applications, ranging from energetics usage[11,12] to life science applications such as coupling reagents[13] and herbicides[14] or in drug discovery as dendrimers, antibacterial, antiviral, antifungal, and anticancer agents, among others.[15,16]

In our earlier work,[5] we demonstrated the sequential introduction of the three nucleophiles [N (amine), O (alcohol), S (thiol)] most commonly found in biological systems onto TA. However, the replacement of the third Cl required elevated temperature. On the other hand, O and S can be introduced in any order; however, triorthogonality could not be achieved because once the N had been introduced the system accepted only N nucleophiles, and very often this substitution was possible only at elevated temperatures and thus not compatible with biological systems.

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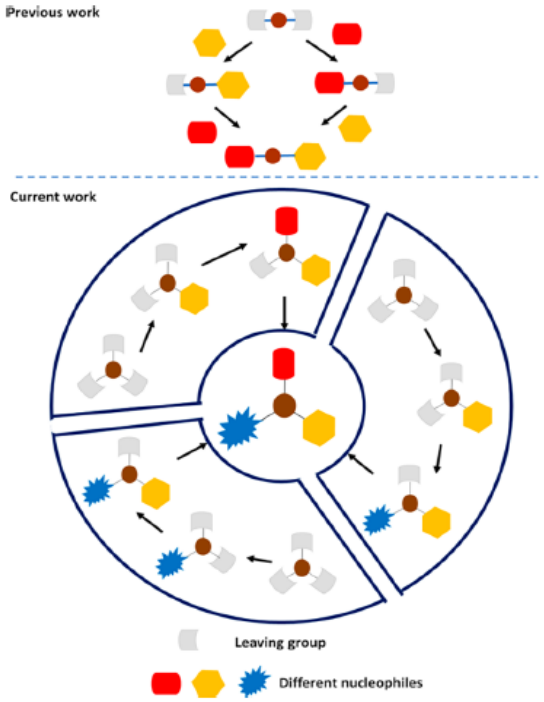

Figure 1. Bi- and triorthogonal chemoselectivity.

Herein, it is investigated whether the introduction of azide (N_3) in one of the positions facilitates a further introduction of S (thiol) or O (alcohol or phenol) nucleophiles at a mild temperature. Moreover, if it is possible to reach the three substituted TA cores using different orders of addition of the nucleophiles, then the same structure can be achieved using distinct strategies. It is important to take into account that N_3 is a resourceful chemical functionality due the ease of its conversion to another moiety, mostly through click chemistry.[17−19] This feature opens up the possibility of using the TA as a versatile linker for the preparation of bioconjugates,[20] which are key for the development of drug discovery strategies, such as antibody drug conjugates (ADCs),[21] and other fields, such as chemical biology[22] and nanobiotechnology.[23]

For *triorthogonal chemoselectivity*, the presence of N_3 is key due to its electron-withdrawing behavior, a capacity that contrasts with other nucleophiles. This behavior is responsible for enhancing the reactivity of the TA core in response to the next nucleophilic substitutions, thereby allowing them to occur at mild temperatures. Thus, in addition to N_3, O (alcohol and phenol) and S (thiol) nucleophiles were used (Figure 2).

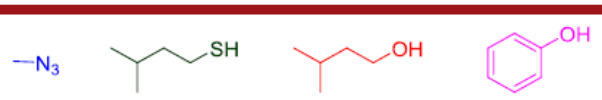

Figure 2. Nucleophiles selected for the substitution on TA.

The corresponding amine nucleophile was not used in this study, as its substitution allows the introduction of other amine nucleophiles only at elevated temperatures.[5] The introduction of N_3 onto TA has been reported in literature,[18,24,25] but herein, N_3 was explored at all three positions on TA (Scheme 1).

