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Coupling reactions between aromatic carbon- and nitrogen- nucleophiles and electrophiles: reaction intermediates, products and their properties

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INTRODUCTION

The aromatic substitution reactions

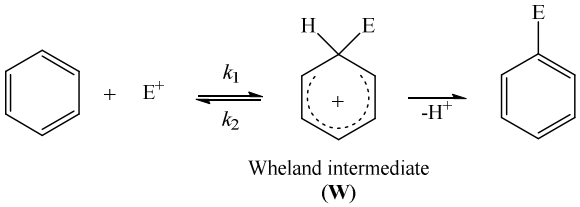
The Electrophilic and Nucleophilic aromatic substitution reactions have been extensively studied over the years[1,2] and their mechanism is well known and widely reported in the literature.

I. ELECTROPHILIC AROMATIC SUBSTITUTION REACTION (S_EAR)

The first example of the S_EAr concerns the substitution of a hydrogen atom on the benzene ring with an atom or group (indicated as E in Scheme1).

Benzene is, in fact, the parent of the aromatic compounds; it has a very high thermodynamic stability due to the delocalization of its pairs of electrons (π electrons) and a lower reactivity compared with a system containing isolated double bonds.

Considering the simplified mechanism of the electrophilic aromatic substitution reaction (S_EAr), reported in Scheme 1, the first interaction occurs between the aromatic ring and the electrophilic species affording a positively charged intermediate, named Wheland intermediate (σ–complex).[3]



Scheme 1. General simplified scheme for the electrophilic aromatic substitution reaction.

The cationic intermediate (or Wheland) derives from the attack of the electrophilic species to one carbon atom of the aromatic ring with a change of its hybridization from sp^2 to sp^3, as a consequence of the addition to the double bond, and the break of the aromatic conjugated system; the resulting σ–complex, is an high energy intermediate[4] (Figure 1).

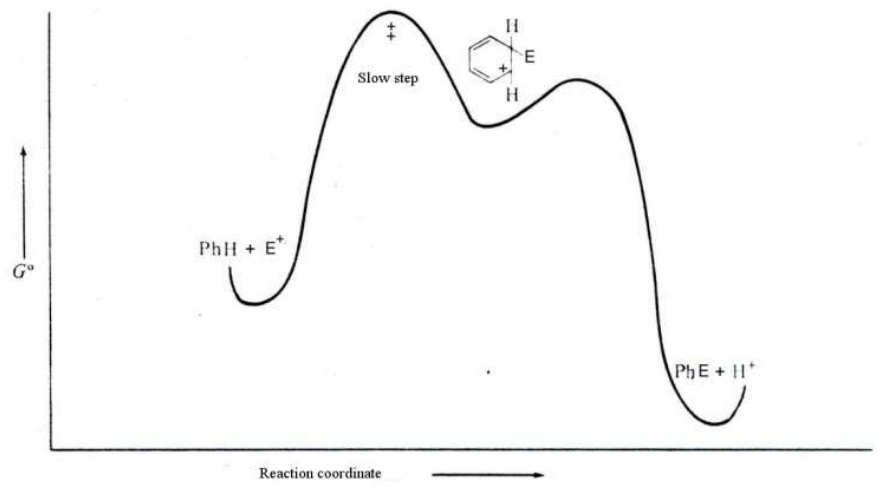
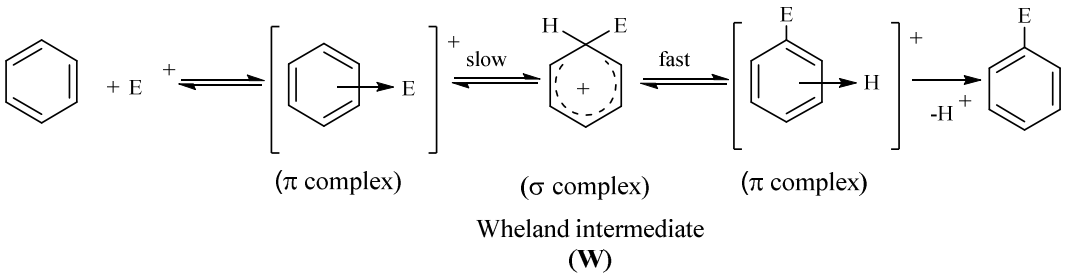


Figure 1. Energetic levels along the reaction coordinate for the electrophilic aromatic substitution reaction.

Finally, the substitution product is obtained by proton loss in the rearomatization step, conventionally considered as the fast and irreversible step of this reaction while the rate-determining step of the overall reaction is considered the formation of the σ intermediate.

Based on the above reported, the isolation of the σ–complex is not a very simple goal and it is complicated by the short lifetime of this species and its low concentration during the reaction.[5]

Actually the general Scheme of the S_EAr depicted in Scheme 1 showing only one intermediate of this reaction, can be considered a simplified Scheme because a lot of investigations carried out principally by J. K. Kochi showed the presence of four steps and three intermediates in the electrophilic aromatic substitution reaction pathway, as reported in Scheme 2.[6-9]



Scheme 2. The general mechanism of the aromatic substitution reaction.

The reaction's pathway reported in Scheme 2 shows that a first step involves a donor-acceptor (**DA**) interaction, in which the electrophile get close to the π–electron cloud of the aromatic system, to obtain a non-covalent complex, usually called π–complex.

In a **DA** complex the electrophile is not localized on a particular atom but is close to the π cloud of the aromatic ring. However some experimental studies, involving electrophiles such as Br^+ or NO_2^+, showed their preferential localization near to a specific C-C bond before to obtain the final σ–complex.[10]

The interaction in the π–complex is weak in nature, and for this reason the activation energy for its formation, is low; this implies that the formation rate for the π–complex is weakly influenced from the substituent groups on the aromatic ring.

The identification of some π–complexes has been possible because of their electronic transition in the visible region of the electromagnetic spectra, giving the typical intense color of these complexes; furthermore, under some experimental conditions, these complexes have been crystallized and analyzed by X-Ray diffraction spectroscopy.[5,8,9,11]

The next step of the reaction allows the formation of a new σ bond between the two substrates, giving the formation of a covalent complex, the σ-complex.

The cyclohexadienic system as the evolution of the π–complex is higher in energy with respect to the starting aromatic compound; this means that the reaction can go in both the directions, depending from the activation energy required to return back to starting materials (loss of the just entered electrophile) or to evolve to substitution product (loss of proton); usually, the proton elimination is favored.

Finally, in the third step, immediately after the rearomatization process, the leaving group forms again a π–complex with the aromatic ring before to be finally turned away; a simplified energetic trend for the four steps of the S_EAr is reported in Figure 2.

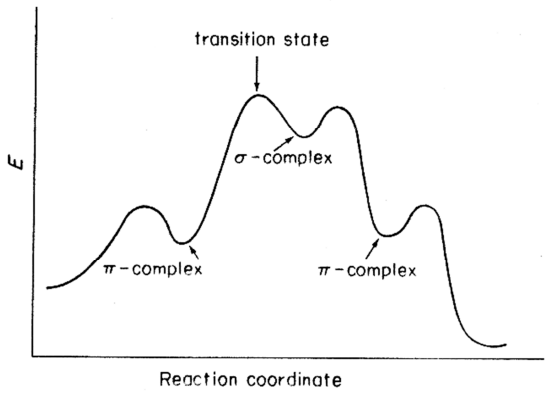


Figure 2. Energetic profile for S_EAr.

The existence of the Wheland intermediate does not legitimate its direct correlation with the transition state. Dewar was the first to deduct the existence of π–complexes along the reaction's coordinate and hypothesized that the reaction's rate could depend also from their stability.[12]

In accordance with Hammond's postulate, assuming that species with similar energies along the reaction's coordinate will also have similar geometry, it is clear that the transition state higher in energy will be similar to the species with a comparable energy.

Thus it is possible to have three different situations:[13]

1. Formation of the π–complex: in this case the transition state higher in energy is similar to the charge-transfer complex (π–complex).[14] If the formation of the π–complex is the rate-determining step there is no isotopic effect.

2. Formation of the Wheland intermediate: the transition state highest in energy is before the Wheland formation. It has been demonstrated that some reactions exhibit a linear relationship between the rate of substitution and the relative stability of the σ–complex; this observation indicates a correlation between the transition state higher in energy and the Wheland intermediate.

3. Proton elimination: the conventional assumption supposes that the proton departure occurs in a fast step, even if is also possible to observe that the transition state higher in energy precedes the proton elimination. A strong isotopic effect (H/D) is characteristic of this reaction as demonstrated by changing the proton with deuterium; in this case the reaction's rate changes. On the assumption that the constant for the proton elimination is k_H and the one for deuterium is k_D, if their ratio k_H/k_D is high (>2), an isotopic effect is present;[2] in this case the reaction can be affected by basic catalysis phenomena.

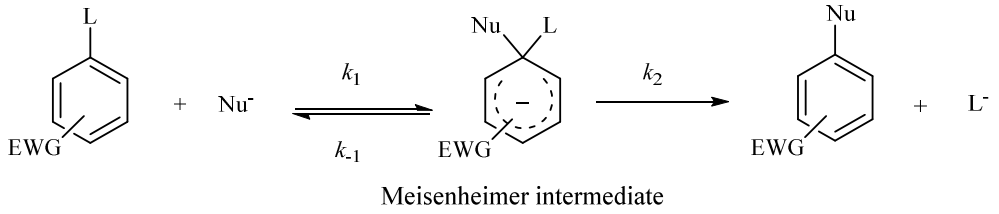
In conclusion, considering a S_EAr, the evaluation of the slow step of the overall reaction is not really simple because a lot of factors can influence the reaction progress as the nature of the electrophiles and of the other substrates in solution and also the effect of different substituents on the aromatic ring that play a fundamental role on the regioselectivity of the reaction.

II. NUCLEOPHILIC AROMATIC SUBSTITUTION REACTION (S_NAR)

Benzene is an electron rich system and this is the reason for its deactivation towards nucleophilic substitution reactions; basically this behaviour depends from the electrostatic repulsion between the π cloud and the nucleophile.

However, the presence of some electron-withdrawing substituents on the aromatic ring, reduces the electron density on it, allowing the interaction with the nucleophile.

According to the simplified mechanism proposed by Bunnett,[15] the nucleophilic aromatic substitution reaction involves two steps: the addition of the nucleophile and the elimination of the leaving group (Scheme 3).



Scheme 3. General simplified mechanism for the nucleophilic aromatic substitution reaction.

It is a bimolecular reaction in which the first step is characterized by the formation of a negatively charged intermediate usually called Meisenheimer complex or σ–complex. In this first step a new σ–bond between the nucleophile and the aromatic ring is formed, then, in the second step of the reaction the Meisenheimer complex evolves to the substitution product by departure of the leaving group and the rearomatization of the ring.

If the nucleophile is a neutral species as in the case of alcohols or amines, a zwitterionic σ–complex, in which the positive charge is localized on the heteroatom, can be obtained (Figure 3).

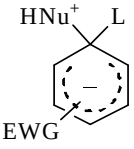


Figure 3. Meisenheimer intermediates from neutral nucleophiles.

Some studies on the nucleophilic aromatic substitution reaction, show that, as in the case of the S_EAr, the formation of the Meisenheimer complex is preceded by a donor-acceptor interactions with formation of a π–complex, that has been characterized in some cases.[16,17]

Examining the possible resonance structures of the σ–anion we can observe that the negative charge is localized in the *ortho* and *para* positions, so the presence of electron-withdrawing groups in these positions helps to delocalize the negative charge, resulting in a stabilization of the σ–complex (Figure 4).

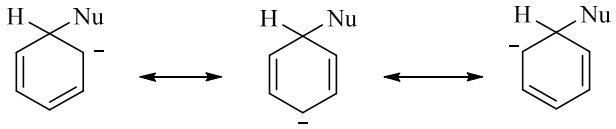


Figure 4. Resonance structures on the Meisenheimer intermediate.

In this kind of substitution reaction, the hydride ion is a bad leaving group and the substitution of a hydrogen atom is not a favoured process; as a consequence in the literature there are some examples of the detection of the Meisenheimer intermediates derived from the attack of the nucleophile onto an electrophilic species that does not possess good leaving groups, working in different experimental conditions.[1b]

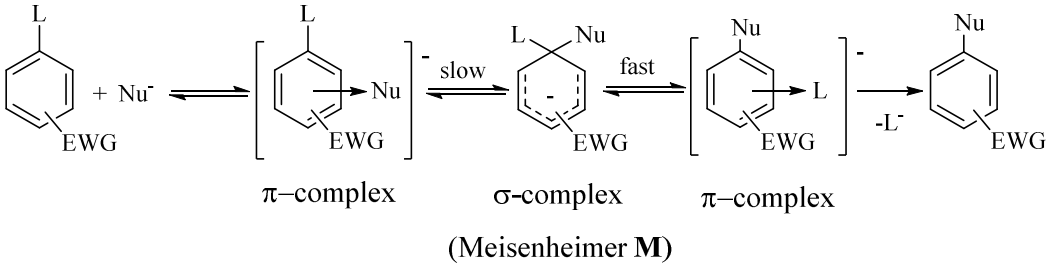
III. S_EAr/S_NAr REACTIONS BETWEEN STRONGLY ACTIVATED NEUTRAL CARBON ELECTROPHILES AND NUCLEOPHILES

The Electrophilic and Nucleophilic aromatic substitution reactions are usually discussed separately because generally only one reagent is aromatic and it is the one who undergoes the substitution.