For the introduction of N_3 in position 1 and given the explosive nature of 2-azido-4,6-dichloro-1,3,5-triazine (TA-N_3), which can make the scaling up and purification cumbersome, a safe method was developed. This method considers the high reactivity of NaN_3 toward TA and limits its introduction to only one position. Thus, NaN_3 in H_2O was added dropwise onto a cooled vigorously stirred solution of TA in acetone. After 30 min, acetone was removed using a rotary evaporator under cold conditions (to prevent unreacted N_3 from reacting further). The aqueous solution remaining was extracted using cold dichloromethane (DCM) to afford the crude product (to avoid explosion in bigger reaction scale due to generation of heat in separatory funnel), which was subjected to isocratic silica gel column purification using *n*-hexane as eluent, achieving an overall yield of 90.4%.

Taking TA-N_3 as the scaffold, the reactivity in front of the above-mentioned nucleophiles [3-methylbutan-1-ol (alcohol/OH), 3-methylbutane-1-thiol (thiol/SH), and phenol (pOH)] was studied. The substitution of the second chlorine was achieved using DIEA as base at 0 °C for the thiol and −20 °C for phenol to avoid double incorporation. In the case of alcohol, only starting material was found even after the reaction was run at rt. This result can be explained by the lower nucleophilicity of alcohols. In general, all of the reactions were carried out in DCM due to the character of the nucleophiles used in this study, but these reactions can be also carried out in acetone, THF, EtOAc, DMSO, and mixtures of the same.

The double-substituted TA (d-TA) with N_3 and thiol or phenol was reacted again with the previous nucleophiles for the last Cl present on the scaffold.[26] In case of thiol and phenol, the reactions were performed at rt for 3−12 h. Also, in this case no reaction was observed with the alcohol, even at 40 °C for 12 h (Scheme 1, Table 1).

After successful replacement of all of the Cl in TA with N_3 at position 1, N_3 was explored at position 2 in TA. First, TA was reacted with the nucleophiles mentioned above in the presence of DIEA at 0 °C (phenol at −20 °C) for 30 min affording pure mono substituted TA derivatives (m-TA) in all of them. In order to introduce N_3 at position 2, m-TAs were treated with NaN_3 at 0 °C for 30 min. The disubstituted TA (d-TA) derivatives were obtained with excellent purity. These derivatives were then treated with the three nucleophiles as explained earlier. The derivatives bearing thiol or phenol along with N_3 were successful at rt to incorporate thiol or phenol; meanwhile, the reaction of d-TA bearing alcohol-N_3 with thiol or phenol required 40 °C for 12 h to reach completion. Alcohol did not react with d-TA in any of the cases, even under heating conditions (Scheme 1, Table 1). We inferred that it is difficult to introduce alcohol as a nucleophile onto N_3-substituted TA under the described reaction conditions.

With these promising results, the reactivity of N_3 at position 3 was checked. In this regard, NaN_3 was reacted with d-TA at rt. However, no product formation was observed. The reaction was therefore heated at 40 °C and monitored by TLC until d-TA had been consumed. The incorporation of N_3 at position 3 was successful in all cases except the molecules where alcohol was present (Scheme 1, Table 1).

For a better understanding of the electronic effect of the nucleophilic substitution on the TA ring, geometry optimization using the density functional theory (DFT) was performed in the gas phase. To this end, we used the Gaussian09 program package, employing the B3LYP (Becke three-parameter Lee−Yang−Parr exchange correlation functional) and the 6-311G++(d,p) as basis set.[27] Geometries were optimized, and the frequency calculations showed no negative eigenvalue. The optimized geometries were used to perform natural bond

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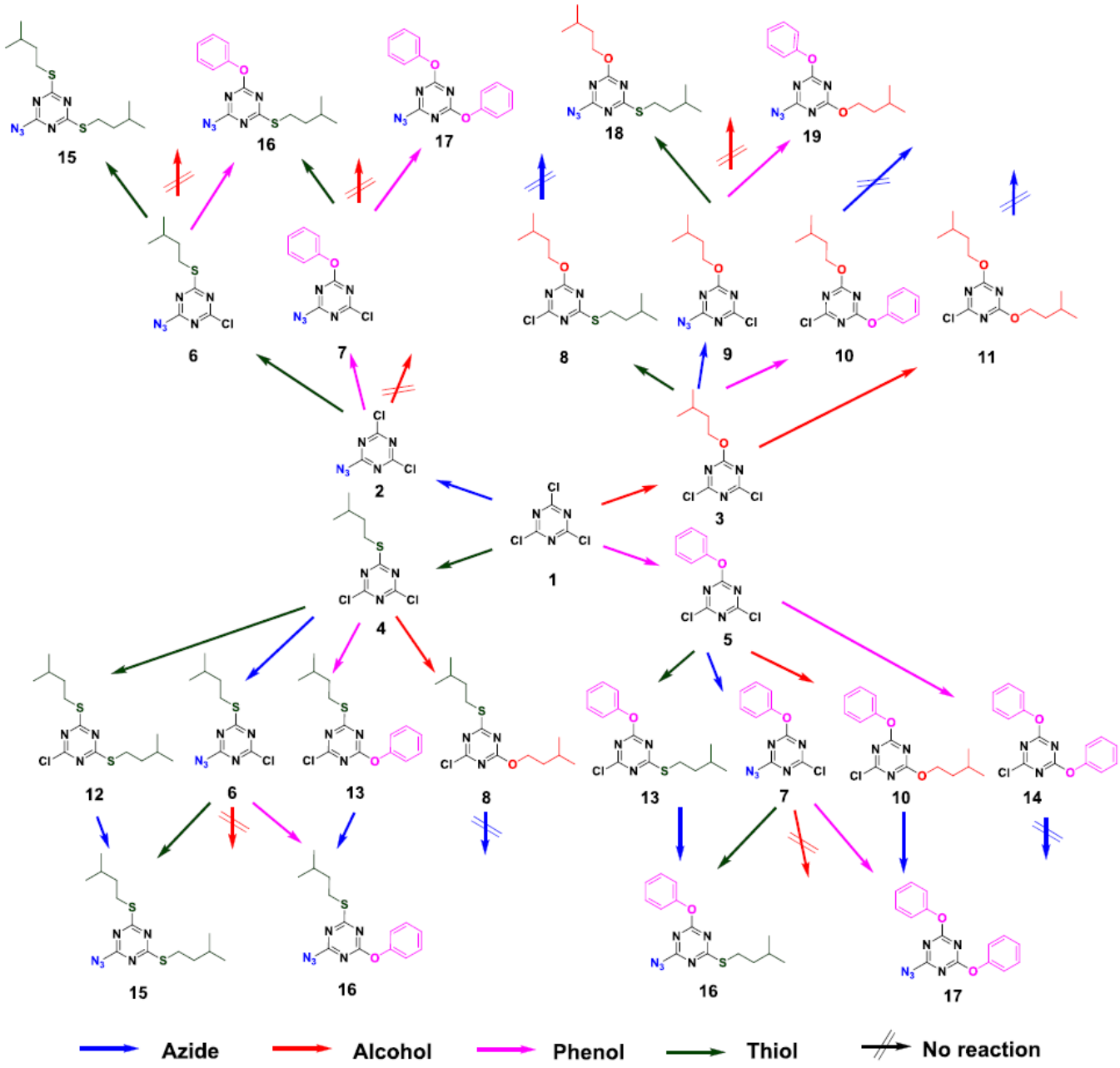
Scheme 1. Possible Routes To Obtain Trisubstituted TA with N_3 in Each Position



orbital (NBO) calculations[28] to calculate the atomic charges present in the molecule. These charges were then compared with those present on "−Cl".