It should be noted that both reactions show a similar behaviour: after the interaction between the reagents, the σ–complex is obtained; this intermediate possess, for both reactions, a carbon atom of the aromatic ring which changes hybridization from sp^2 to sp^3.

The change of hybridization as a result of the addition to a double bond and the breaking of the π aromatic system, generates the σ–complex that is a high-energy intermediate.

The next step provides the elimination of the leaving group and the subsequent rearomatization to obtain the final product. In both cases (S_EAr and S_NAr) many steps are involved in the reaction, but only recently it has been possible to isolate π–complexes also in nucleophilic aromatic substitution reactions (Scheme 4).[16-18]



Scheme 4. Classic mechanism of nucleophilic aromatic substitution reactions.

Using different nucleophile/electrophile combinations and modulating the steric and electronic properties of the substrates, it has been possible in the research group where I worked during my PhD, to isolate and characterize new σ–complexes of the electrophilic and nucleophilic aromatic substitution reactions, showing that sometimes the difference between the two typologies of reaction is simply a formality.

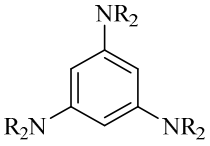
During my PhD I worked in the Boga's research group; from many years the group was involved in many studies concerning the nucleophilic (S_NAr) and electrophilic (S_EAr) aromatic substitution reactions.

The main interest of the research group was focused on the study of different electrophile/nucleophile combinations, between strongly activated species, with the purpose to investigate on their reactivity, to detect new intermediates of the aromatic substitution reaction and to obtain new higly conjugated structures bearing contemporaneously electron donor or acceptor moiety on the same unity.

It is known that the observation of the sigma intermediates in the electrophile-nucleophile combination involving aromatic substrates usually requires that at least one of the two reagents is strongly activated; in fact, the presence of strong electron-donating groups on the aromatic ring enhances the Wheland complex stability, while Meisenheimer complex stability is improved by the presence of electron-withdrawing groups.

In the past, the research group performed lot of reactions involving neutral partners bearing electron-donating and electron-withdrawing groups that allowed to the formation of different σ-complexes of the aromatic substitution reaction.

Among the different nucleophilic species studied during the last years, 1,3,5-tris(*N*,*N*-dialkylamino)benzenes[19] (Figure 5), were involved in a large number of electrophile/nucleophile combinations.



NR_2 = piperidinyl
NR_2 = morpholinyl
NR_2 = pyrrolidinyl

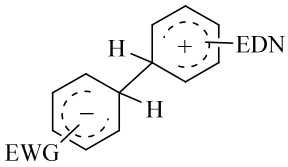
Figure 5. 1,3,5-tris(*N*,*N*-dialkylamino)benzenes structure.

1,3,5-tris(*N*,*N*-dialkylamino)benzenes are arylamines that possess peculiar structural and electronic properties; they are highly symmetrical systems, due to the distribution of the three dialkylamino groups on the aromatic ring (Figure 5), able to stabilize the positive charge of the σ intermediate generated after the electrophilic attack on them.

Thanks to their structure these compounds can react also with "weak" electrophilic species, so they are considered strong nucleophiles (*i.e.* "supernucleophiles"),[20-23] and also potentially "bidentate" nucleophiles, because both carbon and nitrogen atoms can react with electrophilic species; usually, these compounds act as "neutral aromatic carbon supernucleophiles".

In the past, triaminobenzene derivatives were used to obtain moderately stable σ-cationic complexes (the Wheland intermediates **W**) and, in particular, tris(*N*-pyrrolidinyl)benzene afforded σ-complexes not only in protonation reactions,[24-26] but also in alkylation reactions with alkyl halides[19,27] and in halogenation reactions.[28]

Moreover the research group obtained very interesting information on the separate steps of the electrophilic aromatic substitution reaction,[29] coupling triaminobenzene derivatives and different electrophilic species;[20,21] a very interesting result was obtained when performing the reaction between strongly activated reagents, the research group was able to detect and characterize the first Wheland-Meisenheimer -es of the aromatic substitution reaction.[22,23] This new kind of sigma intermediate reported in Figure 6, is a zwitterionic species, contemporaneously Wheland and Meisenheimer, and it was only hypothesized but never observed until these studies.

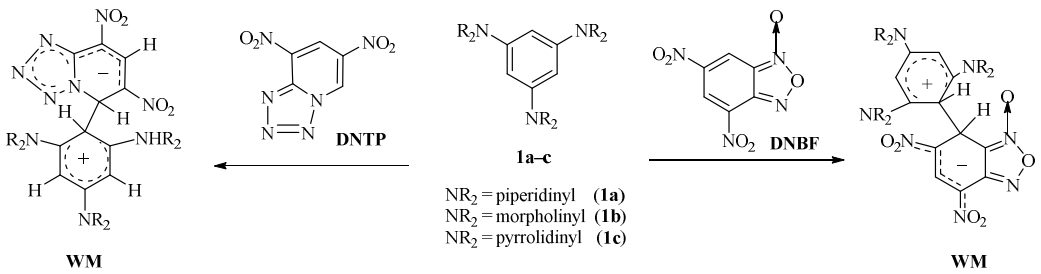


WM

EWG = electron withdrawing groups
EDN = electron donating groups

Figure 6. Example of a Wheland-Meisenheimer complex.

In particular, when the 1,3,5-tris(*N*,*N*-dialkylamino)benzene derivatives **1a-c** were coupled with 4,6-dinitrobenzofuroxan (**DNBF**) or 4,6-dinitrotetrazolepyridine (**DNTP**), as reported in Scheme 5, the new Wheland-Meisenheimer complexes (**WM**) were obtained.[22,23]



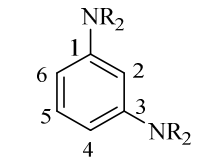
Scheme 5. Nucleophile/electrophile combination between neutral aromatic species giving detectable **WM** intermediates.

DNBF and **DNTP** have an heteroaromatic 10π-electron ring structure,[30-33] and thanks to the presence of the nitro groups on their carbocyclic ring, they are considered as superelectrophilic heteroaromatic compounds,[34,35] able to stabilize the negative charge on their ring in a Meisenheimer complex.

The obtained zwitterionic complexes resulted moderately stable at low temperature and they were characterized by NMR spectroscopy methods.[20]

After these results, different studies were carried out by the research group using triaminobenzenes as supernucleophiles with different electrophilic species,[20-22] and depending from the electrophilic power of the involved electrophile, new substitution products or new σ–intermediates of the aromatic substitution reactions were obtained.

In the last years of my PhD, I also started to investigate on the reactivity of the 1,3-bis(*N*,*N*-dialkylamino)benzene derivatives[36,37] (Figure 7).



NR_2 = piperidinyl
NR_2 = morpholinyl
NR_2 = pyrrolidinyl

Figure 7. 1,3-bis(*N*,*N*-dialkylamino)benzene structure.

Also these species might behave as ambident nucleophiles able to give products from nitrogen or carbon attack, but very few studies on their reactivity are reported in the literature.

Potentially these nucleophiles possess two carbon atoms that can undergo attack, C-2 and C-4; the position 2 should be the more activated for the presence in *ortho* position, of both the electrondonor dialkylamino groups, but it is also a hindered position.

Furthermore, even if the amino-substituted arenes are strong nucleophilic species, in the literature there are no data about their nucleophilicity parameters.

So, the last year of my PhD course, I spent three months in the Department of Chemistry, Ludwig-Maximilians-University of Munich, in collaboration with Prof. Herbert Mayr's group, with the aim to investigate on the nucleophilic reactivities of di- and triaminobenzene derivatives performing their combination with different reference electrophiles, selected from the Mayr's reactivity scales.[34,35,38,39]

During this period we started to develop a methodology to measure the rate constants of these substitution reactions and calculate the nucleophilicity of di- and triaminobenzene derivatives but work is still in progress on this topic.

The next Chapters of this thesis will be a dissertation about the research activity that I have carried out during my period as a PhD student.

In particular, during my PhD I was involved in the study of the aromatic substitution reaction between different electrophile/nucleophile combinations and I was able to synthesize new products for applications in different fields (*e.g.* medicine, biology and materials), and to detect and characterize new intermediates of these reactions (e.g. Wheland

(**W**), Meisenheimer (**M**), and even Wheland-Meisenheimer (**WM**)), mainly using NMR spectroscopic techniques.

IV. MAYR'S ELECTROPHILICITY SCALE

To select the electrophilic species, usually we refer to the Mayr's Reactivity scales; for this reason before to report my results I will briefly introduce how this scales were developed and how is possible to use them to predict if a reaction between an electrophile and a nucleophile could take place.

Since 1950*s* there was an increasing interest in quantify nucleophilicity scales; first Swain-Scott[40] then Edwards[41,42] have proposed the first equations to derive values for the nucleophilicity of some substances and in the 1960s also Pearson and Ritchie enhanced this subject.[43] Finally, in 1994, Prof. Herbert Mayr developed a linear free energy relationship[35,36,38,39] based model for polar organic reactions, which uses eq 1 to predict rates and selectivities for these reactions thus demonstrating that one parameter for electrophiles (*E*) and two parameters for nucleophiles (*N* and *s*) are sufficient to quantitatively describe the rates of a large variety of electrophile/nucleophile combinations:

$$\log k_{20^\circ C} = s_N(E + N) \qquad \text{equation 1}$$

where s_N is a nucleophile-specific parameter, *N* is a nucleophile-specific parameter, and *E* is an electrophile-specific parameter.

To obtain the final equation 1, a series of reactivity scales for electrophiles and nucleophiles were constructed by Mayr and coworkers.[44]

In particular, a set of 29 *para*- and *meta*-substituted benzhydrylium ions and structurally related quinone methides as reference electrophiles, were selected, and the kinetics of their reactions with a variety of carbon nucleophiles in different solvents, were studied by spectrophotometric monitoring of the consumption of the electrophiles.[38,39,45,46]

From the combinations between strong electrophiles with weak nucleophiles and weak electrophiles with strong nucleophiles, they derived a series of second-order rate constants varying from 10^{-5} to 5×10^{7} M^{-1} s^{-1} at 20°C (Figure 8).

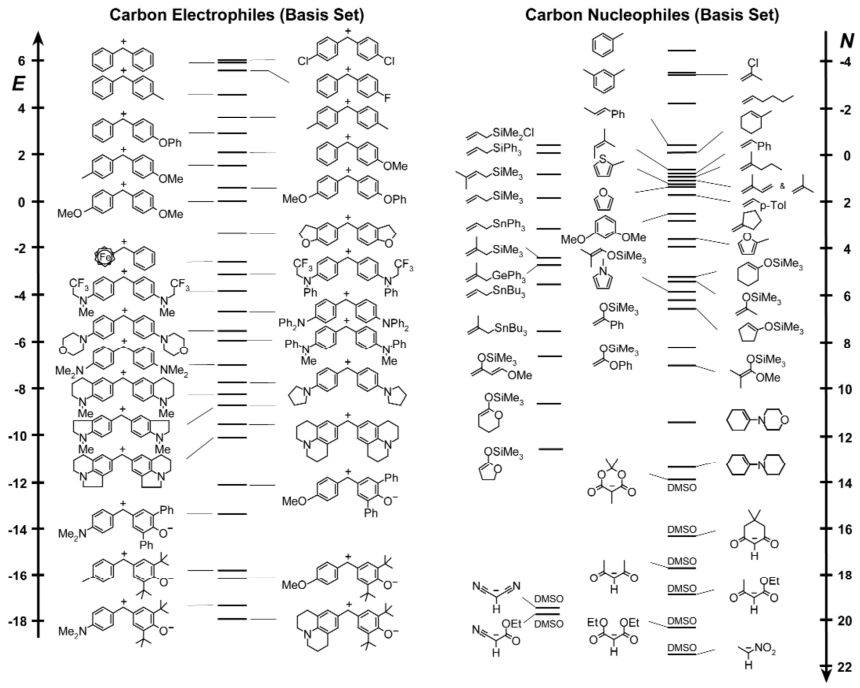


Figure 8. Carbon electrophiles and carbon nucleophiles used for the construction of the reactivity scales.[44]

In this way, 29 nucleophilicity scales were obtained, one for each electrophiles and some of them are depicted in Figure 9.