From Table 2, it was inferred that TA, due to its high charge on "−Cl" (0.088 units), is highly reactive toward nucleophilic substitution. A high charge on "−Cl" indicates less covalency of the C−Cl bond, thereby making the replacement by a nucleophile much easier and explaining the reaction of TA with the nucleophile at 0 °C. In the present study, we examined four nucleophiles, namely, azide, alcohol, thiol, and phenol. Upon the first replacement by these nucleophiles, the charge on "−Cl" decreased from 0.088 units to 0.078, 0.065, 0.068, and 0.069 in the case of azide, alcohol, thiol, and phenol, respectively. These observations indicate that m-TAs require a higher temperature to react with the nucleophiles. Of all of the m-TAs, the azide derivative (TA-N_3) had the highest charge, thus explaining the feasibility of the next nucleophilic substitution at lower temperature (0 °C). In contrast, in the other cases, rt was required. However, for the second substitution in the case of TA-N_3, only thiol and phenol showed acceptable reactivity at 0 °C, whereas alcohol showed no reactivity even at rt. In the case of the other m-TAs (for alcohol, thiol, and phenol), rt was required for the second substitution as the charge carried by the second "−Cl" was lower than that of TA-N_3. Upon the second substitution, the charge present on the third "−Cl" was found to be further reduced, thereby making the derivatives less reactive toward further nucleophilic substitution. The azide-substituted derivatives (d-TAs) showed reactivity toward nucleophiles at rt. From Table 2, it can be inferred that when N_3 is present at one of the positions in TA the charge carried by a third "−Cl" (0.051, 0.051, and 0.058 units in case of alcohol, thiol, and

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Table 1. Triorthogonal Chemoselectivity for TA

order[a]			compound[b]				
1st	2nd	3rd	15	16	17	18	19
N_3	SH	SH	√				
SH	N_3	SH	√				
SH	SH	N_3	√				
N_3	SH	pOH		√			
N_3	pOH	SH		√			
SH	N_3	pOH		√			
SH	pOH	N_3		√			
pOH	N_3	SH		√			
pOH	SH	N_3		√			
pOH	N_3	pOH			√		
pOH	pOH	N_3			√		
N_3	pOH	pOH			√		
N_3	SH	OH				n.o.	
OH	SH	N_3				n.o.	
SH	N_3	OH				n.o.	
SH	OH	N_3				n.o.	
OH	N_3	SH				√	
N_3	pOH	OH					n.o.
OH	pOH	N_3					n.o.
pOH	N_3	OH					n.o.
OH	N_3	pOH					√

[a]Indicates the order of incorporation of nucleophiles onto TA. [b]√ = product observed; n.o. = product not observed.

Table 2. NBO Calculation to Show Charges Present on "−Cl"

	natural atomic charges on Cl		
compound	third Cl	second Cl	first Cl
TA	0.088	0.088	0.088
TA-N_3		0.078	0.077
TA-SH		0.068	0.062
TA−OH		0.065	0.061
TA-pOH		0.069	0.069
TA-N_3-OH			0.051
TA-N_3-SH			0.051
TA-N_3-pOH			0.058
TA-SH-SH			0.043
TA-OH-SH			0.040
TA-pOH-SH			0.048
TA-OH-OH			0.039
TA-pOH-OH			0.043
TA-pOH-pOH			0.051

phenol, respectively) is higher than that of d-TA without N_3, except in the case of disubstituted phenol, thus explaining the reactivity of these derivatives at rt for the replacement of the third "−Cl". The charge carried by "Cl" in the case of disubstituted phenol (0.051 unit), disubstituted thiol (0.043 unit), and one phenol and one thiol (0.048 unit) explains the higher reactivity of N_3 feasible at 40 °C. A lower charge present on "−Cl" explains the requirement of 40−60 °C for the completion of the reaction of d-TA with N_3.

In conclusion, using the tridentate TA core, we have demonstrated, for the first time, the concept of *triorthogonal chemoselectivity*, understood as the incorporation of three distinct substituents in any order, using N_3 and two other substituents, either phenols or thiols, as nucleophiles. The TA derivative containing one each of N_3, SH, and pOH (blue) has been prepared by six different strategies, showing the versatility and flexibility of our system. Other derivatives have been prepared by three different strategies. Although N_3 can be introduced in any position, its placement in either position 1 or 2 facilitates the introduction of the remaining nucleophiles. In these cases, the three substitutions can be carried out at temperatures compatible with biological systems. Although the selection of amines did not allow us to prove the concept that was the object of this study, these nucleophiles can be fully compatible with this system having an N_3 and other substituents, preferably phenols or thiols.

Of note, this strategy will allow the preparation of conjugates from O-type nucleophiles. This advance will broaden the bioconjugation field as there are currently no straightforward methods for introducing O-type nucleophiles. All the examples described in the literature are through N,S-type nucleophiles or Click reactions, which can be also used in the strategy described herein.

N_3-TA is explosive and therefore has been prepared with great care.

The procedure for the synthesis of N_3-TA was modified from the previous published procedures to safely optimize its synthesis at lab scale.[23,24] A 27.3 mmol portion of *s*-triazine (TA) was dissolved in 100 mL of acetone and cooled to 0 °C. Sodium azide (27.3 mmol) was dissolved in water (50 mL) and cooled to 0 °C. A solution of sodium azide was added dropwise to a vigorously stirred solution of TA. The reaction was stirred at 0 °C for 30 min, after which acetone was removed under vacuum under ice-cold conditions. After complete removal of acetone, the remaining water layer was extracted using cold DCM (3 × 50 mL). EtOAc can also be used but is slightly slower in separation in separatory funnel. Another disadvantage associated with EtOAc is removal to obtain crude (EtOAc takes longer time compared to DCM). The organic layer was collected, dried over $MgSO_4$, filtered, and concentrated to afford almost pure TA-N_3. This compound was further purified using silica gel column chromatography and *n*-hexane as mobile phase, affording 4.7 g of pure product, which was confirmed by ^{13}C NMR.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02878.

General procedures for the synthesis of TA derivatives, ^1H and ^{13}C NMR spectra, and HPLC of synthesized compounds and theoretical calculations (PDF)

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Notes

The authors declare no competing financial interest.

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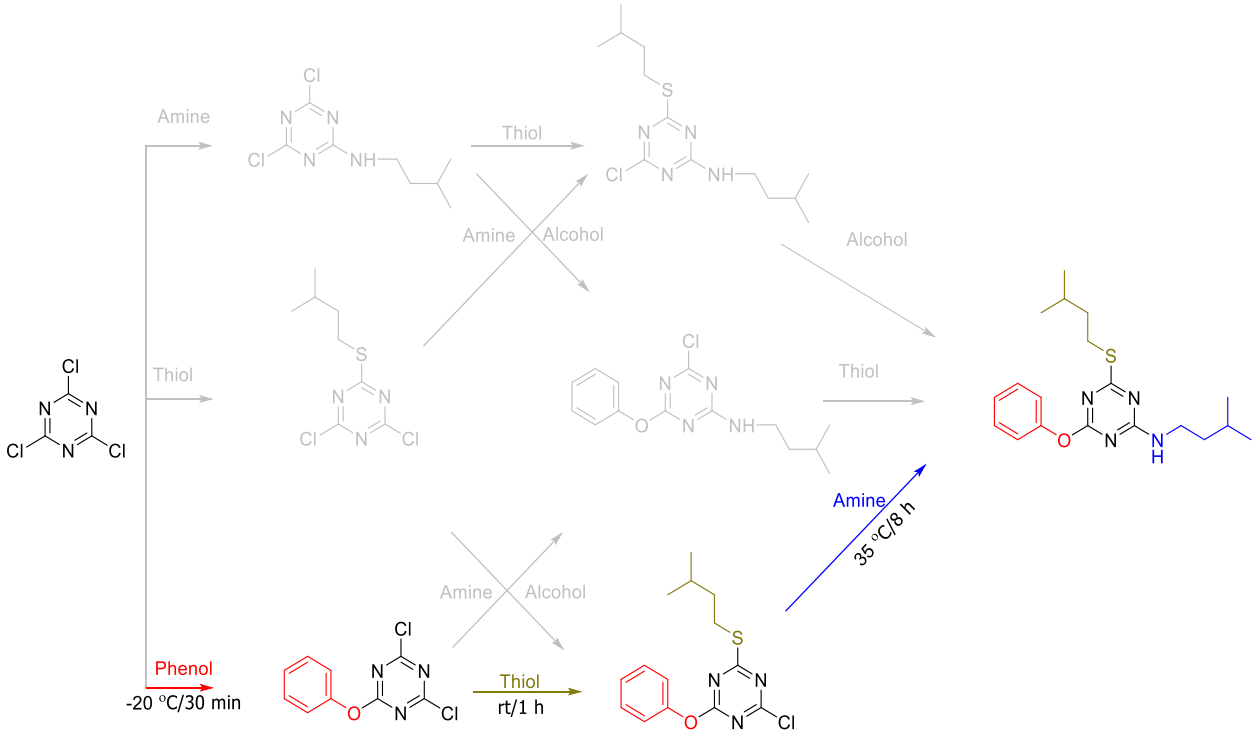
CHAPTER 4