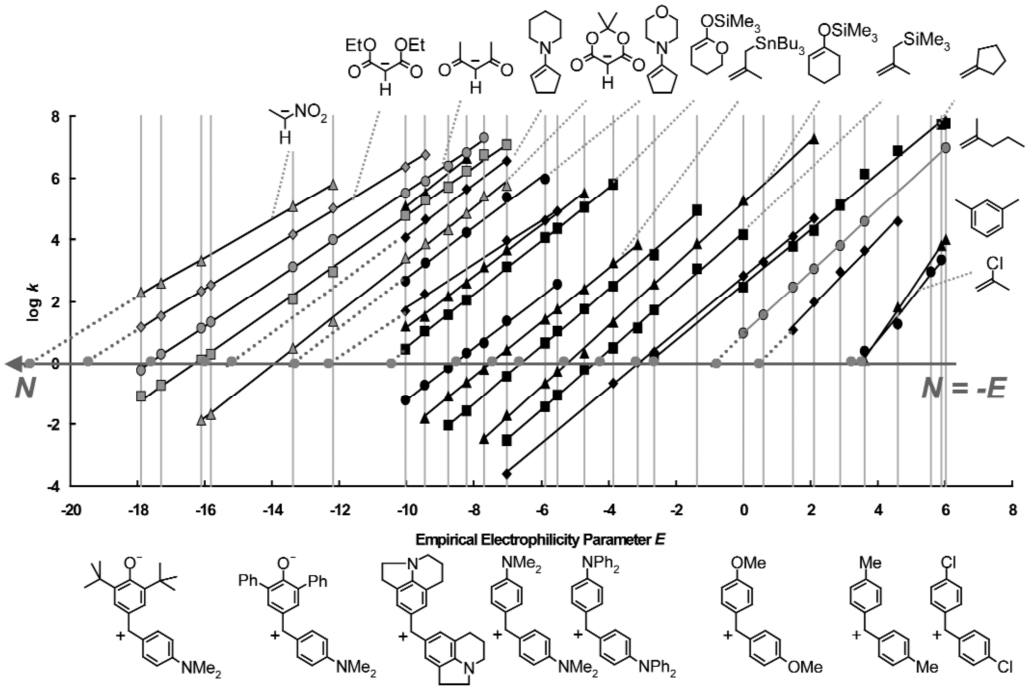


Figure 9. Second-order rate constants for electrophile-nucleophile combinations (20°C).[44]

The reported correlation lines shown in Figure 9 were obtained by a least-square analysis of the rate constants for the reactions of the 29 reference electrophiles with selected carbon nucleophiles; each electrophile is characterized by one parameter *E* [where *E* values for *p*-$MeOC_6H_4)_2CH^+$=0], while nucleophiles are characterized by two parameters *N* and *s* (*s*=1 for 2-methyl-1-pentene).

The previously introduced equation 1 defines nucleophilicity *N* as the negative intercept of a correlation line with the abscissa. So, the *N* and *E* parameters above defined and employed to order the nucleophiles and electrophiles reported in Figure 8, have been obtained from the explained analysis.[38,39,46]

The benzhydrylium ions and quinone methides, thus characterized by *E*, are finally considered as reference electrophiles and are employed to characterize other types of nucleophiles.

Therefore, plotting log *k* (20°C) *versus E* for the reaction of a nucleophile with different electrophiles, the *N* values can be simply calculated and in the same way, also the *E* parameter of an electrophile respect to a reference nucleophile can be determined using equation 1.

The *E*, *N*, and *s* parameters thus obtained can be used for predicting rates and selectivities of polar organic reactions. In fact by ordering nucleophiles with increasing reactivity parameter *N* from left to the right and electrophiles with increasing values of *E* from top to bottom, one arrive at Figure 10, where combinations of electrophiles and nucleophiles on the diagonal are calculated to proceed with a rate constant of 1 M^{-1} s^{-1} (log *k*=0, independent of s_N, equation 1).[47]

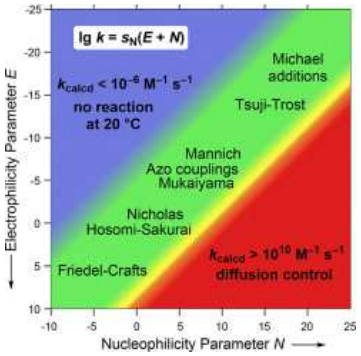


Figure 10. Semiquantitative prediction of reactions of electrophiles with electrophiles.[47]

Moving from any point upwards (*i.e.*, toward weaker electrophiles) or to the left (*i.e.*, toward weaker nucleophiles), on the diagonal, one enters the blue sector where k_2<10^{-6} M^{-1}

s^{-1}, which are not synthetically useful. As a rough guide, Prof. Mayr and coworkers, suggested that the electrophile-nucleophile combinations can be expected to be observable at room temperature, if $E+N>-5$. On the other hand, moving from the diagonal to the right or downwards, one enters the red sector, where diffusion control will be reached [$s(N+E)>9$], which results in a loss of selectivity, and undesired side reactions will again importance. As a result, most synthetically used reactions are located in the green sector of Figure 10.[47]

The benzhydrylium methodology has provided, during the past three decades, the most comprehensive nucleophilicity and electrophilicity scales presently available, constantly updated by the Prof. Mayr's research group and fully available on the Mayr's database of reactivity parameters .[48]

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CHAPTER 1
Reactions between aryldiazonium salts and neutral aromatic carbon nucleophiles

1.1 AZO COUPLING BETWEEN AMINOTHIAZOLE DERIVATIVES AND ARENEDIAZONIUM SALTS: NEW PRODUCTS AND UNEXPECTED LONG RANGE SUBSTITUTENTS TRANSMISSION EFFECT.

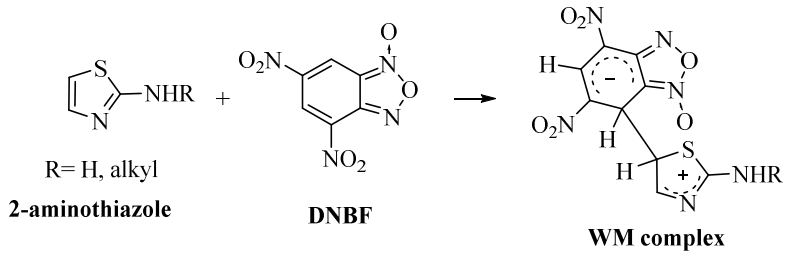
1.1.1 Introduction

2-Aminothiazole is considered to be an interesting compound due to its application in different fields; It is present in a broad range of pharmaceuticals,[1] agrochemicals[2], and materials.[3] Aromatic azo compounds are widely used as commercial dyes and some arylazo-2-aminothiazole derivatives are of interest especially as disperse dyes for dyeing polyester fabrics.[4]

So the synthesis of new 2-aminothiazole derivatives is interesting, and since 2-aminothiazole derivatives possess three nucleophilic sites, *i.e.* the endo- and exocyclic nitrogen atom and the C5-carbon atom, their reactions with a number of different electrophiles are of interest also in mechanistic studies.

As reported in the Introduction of this thesis, in the past, the first Wheland-Meisenheimer (**WM**) complexes were obtained by the research group combining *sym*-triaminobenzene derivatives (strongly activated neutral carbon nucleophiles) with different electrophiles, including 4,6-dinitrobenzofuroxan (**DNBF**).[5,6]

Later, the reactivity of 2-aminothiazole derivatives towards **DNBF** was investigated by the research group (Scheme 1).[7]



Scheme 1. WM complex from the reaction between 2-aminothiazole and its derivatives and **DNBF**.

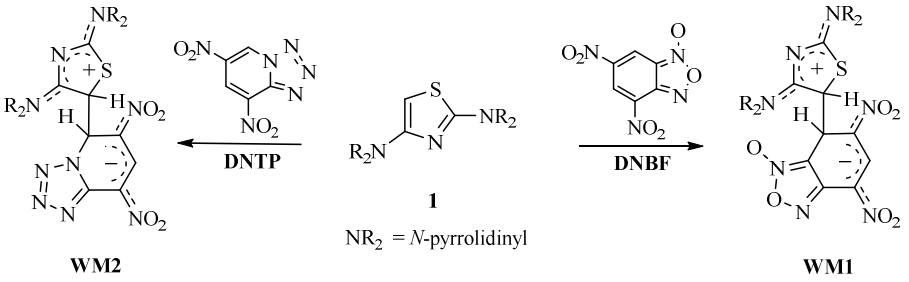
This investigation permitted to detect **WM** complexes derived from the coupling between the C-7 carbon atom of the electrophile and the C-5 carbon atom of the thiazole ring;[8] the very short life-time of these intermediates allowed the research group to investigate on the 2,4-diaminothiazole and its derivatives, more nucleophilic substrates with respect to 2-aminothiazole and more able to stabilize the positive charge in the thiazole ring as reported in the literature for the reaction with the proton.[9]

It is known that 2,4-diaminothiazole is an electron-rich molecule able to complex electrophilic species, such as bromine[10] but generally their derivatives possess further properties complicated by the tautomerism of both amino groups.

Clearly, 2,4-bis(dialkylamino)thiazole derivatives, don't have this complication, and their strong carbon nucleophilicity was also discussed by Gompper and coworkers[11] in a previous work in which a zwitterionic complex between *N*,*N*,*N'*,*N'*-tetramethyl-1,3-thiazole-2,4-diamine and 1,3,5-trinitrobenzene was obtained.

Based on these considerations we decided to prepare a very poorly studied 2,4-diaminothiazole derivative, the 2,4-dipyrrolidin-1-yl-1,3-thiazole (**1**),[9] with the idea that it might be a promising candidate to behave as carbon supernucleophile.

The reactivity of 2,4-dipyrrolidin-1-yl-1,3-thiazole (**1**) was studied combining it with the superelectrophiles **DNBF** and 4,6-dinitrotetrazolepyridine (**DNTP)**, and in both cases were obtained ultrastable **WM** complexes (Scheme 2), whose structure was also confirmed by X-ray diffraction analysis.[12]



Scheme 2. Reactions between the diaminothiazole derivative **1** and **DNBF** or **DNTP** with formation of new Wheland-Meisenheimer complexes **WM1** and **WM2**.

The obtained intermediates (**WM1** and **WM2** in Scheme 2) were the first examples of Wheland-Meisenheimer complexes so stable to permit a study on their crystal structure.

Thus, one can affirm that the two pyrrolidininyl groups in 2 and 4 position of the aminothiazole derivative enhance the nucleophilic power at 5 position of the thiazole ring,

making this compound a supernucleophile at the neutral carbon atom, comparable to the triaminobenzene derivatives.

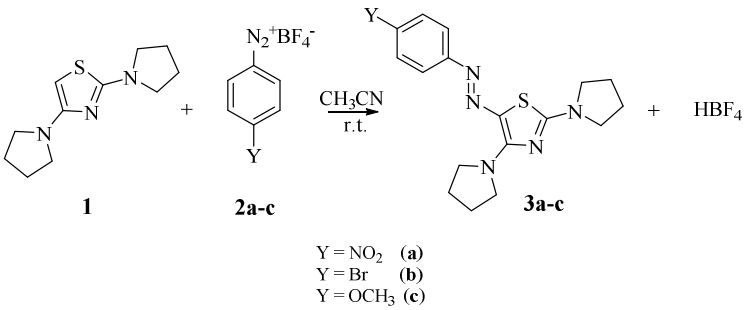
1.1.2 Results and Discussion

- *Reaction between 2,4-dipyrrolidinylthiazole and arenediazonium salts*[13]

Based on the above reported results, and owing the importance to synthesize new aminothiazole derivatives for applications in different fields, we turned our attention on the 2,4-dipyrrolidin-1-yl-1,3-thiazole reactivity towards another electrophilic species, the arenediazonium ions.

The reactions between 2,4-dipyrrolidinylthiazole (**1**) and the arenediazonium salts **2a-c** were carried out in acetonitrile at room temperature with a two-fold excess of **1** to neutralize the tetrafluoroboric acid produced during the reaction (Scheme 3).

In the case of the reactions of **1** with **2a** and **2b**, after about 30 min, a solid precipitated from the crude reaction mixture while TLC and ^{1}H NMR analysis of the mother liquor showed presence of the protonated form of **1** and of several unidentified compounds. The precipitates were analyzed and their NMR and mass spectral data agreed with those of compounds **3a** and **3b**, recovered in 50% and 48% yield, respectively.



Scheme 3. Reactions between 2,4-dipyrrolidinylthiazole and arenediazonium salts

When the reaction was carried out with the 4-methoxybenzenediazonium tetrafluoroborate salt (**2c**) no precipitate was obtained and any attempts to isolate **3c** from the reaction mixture failed.

During this study we observed that the ^{1}H-NMR spectrum in $CDCl_3$ of compounds **3a** and **3b** showed separate signals for the four methylene groups bound to each carbon atom situated in α position to the pyrrolidinyl nitrogen atom; furthermore, in the case of compound **3a,** the ^{1}H-NMR spectrum in acetonitrile, showed well separated signals for each

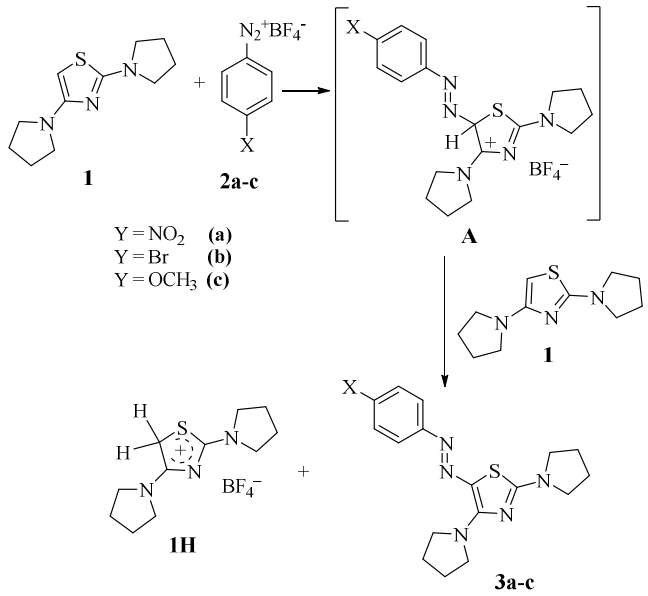
hydrogen atom bound to the carbon atoms in α position to the pyrrolidinyl moieties; an analogous solvent effect was reported in a study on enaminonitriles.[14]

These NMR data, indicate that in all cases, the methylene protons in α position to the nitrogen atom, are not equivalent, thus suggesting a hindered rotation around the C2-N and C4-N bonds.

This finding can be ascribed to a strong mesomeric effect that induces a partial double bond character for both C2-N and C4-N bonds through a conjugated system involving the lone pair of the pyrrolidinyl nitrogen atom with the π electrons of the thiazole ring and the azo substituent in position 5.

Given that 2,4-dipyrrolidinylthiazole (**1**) has demonstrated to be able to stabilize a positive charge in position 5 of the thiazole moiety in reactions with **DNBF**, **DNTP**[12] and the proton,[11] we tried to see if also in the present case the σ-cationic intermediate derived from the addition of the diazonium ion to **1** might be detected.

For this purpose the reactivity of 2,4-dipyrrolidinylthiazole (**1**) with arenediazonium salts **2a-c** was also investigated performing their reactions directly in the NMR spectroscopy tube, under different experimental conditions and no evidence of a σ-cationic intermediate (like **A** in Scheme 4), derived from the addition of the diazonium salts to **1**, was obtained; the only species in solution were the substitution products **3a-c** and compound **1H** (Scheme 4); signals indicating the presence of some unknown species were also detected.



Scheme 4. Products from **1** and **2a-c** observed carrying out the reaction in the NMR spectroscopy tube.

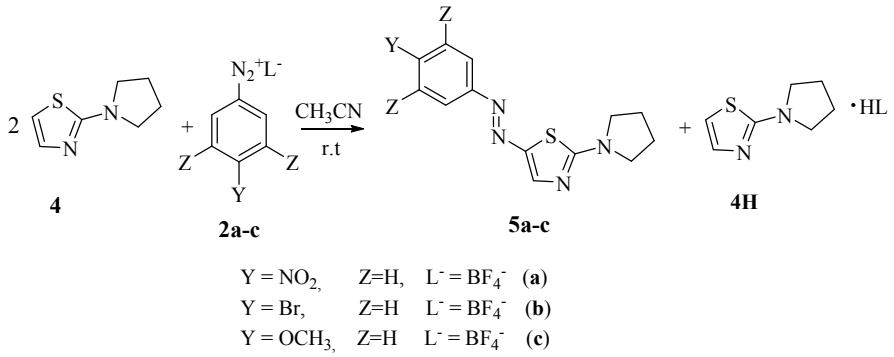
Probably, the high reactivity of compound **1** might be the cause of the formation of numerous species; actually, the recovery of the azo compounds **3a** and **3b** in almost 50% yield was possible owing to their poor solubility in the reaction medium that caused their precipitation and, likely, shifted the reaction outcome towards their further formation.

These results allowed us to start a new investigation involving the arenediazonium salts as the electrophiles and a new nucleophilic species, the 2-pyrrolidinylthiazole (**4**), that we presumed to be less reactive respect to the 2,4-dipyrrolidinylthiazole, due to the presence of only one pyrrolidinyl group on the thiazolic ring.

- *Reactions between 2-pyrrolidinylthiazole and arenediazonium salts*[13]

2-Pyrrolidinylthiazole (**4**), whose reactivity has been very poorly investigated so far,[15] was synthesized by us under solvent-free conditions at room temperature from 2-bromothiazole and pyrrolidine.

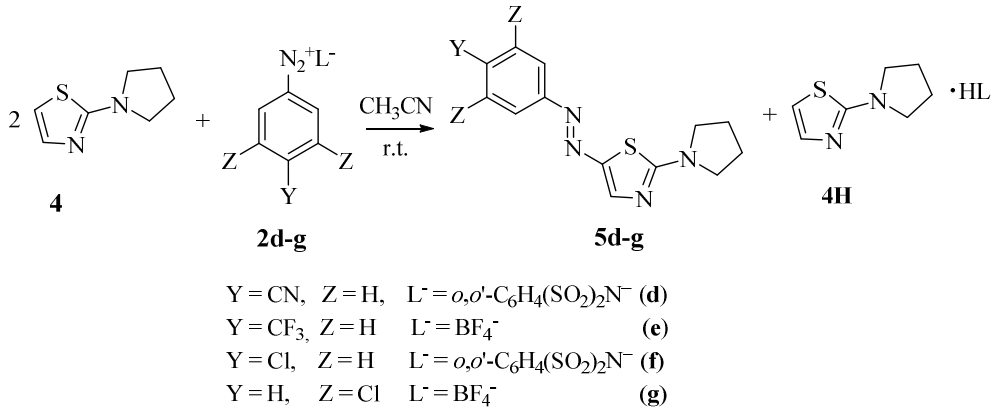
The reactions between **4** and **2a-c** (Scheme 5) were carried out in relative molar ratio 2/1 at room temperature, in acetonitrile and the substitution products **5a-c** were obtained in high yields; these results were a confirmation that the low yields for the azo compounds **3a,b** are due to the occurrence of concomitant reactions when the highly reactive 2,4-dipyrrolidinylthiazole (**1**) was combined with arenediazonium salts **2a-c**.



Scheme 5. Reactions between 2-pyrrolidinylthiazole (**4**) and arenediazonium salts **2a-c**.

In the present case, from the reaction between the mono-pyrrolidinylthiazole **4** and the 4-methoxy derivative **2c**, the corresponding substitution product **5c** was obtained, opposite respect to the reaction of the same compound with the di-pyrrolidinylthiazole **1**; in that case no azo product was obtained.

To extend this study, we decided to perform the reaction between **4** and others benzenediazonium salts (**2d-g**), with different substituents in *para* position, as reported in Scheme 6.



Scheme 6. Reactions between 2-pyrrolidinylthiazole (**4**) and arenediazonium salts **2d-g**.

The reactions were carried out under the above reported experimental conditions, and the azo compounds **5d-g** were obtained. In many cases the reaction products were easily separated from the reaction mixtures in almost pure form by simple filtration and the ^{1}H NMR analysis of the residues from mother liquors showed the presence of the protonated thiazole (**4H**) and of further amount of the azo compounds **5d-g**.

All the new synthesized compounds **5a-g** were fully characterized and in the cases of **5a** and **5c**, we were able to obtain single crystals suitable for X-Ray diffraction analysis; Figures 1 and 2 show a graphic representation of the crystal structures of compound **5a** and **5c**, respectively.

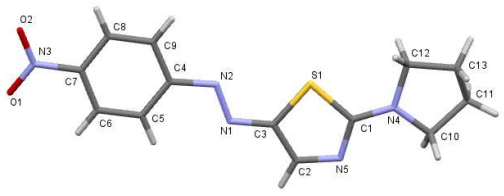


Figure 1. Graphic representation of the crystalline structure of compound **5a**.

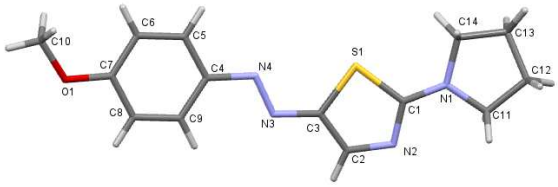


Figure 2. Graphic representation of the crystalline structure of compound **5c**.

For both compounds **5a** and **5c** the X-Rays structure shows a trans geometry around the N=N bond and the coplanarity of the two aromatic rings, the azo bond, and, for compound **5a**, also the nitro group. Table 1 reports selected bond lengths for compound **5a**.

Table 1. Selected bond lengths for compound **5a**.

Bond	Length(Å)	Bond	Lengt(Å)
C1-N4	1.330	C3-N1	1.350
C1-N5	1.331	N1-N2	1.281
C2-N5	1.355	C4-N2	1.414
C2-C3	1.375	C10-N4	1.475

As it can be seen, all the reported C–N bond length values are very close one together and in particular the C1–N4 and the C3-N1 distances (1.33 and 1.35 Å, respectively) are shorter than a standard C-N single bond distance (*e.g.* C10-N4 = 1.475 Å) thus indicating a marked double bond character of the exocyclic C-N bond, due to the electron delocalization by resonance over the all-conjugated moiety present in the molecule; analogous considerations can be made for data of compound **5c**, reported in Table 2.

Table 2. Bond lengths for some C-N bond of **5c**.

Bond	Lenght (Å)	Bond	Lenght (Å)
C1-N1	1.333	C2-C3	1.366
C1-N2	1.322	C3-N3	1.362
C2-N2	1.355	C11-N1	1.462

The reactions between **4** and **2a-c** were also performed directly in the NMR spectroscopy tube, in equimolar amount of reagents, in $CDCl_3$ and their progress was monitored over time by NMR spectroscopy.

The ^{1}H-NMR spectrum of the reaction mixture, recorded when the reagents conversion was not complete, showed signals ascribed to the compound **2**, those of the substitution product **5** (in relative ratio dependent form the reaction time) and only two signals (splitted into doublets) for the 2-pyrrolidinylthiazole ring, belonging to the H-4 and H-5 hydrogen atoms.

Since during the reaction, both presences of the unreacted **4** and of its salt **4H** could be expected, while in the spectrum were present signals ascribed to only one species, our suggestion was that a protonation phenomenon involving both **4** and **4H** occurred. This behaviour might be an indication that the proton is not located onto a defined position but it is involved in a sort of equilibrium between **4** and **4H**; a similar situation was observed, in past studies between triaminobenzene derivatives and the proton.[16,17]

The observed behaviour in the interaction between **4** and the proton is in agreement with the nucleophilicity difference between the mono- and the di-pyrrolidinylthiazole; in fact, due to the very strong nucleophilicity of **1** and to the ability of the pyrrolidinyl group to stabilize the positive charge in the ring, the proton is localized at the C-5 while in compound **4** the proton in not located in a preferential position.

During the NMR characterization of compounds **5a-c**, a peculiarity was observed in the recorded ^{1}H-NMR spectra, in $CDCl_3$, at room temperature: the signals belonging to the methylene protons in α position to the nitrogen atom of the pyrrolidine ring appeared to be broad, as close to a coalescence situation; this was ascribed to a constricted rotation of the pyrrolidinyl ring in the molecule.

Moreover, by comparing the spectra, we noted that the signals belonging to the methylene protons in the spectra of **5a**, **5b** and **5c** recorded at 27 °C, gradually broadened on going from **5c** to **5b** to **5a** (Figure 3).

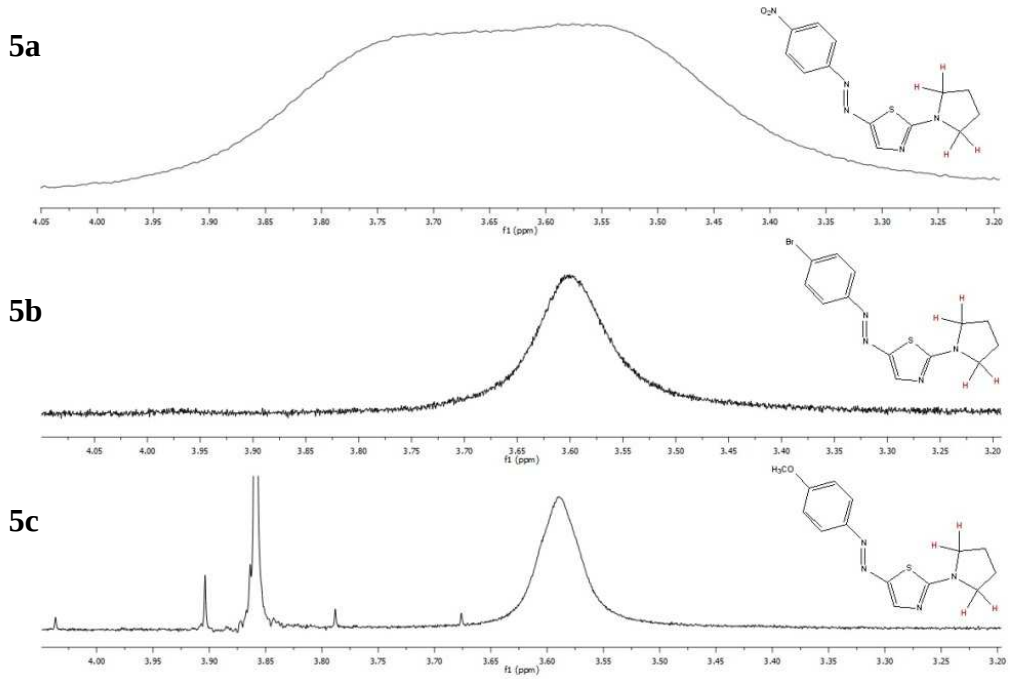


Figure 3. ^{1}H NMR signals in $CDCl_3$ at 25°C of methylene protons in position adjacent to the pyrrolidinyl nitrogen of compounds **5a-c** (ordered from up to bottom).

Given that a similar signal broadening was not observed in the spectrum of compound **4** and that, comparing compounds **5a-c** the only difference is the *para*-substituent on the benzene ring of the azo moiety, we hypothesized that a different contribution of the mesomeric electronic effects, due to the *para*-substituent, might induce a different double bond character of the exocyclic C2-N bond.

This effect might be more pronounced on going from less to more electron-withdrawing substituents of the azo moiety; in other words, the involvement of the mesomeric electronic effect of the substituent on the benzene ring might influence the rotational freedom around the C2-N bond.

To complete the NMR study, the reactions between **4** and **2d-g** were also performed directly in the NMR tube, working under the same experimental conditions used for the reactions involving compounds **2a-c**.

It must be remarked that the above-hypothesized effect might sound ’unexpected’ since the distance from the site of the restricted rotation and the substituent on the benzene ring is huge.