Conclusion

A range of new triazine derivatives have been successfully synthesized and characterized using TCT as starting product. Mono, di and tri substituted triazine derivatives were respectively obtained by temperature-dependent nucleophilic substitution of TCT chlorine atoms.

For the first time, we were able to successfully show in a sequential order the trisubstitution of TCT chlorine atoms under an ambient temperature compatible with the biological system. The nucleophiles used for this study are phenol (O), isopentyl amine (N), 3-methylbutane-1-thiol (S), and the best order of incorporation was found to be first phenol at -20 ºC, second thiol at room temperature and third amine at 35 ºC as shown in Scheme 7. Taking advantage of the high chemical reactivity of phenol, the third substitution with amine was achieved at 35 ºC instead of the reflux condition reported in literatures. Also, in all our attempts to carry out competitive test on the nucleophiles in one pot reaction, result shows that the presence of amine blocks the incorporation of other nucleophiles, despite the high reactivity of phenol. Replacement of the third "Cl" at 35 ºC therefore extends the use of TCT beyond a triorthogonal linker in the biological context, thereby paving the way for nucleophilic reactions involving various peptides, antibodies, and drugs.



Scheme 7: Preferential route for the synthesis of trisubstituted-triazine under ambient temperature

In Chapter 3 of this work, we explored TCT as a triorthogonal chemoselective linker in a series of reaction. In all of the possible cases, the three substitutions were carried out using different nucleophiles at a temperature compatible with biological systems. The nucleophiles used for this study are: phenol, isopentyl amine, 3-methylbutane-1-thiol, 3-methylbutan-1-ol and sodium azide, and all products were obtained in good yields. The introduction of azide (which has high electron withdrawing ability) in this chapter help to check the possibility of incorporating other nucleophiles at a biological friendly condition.

Synthesized TCT derivatives were successfully characterized using ^{1}H NMR, ^{13}C NMR, and HRMS. Furthermore, theoretical calculations were performed and NBO calculations helped to determine the atomic charges of “Cl” in each molecule. The charges found supported the experimental results, thus validating the findings.

Supporting Information

Comprehensive list of the supporting information is available online, however, present in the CD is the characterization data of all synthesized TCT derivatives attached,

This include:

- High Performance Liquid Chromatography (HPLC)
- Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR)
- Carbon Nuclear Magnetic Resonance spectroscopy (^{13}C NMR)
- Theoretical calculations