To support our hypothesis we decided to derive the activation energy parameter $\Delta G^{\neq}$ of the rotational process for all compounds **5a-g** in order to verify if these data might be related to the Hammett substituent parameters. For this purpose, we carried out dynamic-NMR

simulations for **5a-g** and these results were compared with the experimental data obtained from the variable temperature NMR experiments; Figure 4 shows, as an example, the experimental and simulated spectra for compound **5a**, including the temperatures and the rotational rate constants (*k*) extracted from the line-shape simulation.

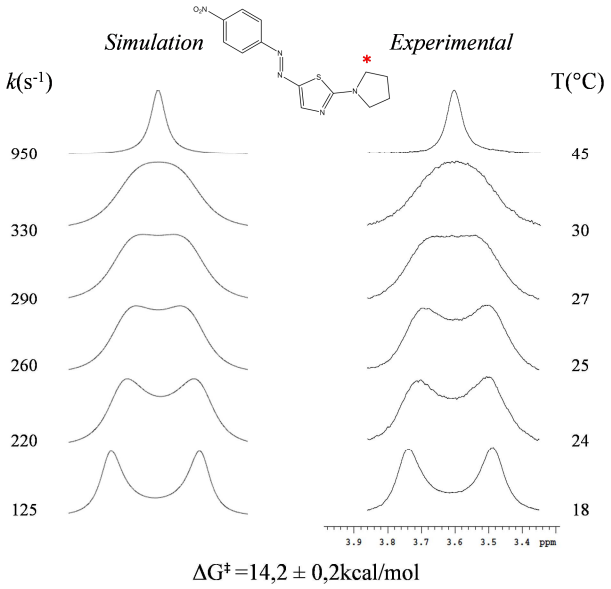


Figure 4. Variable temperature ^{1}H NMR spectra in $CDCl_3$ and dynamic-NMR simulations for methylene signals of **5a**.

In Table 3 are collected the $\Delta G^{\neq}$ values for compounds **5a-g** obtained from dynamic ^{1}H NMR data using the Eyring equation;[18,19]for all compounds the experimental free energy activation rotation was found to be invariant with the temperature, thus implying a very small activation entropy, as usually happens in conformational processes.

The values reported in Table 3 show that according with the σ Hammett substituent constants, the $\Delta G^{\neq}$ values decrease on going from the more electron withdrawing substituents to the less one.

Table 3. $\Delta G^{\neq}$ Parameters for C–N rotation from dynamic ^{1}H-NMR data and σ substituent constants[a].

Compound	Substituent	$\Delta G^{\neq}$ (Kcal/mol)[a,b]	σ[c]
5a	4-NO_2	14.2	0.81[d]
5b	4-Br	13.5	0.22[d]
5c	4-OCH_3	12.9	–0.28[d]
5d	4-CN	14.2	0.71[d]
5e	4-CF_3	13.7	0.53[d]
5f	4-Cl	13.6	0.22[d]
5g	3,5-dichloro	13.9	0.37 (x 2)[e]

a. As the mean of $\Delta G^{\neq}$ calculated at each temperature. b. ±0.2 kcal/mol. c. O. Exner, Correlation Analysis of Chemical Data, Plenum Press, N.Y., pp. 61-62, 1988.[20] d. σ_p value. e. σ_m value.

The calculated $\Delta G^{\neq}$ values for compounds **5a-g**, were plotted *versus* the Hammett σ substituent constants, and reported in Figure 5.

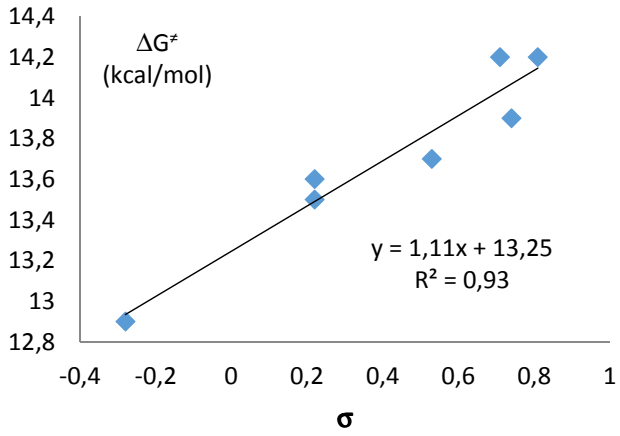


Figure 5. Plot of $\Delta G^{\neq}$ values for compounds **5a–g** *vs.* σ substituent constants.

A good linear correlation was found plotting $\Delta G^{\neq}$ *versus* the Hammett σ substituent constants (Figure 5), thus supporting the hypothesis that the rotation around the C–N bond

between the thiazole C-2 carbon atom and the pyrrolidinyl substituent can be subjected to a 'remote' influence of the substituent in *para*-position to the azo-moiety by mesomeric effect. It must be remarked that a significant electronic effect ($\rho > 1$) refers to a transmission of these effects through more than ten bonds, and the obtained results, appears quite 'unusual'. Moreover, the correlation using σ^- values also resulted quite good ($y = 0.91x + 13.2$; $R^2 = 0.91$): clearly, the very close correlations obtained by using σ or σ^- constants can be considered an indication that the extra-conjugation contribution becomes negligible likely due to the remote position of the substituent.

1.1.3 Conclusions

The reaction between the 2-*N*-pyrrolidinylthiazole, a very poorly studied compound, with different arenediazonium salts, gave a series of new azo compounds, in good yields that could be interesting and promising products for application in different fields.

An NMR spectroscopic study of these compounds, in $CDCl_3$ solution, revealed a peculiarity for the methylenic protons in alpha position to the nitrogen atom of the pyrrolidinyl ring: a broadening of their signals was observed in different extent, depending on the substituent in *para*-position of the benzene ring of the azo moiety; the observed behaviour indicate an hindered rotation around the C2–N bond.

The energy activation parameters of this process were calculated through ^{1}H-NMR experiments carried out at different temperatures and the results obtained showed a good correlation with the Hammett substituent constants. These findings indicate an influence (by mesomeric effect) of the 'remote' substituent on the rotational freedom around the C-N bond, due to its significant double bond character.

1.1.4 Experimental Section

The ^{1}H and ^{13}C NMR spectra were recorded with a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H NMR) and 100.56, or 150.80 MHz (for ^{13}C NMR), respectively. *J* values are given in Hz. Signal multiplicities were established by DEPT experiments. Chemical shifts were referenced to the solvent [δ =7.26 and 77.0 ppm for $CDCl_3$), (δ=2.0 and 118.20 ppm for CD_3CN), (δ=4.3 and 57.3 ppm for CD_3NO_2) for ^{1}H and ^{13}C NMR, respectively]. Chromatographic purifications (FC) were carried out on silica gel columns at medium pressure.

The arenediazonium tetrafluoroborate salts **2a-c** and **2g** are commercially available, 4-cyanobenzenediazonium benzo[*d*][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide (**2d**)[21], 4-(trifluoromethyl)benzenediazonium tetrafluoroborate (**2e**)[22] and 4-

(chloro)benzenediazonium benzo[*d*][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide[21](**2f**), were prepared as reported in ref. 21 and 22, and their spectral data agree with those in the literature.

Synthesis of 2-pyrrolidinylthiazole (4):

Pyrrolidine (0.25 mL, 3.05 mmol) was added to 2-bromothiazole (200 mg, 1.22 mmol), then the mixture was magnetically stirred, at room temperature, without solvent; immediately after the mixing of the reagents, the development of gas was observed (likely HBr). The reaction was monitored by TLC, using a mixture of ethyl ether/light petroleum in 8/2 ratio, and by GC-MS. After 48 hours the 2-pyrrolidinylthiazole **4** was isolated by purification on column chromatography on silica gel (FC) using as eluent the solvent in the same ratio as used for the TLC analysis.

Compound **4** was obtained in 80% yield and it was stored at -18°C.

2-pyrrolidinylthiazole (4): **^{1}H NMR:** ($CDCl_3$, 400 MHz) δ (ppm): 7.18 (d, *J* = 3.6 Hz, 1 H), 6.44 (d, *J* = 3.6 Hz, 1 H), 3.46 (t, *J* = 6.7 Hz, 4 H), 2.03 (t, *J* = 6.7 Hz, 4H); **^{13}C NMR:** (CD_3Cl, 100.56 MHz) δ (ppm): 168.4, 139.9, 105.6, 49.5, 25.7; **GC-MS (m/z):** 154 [M^+, 77], 126 (100), 112 (43), 99 (86), 85 (23), 70 (11), 58 (29).

General procedure for the synthesis of compounds 5a–g:

A solution of 4-nitrobenzendiazonium tetrafluoroborate (**2a**, 0.050 g, 0.21 mmol) in CH_3CN (2.5 mL) was added dropwise to a solution of **4** (0,065 g, 0.42 mmol) in CH_3CN (2.5 mL) and the mixture was stirred at room temperature. In all cases, except case **e**, the formation of a precipitate was observed after 30 min; the solid was collected by filtration over a Buchner funnel, washed with cold acetonitrile and dried under vacuum. Further amount of compounds **5c**, **5d**, **5g** was obtained after FC of the concentrated mother liquor. The yields reported for **5a**, **5b**, and **5f** were obtained collecting the solid precipitated from the crude reaction mixture; for cases **5c**, **5d**, and **5g** they are the sum of the yield of the solid precipitated and of that obtained after FC of the concentrated mother liquor. In case of **5e** the conversion was 70% after 60 min and the yield reported was obtained by FC.

(*E*)-5-((4-Nitrophenyl)diazenyl)-2-(pyrrolidin-1-yl)thiazole (5a): bordeaux solid 0.057 g 90% yield. mp> 240 °C (dec.). **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 8.28 (d, *J* = 9.0 Hz, 2 H), 8.16 (s, 1 H), 7.81 (d, *J* = 9.0 Hz, 2 H), 4.00-3.20 (m, 4 H, NCH_2), 2.24-2.04 (m, 4 H, $NCH_2C\underline{H_2}$); **^{13}C NMR**: (100.56 MHz, $CDCl_3$, 45 °C) δ (ppm): 170.0 (C), 156.8 (C),

152.0 (CH), 147.0 (C), 145.8 (C), 124.7 (CH), 122.3 (CH), 49.5 (br., NCH_2), 25.5 ($NCH_2C\underline{H}_2$). **ESI MS (ES^+) m/z**: 304 $[M+H]^+$, 326 $[M+Na]^+$, 342 $[M+K]^+$.

5-((4-Bromophenyl)diazenyl)-2-(pyrrolidin-1-yl)thiazole (5b): orange solid 0.053 g 75% yield. mp 213-215 °C(dec.). **^{1}H NMR** ($CDCl_3$, 600 MHz, 25 °C) δ (ppm): 8.03 (s, 1 H), 7.59 (d, *J* = 8.9 Hz, 2 H), 7.54 (d, *J* = 8.9 Hz, 2 H), 3.87–3.32 (m, 4 H, NCH_2), 2.14–2.07 (m, 4 H, $NCH_2C\underline{H}_2$); **^{13}C NMR**: (150.8 MHz, $CDCl_3$, 25 °C) δ (ppm): 168.6 (C), 151.5 (C), 148.5 (C), 145.5 (C), 133.1 (CH), 123.5 (CH), 122.8 (C), 49.7 (br., NCH_2), 25.5 ($NCH_2C\underline{H}_2$). **ESI MS (ES^+) m/z**: 337, 339 $[M+H]^+$, 359, 361 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{13}BrN_4S$: C, 46.30; H, 3.89; Br, 23.69; N, 16.61; S, 9.1. Found: C, 46.35; H, 3.90; N, 16.59.

(*E*)-5-((4-Methoxyphenyl)diazenyl)-2-(pyrrolidin-1-yl)thiazole (5c): orange solid 0.030 g 50% yield. mp >199 °C (dec). **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 7.94 (s, 1 H), 7.71 (d, *J* = 8.9 Hz, 2 H), 6.94 (d, *J* = 8.9 Hz, 2 H), 3.85 (s, 3 H, OCH_3), 3.66–3.46 (m, 4 H, NCH_2), 2.13–2.04 (m, 4 H, $NCH_2C\underline{H}_2$); **^{13}C NMR**: (100 MHz, $CDCl_3$, 25 °C) δ (ppm): 167.8 (C), 160.5 (C), 146.9 (C), 146.1 (CH), 145.9 (C), 123.6 (CH), 114.2 (CH), 55.5 (OCH_3), 49.5 (NCH_2), 25.5 ($NCH_2C\underline{H}_2$). **ESI MS (ES^+) m/z**: 289 $[M+H]^+$, 311 $[M+Na]^+$, 327 $[M+K]^+$.

4-((2-(Pyrrolidin-1-yl)thiazol-5-yl)diazenyl)benzonitrile (5d): metallic bordeaux solid 0,043 g, 72% yield. mp>200 °C (dec.). **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 8.12 (s, 1 H), 7.77 (d, *J* = 9.2 Hz, 2 H), 7.69 (d, *J* = 9.2 Hz, 2 H), 4.01–3.11 (m, 4 H, NCH_2), 2.15–2.08 (m, 4 H, $NCH_2C\underline{H}_2$); **^{13}C NMR**: (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm): 169.6 (C), 155.2 (C), 151.3 (CH),145.5 (C), 133.0 (CH), 122.4 (CH), 119.0 (C), 111.1 (C), 49.8 (br., NCH_2), 25.5 ($NCH_2C\underline{H}_2$). **ESI MS (ES^+) m/z**: 284 $[M+H]^+$, 306 $[M+Na]^+$, 322 $[M+K]^+$. Anal. Calcd for $C_{14}H_{13}N_5S$: C, 59.34; H, 4.62; N, 24.72; S, 11.31. Found: C, 59.41; H, 4.63; N, 24.67.

2-(Pyrrolidin-1-yl)-5-((4-(trifluoromethyl)phenyl)diazenyl)thiazole (5e): red solid 0.028 g, 41% yield. mp>136 °C (dec.). **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 8.11 (s, 1 H), 7.81 (d, *J* = 8.3 Hz, 2 H), 7.68 (d, *J* = 8.3 Hz, 2 H), 3.90–3.40 (m, 4 H, NCH_2), 2.20–2.08 (m, 4 H, $NCH_2C\underline{H}_2$); **^{13}C NMR**: (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm): 168.8 (C), 154.5 (C), 148.7 (C), 145.1 (C), 130.2 (C, q, $^2J_{C\text{-}F}$=33.3 Hz), 126.1 (CH, q, $^3J_{C\text{-}F}$=3.96 Hz), 124.1 (C, q, $^1J_{C\text{-}F}$=272 Hz), 122.1 (CH), 50.1 (br., CH_2), 25.5 (CH_2). **ESI MS (ES^+) m/z**: 327 $[M+H]^+$. Anal. Calcd for $C_{14}H_{13}F_3N_4S$: C, 51.53; H, 4.02; N, 17.17; S, 9.82. Found: C, 51.65; H, 4.03; N, 17.13.

5-((4-Chlorophenyl)diazenyl)-2-(pyrrolidin-1-yl)thiazole (**5f**): orange solid 0.049 g, 80% yield. mp>198 (dec.). **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 8.02 (s, 1 H), 7.65 (d, *J* = 8.8 Hz, 2 H), 7.38 (d, *J* = 8.8 Hz, 2 H), 3.72–3.40 (m, 4 H, NCH_2), 2.15–2.04 (m, 4 H, $NCH_2C\underline{H}_2$); **^{13}C NMR**: (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm): 168.6, 151.1, 148.5,145.5, 134.4, 129.1 (CH), 123.2 (CH), 49.6 (NCH_2), 25.5 $NCH_2\underline{C}H_2$). **ESI MS (ES$^+$) m/z**: 293, 295 $[M+H]^+$, 315, 317 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{13}ClN_4S$: C, 53.33; H, 4.48; Cl, 12.11; N, 19.14; S, 10.95. Found: C, 53.37; H, 4.47; N, 19.19.

5-((3,5-Dichlorophenyl)diazenyl)-2-(pyrrolidin-1-yl)thiazole (5g): orange solid 0.046 g 67% yield. mp 141-143 °C (dec.). **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 8.10 (s, 1 H), 7.62 (d, *J*=1.9 Hz, 2 H), 7.30 (t, *J*= 1.9 Hz, 1 H), 3.93–3.40 (m, 4 H, NCH_2), 2.26–2.08 (m, 4 H, $NCH_2C\underline{H}_2$); **^{13}C NMR**: (100 MHz, $CDCl_3$, 25 °C) δ (ppm): 169.3, 154.2, 150.6 (CH),145.1, 135.3, 127.9 (CH), 120.5 (CH), 49.8 (br., NCH_2), 25.5 ($NCH_2C\underline{H}_2$). **ESI MS (ES$^+$) m/z**: 327, 329 $[M+H]^+$, 349, 351 $[M+Na]^+$, 365 $[M+K]^+$. Anal. Calcd for $C_{13}H_{12}Cl_2N_4S$: C, 47.72; H, 3.70; Cl, 21.67; N, 17.12; S, 9.80. Found: C, 47.81; H, 3.69; N, 17.09.

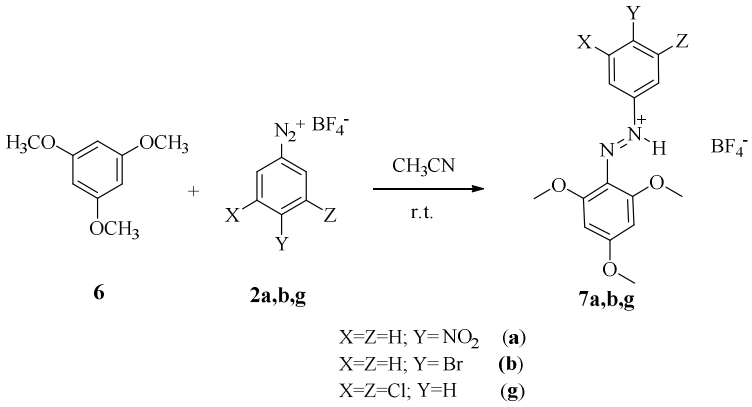
1.2 REACTIONS BETWEEN ARENEDIAZONIUM SALTS AND ANISOLE DERIVATIVES: REACTIVITY, REGIOSELECTIVITY AND FORMATION OF SOLID STATE FLUORESCENT COMPOUNDS

1.2.1 Introduction

As reported in the previous chapter, in the past, interesting mechanistic informations have been obtained by using diazonium salt derivatives as electrophilic substrates and tris(dialkylamino)benzenes as neutral carbon nucleophiles.[23-26]

After these results, the research group decided to continue the mechanistic study of the reactions involving arenediazonium salts, changing the nucleophilic partner, and one of the selected candidates was 1,3,5-trimethoxybenzene due to its symmetry and to the presence of the methoxy substituent with electron-donor effect similar, even if minor, to that of the dialkylamino group in *sym*-triaminobenzenes.

The reaction between 1,3,5-trimethoxybenzene (**6**) and benzenediazonium salts **2** (Scheme 7), carried out in acetonitrile at room temperature, gave the monosubstituted coupling products in saline form **7** (tetrafluoroborate salts), that were isolated by precipitation from the reaction mixture.



Scheme 7. Reactions between 1,3,5-trimethoxybenzene and benzenediazonium salts.

Contrarily to what observed with *sym*-triaminobenzenes, no evidence of the Wheland intermediate for these reactions was obtained likely due to the lower ability of the methoxy group to stabilize the positive charge of the σ intermediate on the ring with respect to the dialkylamino groups of the triaminobenzene derivatives.

During that work, it was observed an interesting property of compounds **7**, that resulted fluorescents in solid state and lose this property after neutralization.[27]

It is really interesting to note that usually azobenzene derivatives are not fluorescent compounds, but if the *cis-trans* photoisomerization is blocked their fluorescence is higher,[28] and this is the case of salts **7**.

This property makes the obtained salts (**7**) interesting for hypothetical future applications and work is still in progress on this topic.

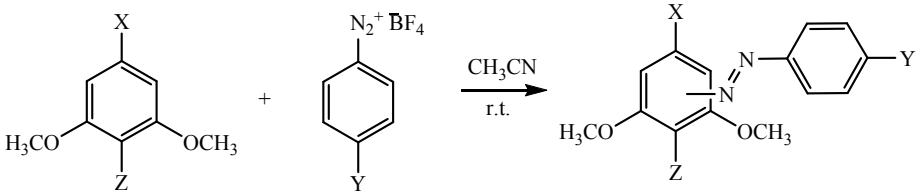
Moreover, respect to previous reactions involving triaminobenzenes as nucleophiles, in this case, when the reaction between **6** and **2** was carried out in 1:2 relative molar ratio, no evidence of the formation of the di-cationic species was obtained.

Recent studies of the research group regarding the reaction between 1,3,5-trihydroxybenzene and **2a-c** in 2:1 molar ratio in favour of the electrophile, gave a mixture of two different products. The first was the product from the attack of one molecule of the electrophile and the second was the product obtained from the attack of three molecules of the electrophile.[29]

The above-discussed results regard a relatively simple investigation, concerning symmetrical systems and thus only a possible mono azo-coupling product. In the current study I started an investigation about the reactivity between the same benzenediazonium salts and other neutral carbon nucleophiles with different groups on the aromatic ring. Herein I will report the obtained results from this investigation.

1.2.2 Results and Discussion

The nucleophilic species **8a-c**, bearing different groups on the aromatic ring were coupled with compounds **2a-c**, in equimolar amount and in acetonitrile at room temperature (Scheme 8).



8a-c **2a-c**

X = OH, Z = OCH_3 **(8a)** Y = NO_2 **(2a)**
X = OH, Z = H **(8b)** Y= Br **(2b)**
X = OCH_3, Z = NO_2 **(8c)** Y= OCH_3 **(2c)**

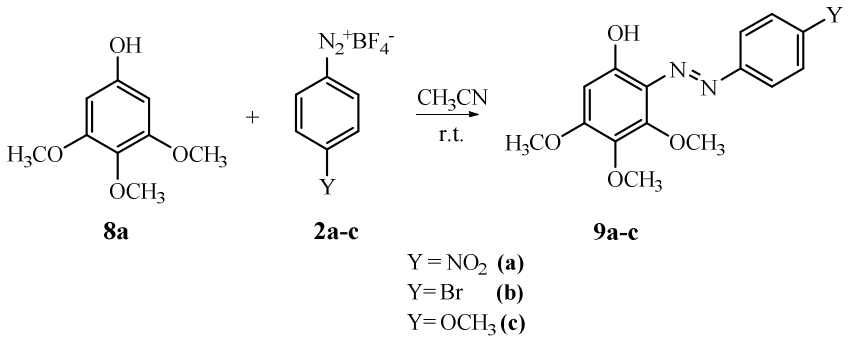
Scheme 8. Reactions between arenediazonium salts and substituted anisole derivatives.

The final azo coupling products were obtained after purification on silica gel column and they were characterized by usual spectroscopic methods.

For the sake of clarity I will discuss the combination between each nucleophile with the three electrophiles, separately, as follows.

- *Reactions between 3,4,5-trimethoxyphenol **8a** and **2a-c***

The reactions between equimolar amount of **8a** and the electrophilic species **2a-c** (Scheme 9), gave the azo-coupling products **9a** and **9b**.



Scheme 9. Reactions between 3,4,5-trimethoxyphenol and benzenediazonium salts.

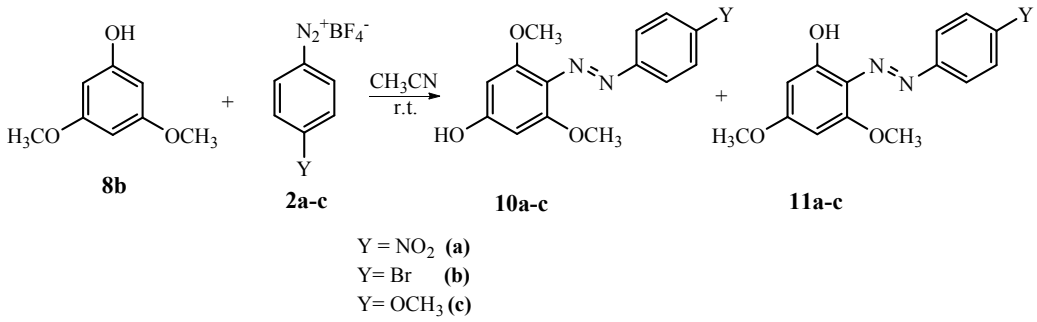
Compound **9a** was obtained in 83% yield after 90 minutes at room temperature and compound **9b** in 41% yield after 24 hours (without a total conversion of reagents); instead, no substitution product was obtained from the reaction between **8a** and **2c**, neither at room temperature nor after heating under reflux for two hours.

This trend can be explained analyzing the electrophilic reactivity of the diazonium salts (**2a-c**) that increases with increasing the electron-withdrawing power of the substituent in *para* position, and this is reflected in the different obtained yields under the above cited experimental conditions.

The reaction between **8a** and **2a** was also repeated working in 2:1 molar ratio in favour of the electrophile, and under these experimental conditions only compound **9a** was obtained: no evidence of the second electrophilic attack on the nucleophile was observed, contrarily to what was observed in the case of the reaction between **2a** and 1,3,5-trihydroxybenzene.[29]

- *Reactions between 3,5-dimethoxyphenol **8b** and **2a-c***

In the case of the reactions between 3,5-dimethoxyphenol **8b** and benzenediazonium salts **2a-c**, two different attack positions are present on the aromatic ring, giving the possibility to obtain two different compounds, as reported in Scheme 10.



Scheme 10. Reactions between 3,5-dimethoxyphenol and benzenediazonium salts.

The reaction between **8b** and 4-nitrobenzenediazonium tetrafluoroborate (**2a)**, performed in CH_3CN at room temperature and with equimolar amount of reagents, immediately after mixing, gave a precipitate; ^{1}H-NMR analysis showed that the solid was a mixture of the two possible compounds, the symmetric (**10a**) and the unsymmetric (**11a**), with **10a** in greater amount with respect to **11a**.

Also in the mother liquor both products were present, but here the unsymmetric compound (**11a**) was the predominant species. After work-up (see experimental), compounds **10a** and **11a** were obtained in 42% and 36% yield, respectively, after purification on silica gel column.

It is interesting to observe that the NMR spectrum of compound **10a** showed broad signals, due to the presence of the methoxy substituents in *ortho* position to the azo group that hindered the free rotation around the C4-N single bond. This hypothesis was confirmed by the NMR spectrum recorded at higher temperature (40 °C) that showed a sharpness of the signals.

The reaction was also repeated mixing reagents directly in the NMR spectroscopy tube, in DMSO-d_6. Under these experimental conditions no precipitate was observed and it was possible to study the reaction progress over time through ^{1}H-NMR spectroscopy; it was also possible to calculate the relative ratio of the two products and **10a** resulted to be the main product (87/13 relative % molar ratio between **10a** and **11a**).

The reaction between **8b** and 4-bromobenzenediazonium tetrafluoroborate (**2b**), was carried out under the above reported experimental conditions and gave again a precipitate. In this

case after chromatographic separation, three different compounds were obtained; the symmetric (**10b**) in larger amount with respect to the others.

The other products were analyzed by NMR spectroscopy in $CDCl_3$ and they showed an unsymmetric structure; one of these compounds was obtained in very low yield (only 3%).

Based on the obtained ^{1}H-NMR spectra, we ascribed structure **11b** to the main unsymmetric compound, whereas it was not possible to obtain detailed NMR information for the second asymmetric compound because it was obtained in very low yield.

The reaction was also repeated mixing reagents directly in the NMR spectroscopy tube, in DMSO-d_6. Under these experimental conditions no precipitate was observed and it was possible to study the reaction progress over time analyzing the NMR spectrum, that showed only signals ascribed to compounds **10b** and **11b** in a 70/30 relative % molar ratio.

The reaction between **8b** and 4-methoxybenzenediazonium tetrafluoroborate (**2c**), was carried out under the above reported experimental conditions and at room temperature it resulted to be very slow; after 24 hours the conversion was only 10%. Therefore, we decided to heat under reflux and after 2 hours the conversion (calculated through ^{1}H-NMR in DMSO-d_6 of a little amount of the concentrated crude reaction mixture) was 56%.

The recorded spectrum evidenced signals ascribed to a single reaction product showing a unymmetric structure; compound **11c** was fully characterized after purification on silica gel column.

Also in this case, the reaction was repeated directly in the NMR spectroscopy tube, in DMSO-d_6. After 24 hours the spectrum showed signals ascribed to both reagents (**8b** and **2c**) and to two different products in agreement with structures **10c** and **11c** in a relative % molar ratio of 72/28 and with a conversion of 10%.

We can observe that at room temperature in DMSO-d_6 a mixture of two products was obtained, instead heating the solution only compound **11c** was isolated; even if the reaction solvent is different, probably this phenomenon could be an indication of a positional isomerization induced by the temperature increase and, more specifically, it could be seen in term of reversibility of the electrophilic attack, as observed in past studies on the azo-copulation reaction with triaminobenzene derivatives as nucleophiles;[23-25] further investigation is needed to confirm this hypothesis.

Comparing the NMR data for the reactions between **8b** and **2a-c** in DMSO-d_6, at room temperature, we can observe that the formation of the products with a symmetric structure (**10**) is favoured respect to the unsymmetric compounds (**11**).

This behaviour might be a consequence of the effect of the OH group, that produces a minor inductive effect (-I) in *para* respect to the *ortho* position, and activates the *para* position by mesomeric effect, in major extent than the *ortho* position, as reported in the literature.[30a]

It is also relevant to consider that the hydroxy group is more activating respect to the methoxy group, in the S_EAr.[30b]

About the N=N bond geometry, all the synthesized compounds have been depicted with a *trans* configuration of the N=N bond, on the basis of the well known stability of this configuration for the azo compounds.[31] Unfortunately, all our attempts to obtain crystals of the obtained azo compounds suitable for X-Ray analysis failed. Finally, no products were obtained from the reactions between **8c** and **2a-c**, likely due to the lower nucleophilic ability of **8c** for the presence of the nitro group on the aromatic ring.

It is interesting to note that some of the synthesized compounds appeared as bright colored solids and under UV lamp (365 nm) they showed an intense solid state fluorescence.

In Figure 6 is reported a picture of the fluorescence in the case of compound **11c**.

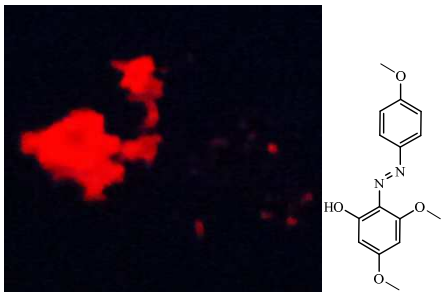


Figure 6. Solid-state fluorescence of compound **11c** under 365 nm UV lamp.

This finding it is really interesting compared with results obtained from the reactions between 1,3,5-trimethoxybenzene and diazonium salts (**2**),[27] in which only the monosubstitued coupling products in saline form (tetrafluoroborate salt) showed the solid state fluorescence and the related neutral compounds didn't show this property.

In this case, the reaction between **8c** and **2a-c** showed solid state fluorescence for neutral compounds (confirmed by absence of the BF_4^- signal in the ^{19}F-NMR spectrum) and we can explain this behaviour by making some observations on the structure of compound **11c**; in this case, the hydroxyl group is adjacent to the azo group and there is the possibility that some interaction, such as an intramolecular hydrogen bond, might simulate the situation of the salts **7** (Figure 7).

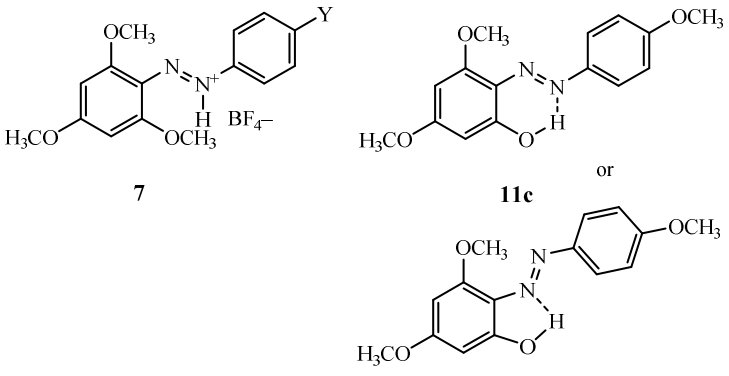


Figure 7. Comparison between salts **7** and the neutral product **11c**.

The hydrogen bond interaction depicted in Figure 7 could block the photoisomerization process, giving only one of the two isomers, thus producing the solid-state fluorescence phenomenon.

Moreover, the fluorescence resulted stronger in the case of compound **11c**, that possess a *para*-methoxy group on the benzenediazonium moiety; this behaviour could depend from the mesomeric effect +M of this substituent, that might help the nitrogen atom of the azo group to give an hydrogen bond interaction. Our hypothesis on the solid state fluorescence of the neutral compounds need to be verified by further and more detailed studies and work is still in progress on this topic.

1.2.3 Conclusions

The azo-coupling reaction between substituted anisole derivatives and aryldiazonium salts, bearing substituents with different electronic demands in position 4 gave new interesting products that were isolated and fully characterized. Their spectroscopy properties will be helpful in future mechanistic studies; moreover, some of these compounds showed solid-state fluorescence and for this reason they could be interesting for applications in many areas of applied chemistry.

1.2.4 Experimental section

The ^{1}H and ^{13}C NMR spectra were recorded with a Varian Inova 300 and a Varian Mercury 400 spectrometers operating at 300, or 400 MHz (for ^{1}H NMR) and 75.46, or 100.56 MHz (for ^{13}C NMR), respectively. *J* values are given in hertz (Hz). Signal multiplicities were established by DEPT experiments. Chemical shifts were referenced to the solvent [δ =7.26

and 77.0 ppm for $CDCl_3$), (δ =2.0 and 118.20 ppm for CD_3CN), (δ =2.50 and 39.50 ppm for DMSO-d_6) for ^1H and ^{13}C NMR, respectively]. ESI-MS spectra were recorded with a WATERS 2Q 4000 instrument. Chromatographic purifications were carried out on silica gel (0.037-0.063 mm, Merck) columns at medium pressure. Thin layer chromatography (TLC) was performed on silica gel 60 F254 coated aluminum foils (Fluka). Melting points were measured on a Stuart SMP3 apparatus and are uncorrected. Solvents and reagents were commercial materials (Aldrich or Fluka) if not specified.

General procedure for the synthesis of the azo coupling products:

To a magnetically stirred solution of the nucleophile (0.2 mmol of the anisole substituted derivative **8a**, or **8b**, or **8c**) dissolved in CH_3CN (2 mL) was added an equimolar amount of the electrophile (benzenediazonium salt **2a**, or **2b**, or **2c**), at room temperature; in the case of the reactions with 4-methoxybenzenediazonium tetrafluoroborate (**2c**), they were carried out under reflux at about 80°C.

The reactions were monitored by TLC, with different eluents (usually CH_2Cl_2) and ^1H-NMR analysis. In some reactions the formation of a precipitate was observed, this solid was collected by filtration and washed with cold CH_3CN; then analyzed by NMR spectroscopy.

Finally, the products were purified by column chromatography on silica gel (FC), using dichloromethane as eluent and methanol as second eluent, when a mixture of products was present. All the products were fully characterized by usual spectroscopic methods; ^{19}F-NMR spectroscopy was also used to confirm the neutral form of the obtained compounds.

Chemico-physical data for the synthesized compounds are reported as follows.

3,4,5-Trimethoxy-2-[(4-nitrophenyl)diazenyl]phenol (9a): red solid, 83% yield (52% by precipitation from the reaction mixture, 31% after FC of the mother liquor), m.p. 208.9-210.0 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz, 25°C) δ (ppm): 8.36 (d, *J* = 9.00 Hz, 2H), 7.95 (d, *J* = 9.00 Hz, 2H), 6.25 (s, 1H), 4.00 (s, 3H), 3.91 (s, 3H), 3.75 (s, 3H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 163.0, 162.7, 151.5, 150.5, 145.9, 136.3, 128.0, 125.4, 120.1, 97.3, 62.8, 61.1, 56.8. **ESI MS (ES^+) m/z:** 334 $[M+H]^+$, 356 $[M+Na]^+$.

3,4,5-Trimethoxy-2-[(4-bromophenyl)diazenyl]phenol (9b): red solid, 41% yield; m.p. 164.3–165.2 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz, 25°C) δ (ppm): 7.79 (d, *J* = 9.02 Hz, 2H), 7.75 (d, *J* = 9.02 Hz, 2H), 6.39 (s, 1H), 3.99 (s, 1H), 3.89 (s, 3H), 3.73 (s, 3H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 159.7, 154.0, 152.1, 148.5, 135.4, 132.6, 126.2, 123.0, 122.9, 96.1, 63.1, 61.0, 56.4. **ESI MS (ES^+) m/z:** 369 $[M+H]^+$, 391 $[M+Na]^+$.

3,5-Dimethoxy-4-[(4-nitrophenyl)diazenyl]phenol (10a): orange solid, 42% yield, m.p. > 228 °C dec. **^{1}H NMR** (DMSO-d_6, 300MHz, 25°C) δ (ppm): 8.29 (d, *J* = 9.17 Hz, 2H), 7.69 (d, *J* = 9.17 Hz, 2H), 5.76 (s, 1H), 3.85 (br.s, 6H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 184.9, 158.9, 148.9, 142.8, 125.5, 125.2, 116.0, 102.0, 56.4. **ESI MS (ES^+) m/z:** 302 $[M-H]^-$.

3,5-Dimethoxy-2-((4-nitrophenyl)diazenyl)phenol (11a): red solid, 36% yield, m.p. > 240 °C dec. **^{1}H NMR** (DMSO-d_6, 300 MHz, 25°C) δ (ppm): 8.36 (d, *J* = 9.10 Hz, 2H), 7.90 (d, *J* = 9.10 Hz, 2H), 6.12 (d, *J* = 2.30, 1H), 6.03 (d, *J* = 2.30, 1H), 3.91 (s, 3H), 3.87 (s, 3H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 169.1, 165.8, 159.9, 151.5, 147.9, 126.2, 125.5, 120.1, 94.6, 93.2, 56.4, 56.4. **ESI MS (ES^-) m/z:** 302 $[M-H]^-$

4-[(4-Bromophenyl)diazenyl]-3,5-dimethoxyphenol (10b): orange-red solid, 45% yield, m.p. 177.4–178.9 °C **^{1}H NMR** (DMSO-d_6, 400 MHz, 25°C) δ (ppm): 7.65 (d, *J* = 8.97 Hz, 2H), 7.55 (d, *J* = 8.97 Hz, 3H), 6.02 (br. s, 2H), 3.80 (s, 6H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 161.1, 157.1, 132.0, 124.0, 121.3, 119.8, 95.7, 93.9, 56.0. **ESI MS (ES^+) m/z:** 336 $[M+H]^+$, 359 $[M+Na]^+$.

2-[(4-Bromophenyl)diazenyl]-3,5-dimethoxyphenol (11b): orange solid, 21% yield, m.p. 193.2-194.1 °C **^{1}H NMR** (DMSO-d_6, 400 MHz, 25°C) δ (ppm): 7.72 (br. s, 4H), 6.20 (d, *J* = 2.20 Hz, 1H), 6.10 (d, *J* = 2.20 Hz, 1H), 3.91 (s, 3H), 3.85 (s, 3H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 166.4, 160.0, 159.3, 148.1, 132.5, 124.0, 122.6, 122.3, 93.9, 91.9, 56.2, 56.0. **ESI MS (ES^+) m/z:** 336 $[M+H]^+$, 359 $[M+Na]^+$

2-[(4-Bromophenyl)diazenyl]-3,5-dimethoxyphenol (12): orange solid, 3%yield. **^{1}H NMR** ($CDCl_3$, 300 MHz, 25°C) δ (ppm): 7.83 (d, *J* = 8.87 Hz, 2H), 7.59 (d, *J* = 8.87 Hz, 2H), 6.16 (d, *J* = 2.12 Hz, 1H), 6.07 (d, *J* = 2.12 Hz, 1H), 3.98 (s, 3H), 3.94 (s, 3H).

3,5-Dimethoxy-2-[(4-methoxyphenyl)diazenyl]phenol (11c): orange solid, 49% yield, m.p. 118.8-119.5 °C. **^{1}H NMR** (DMSO-d_6, 400MHz, 25°C) δ (ppm): 7.75(d, *J* = 8.98 Hz, 2H), 7.08 (d, *J* = 8.98 Hz, 2H), 6.20 (d, *J* = 2.86 Hz, 1H), 6.09 (d, *J* = 2.86 Hz, 1H), 3.89 (s, 3H), 3.839(s, 3H), 3.836(s,3H). **^{13}C NMR** (DMSO-d_6, 100.56 MHz, 25°C) δ (ppm): 164.7, 160.7, 159.6, 157.0, 143.7, 123.2, 122.8, 114.8, 93.6, 91.5, 56.1, 55.8, 55.5 **ESI MS (ES^+) m/z:** 289 $[M+H]^+$, 311 $[M+Na]^+$

1.3 REACTIONS BETWEEN ARYLDIAZONIUM SALTS AND 1,3-DISUBSTITUTED BENZENE DERIVATIVES

1.3.1 Introduction

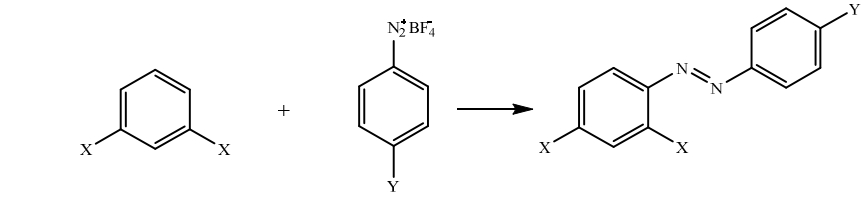
In the frame of our interest about the reactivity of arenediazonium salts, we decided to extend our investigation to their reaction with disubstituted benzenes as nucleophilic species.

In particular, the selected nucleophilic species were 1,3-bis(*N*,*N*-dialkylamino)benzene derivatives and 1,3-dimethoxybenzene. The diamino derivatives are compounds[32-34] very poorly studied so far and their reactions with arenediazonium salts were never reported in the literature; instead, about the 1,3-dimethoxybenzenes, some related azocompounds were reported in the literature, but they were obtained in very strong experimental conditions and not by direct coupling.[35,36]

Herein I report the obtained results from the reactions between the above-introduced disubstituted arenes with some aryldiazonium salts.

1.3.2 Results and Discussion

The reactions between the 1,3-disubstituted benzene derivatives **14a-d** and the aryldiazonium salts **2a-c** (Scheme 11) were carried out in equimolar amount of reagents, in acetonitrile, at room temperature and the substitution products **15-26** were obtained in high yield except for the case of the reaction between **14d** and **2c**, thet did not occurred.



14a-d

X = *N*-piperidinyl (**14a**, DPBH)
X = *N*-morpholinyl (**14b**, DMBH)
X = *N*-pyrolidinyl (**14c**, DPYBH)
X = methoxy (**14d**, DOMeBH)

2a-c

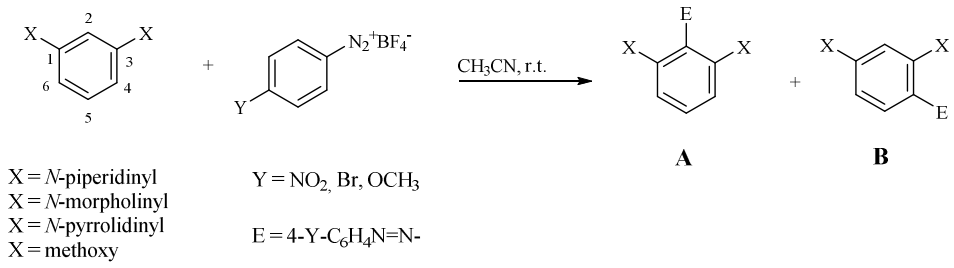
Y = NO_2 (**2a**)
Y = Br (**2b**)
Y = OCH_3 (**2c**)

15-26

X = *N*-piperidinyl	Y = NO_2	(**15**)
X = *N*-piperidinyl	Y = Br	(**16**)
X = *N*-piperidinyl	Y = OCH_3	(**17**)
X = *N*-morpholinyl	Y = NO_2	(**18**)
X = *N*-morpholinyl	Y = Br	(**19**)
X = *N*-morpholinyl	Y = OCH_3	(**20**)
X = *N*-pyrrolidinyl	Y = NO_2	(**21**)
X = *N*-pyrrolidinyl	Y = Br	(**22**)
X = *N*-pyrrolidinyl	Y = OCH_3	(**23**)
X = methoxy	Y = NO_2	(**24**)
X = methoxy	Y = Br	(**25**)
X = methoxy	Y = OCH_3	(**26**)

Scheme 11. Reactions between the disubstituted arenes **14a-d** and the aryldiazonium salts **2a-c**.

It is important to observe that opposite to the reactions involving triaminobenzene derivatives, in which only one substitution product can be obtained due to their symmetry, in the case of the 1,3-disubstituted arenes, considering the electronic effect of both substituents on the aromatic ring, two different products might be obtained; one with the electrophile situated in *ortho* position to both the substituents (position 2, **A** in Scheme 12) and the other with the electrophile in *ortho* with respect to one substituent and in *para* with respect to the other one (position 4 or 6, **B** in Scheme 12).



Scheme 12. Possible products from the reaction involving diaminobenzene derivatives.

In all the performed reactions, only the substitution product derived from the attack of the electrophilic species in 4 position of the nucleophile was obtained, as the **B** form in Scheme 12. This behaviour depends from the lower steric hindrance in position 4 with respect to position 2, and it is also due to the lower inductive effect (-I) of the substituents in position 4 with respect to position 2.

In Table 4 are reported the reaction times and the obtained yields, after purification on silica gel column, of the new synthesized compounds (**15-26**).

Table 4. Reactions between compounds **14a-d** and **2a-c**

Reaction	Nucleophile	Electrophile (substituent)	Reaction time	Product	Yield (%)[a]
1	**14a** (DPBH)	**2a** (NO_2)	30 min	**15**	98%
2	**14a** (DPBH)	**2b** (Br)	30 min	**16**	97%
3	**14a** (DPBH)	**2c** (OCH_3)	30 min	**17**	78%
4	**14b** (DMBH)	**2a** (NO_2)	30 min	**18**	97%
5	**14b** (DMBH)	**2b** (Br)	30 min	**19**	96%
6	**14b** (DMBH)	**2c** (OCH_3)	30 min	**20**	78%
7	**14c** (DPYBH)	**2a** (NO_2)	15 min	**21**	95%
8	**14c** (DPYBH)	**2b** (Br)	15 min	**22**	77%
9	**14c** (DPYBH)	**2c** (OCH_3)	15 min	**23**	73%
10	**14d** (DOMeBH)	**2a** (NO_2)	24 h	**24**	77%
11	**14d** (DOMeBH)	**2b** (Br)	48 h	**25**	26%
12	**14d** (DOMeBH)	**2c** (OCH_3)	72 h	**26**	0%

a. yields calculated after purification on silica gel column.

Analyzing the data reported in Table 4, we can observe that the reactions involving 1,3-trimethoxybenzene (**14d**) as nucleophile and 4-nitrobenzenediazonium tetrafluoroborate (**2a**) or 4-bromobenzenediazonium tetrafluoroborate (**2b**) needed more time, compared to the others, to give the final azo-coupling product, in 77% and 26% yields, respectively, due to the low reagent conversion.

Instead, the reaction between **14d** and the 4-methoxybenzenediazonium tetrafluoroborate (**2c**), didn't show any conversion, neither after three days.

We can explain these results by considering the electrophilic power of the involved arenediazonium salts that decreases from the nitro derivative (**2a)** to the methoxy one (**2c**) as predictable considering the substituent effect and the electrophilic values calculated for compounds **2a**, **2b** and **2c** (E= -5.1, -6.6, e -8.4, respectively), by Professor H. Mayr.[37]

The reactions between the 1,3-diaminobenzene derivatives **14a-c**, more nucleophilic species with respect to **14d**, quickly gave the new azo compounds **15-23**, with almost total conversion; these products were isolated in high yield after purification on silica gel column and were fully characterized by ^{1}H NMR, ^{13}C NMR ed ESI-MS spectroscopy.

Also in this case the obtained results are explained considering the relative electrophilic power of the salts **2a-c**, in fact in the case of reactions 1-6, a decreasing of the yields can be observed on going from the more reactive electrophile (**2a**) to the less one (**2c**).

The ^{1}H-NMR analysis of the crude reaction mixtures for the reaction 7-9 showed the total conversion of the reagents in the azo coupling products in a shorter time with respect to the other reactions, owing to the strong nucleophilic power of the 1,3-di(pyrrolidinyl) derivative (**14c**) with respect to **14a** and **14b**.

Until now the nucleophilic parameters at the carbon or nitrogen atoms for the tri- and diaminobenzene derivatives were not reported, and their quantification is a part of our collaboration with Professor Herbert Mayr's group, but we can explain the stronger reactivity of the pyrrolidinyl derivative analyzing the nucleophilic power of the nitrogen atom for the substituents on the aromatic ring of compound **14a-c**, that are: piperidine, morpholine and pyrrolidine; the nitrogen nucleophilicity values, reported in the literature, for the above secondary amines, in acetonitrile, in decreasing order, are: pyrrolidine 18.64, piperidine 17.35, morpholine 15.65.[38]

In agreement with our results, the pyrrolidine is the stronger nucleophilic species among the involved amines and probably this is an indirect explanation for the shorter reaction time in the case of reactions 7-9.

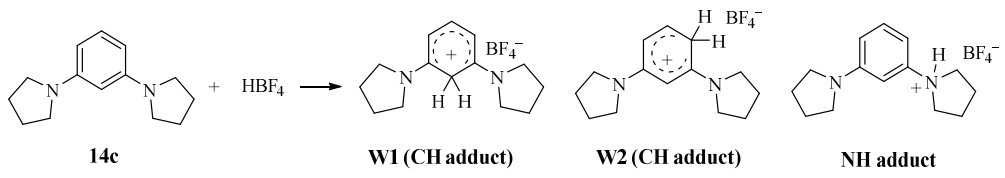
It is interesting to note that the reactions have been carried out with equimolar amount of the reagents and, in the case of diaminobenzene derivatives and aryldiazonium salts, the final products were obtained in high yields (from 73% to 98%), thus indicating that the produced tetrafluoroboric acid in the reaction mixture doesn't react with the nucleophilic reagents producing a salt that might hinder the reaction, but, likely, the proton expelled during the rearomatization process salifies a nitrogen atom of the azo coupling product rather than one belonging to the nucleophilic reagents.

This hypothesis is also supported by the ^{1}H-NMR spectra of the crude reaction mixture, that show in all cases, broad signals low-field shifted with respect to those of the purified compounds, in agreement with a protonation phenomenon, analogous to that observed in past studies of the research group between triaminobenzene and benzofurazan derivatives.[17]

In this context, it has to be noted that there are no data in the literature about the protonation reaction of 1,3-diaminobenzene derivatives, instead, there are a lot of publications about the reactions between triaminobenzene derivatives and different organic and inorganic acids, that report Wheland complexes and/or ammonium salts formation.[16,39,40]

In particular, it has been reported that the protonation of 1,3,5-tris(*N*-pyrrolidinyl)benzene, occurs only on the carbon atom of the aromatic ring, giving the Wheland complex; based on these results, we decided to investigate on the reaction between the 1,3-di(pyrrolidinyl)benzene and tetrafluoroboric acid.

As reported in Scheme 13, from the reaction between **14c** and tetrafluoroboric acid, in principle, is possible to obtain two Wheland complexes (**W1** and **W2**) and one ammonium salts (NH adduct).



14c **W1 (CH adduct)** **W2 (CH adduct)** **NH adduct**

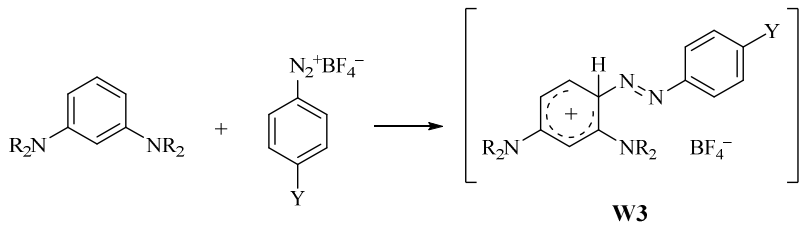
Schema 13. Possible products from the protonation of 1,3-di(pyrrolidinyl)benzene.

The reaction reported in Scheme 13 was carried out mixing equimolar amount of both reagents directly in the NMR spectroscopy tube, in acetonitrile, at room temperature and the recorded ^{1}H-NMR spectrum together with the homonuclear (*g*-COSY) and heteronuclear (*g*-

HSQC) correlation experiments, showed signals in agreement with the formation of two species: the unsymmetric CH adduct (**W2**) and the nitrogen adduct.

This experiment gave evidence of the regioselective formation of a Wheland intermediate in the case of 1,3-diaminobenzene derivatives and, to the best of our knowledge, represents the first instance of a Wheland complex involving diaminobenzene derivatives; these findings suggest further mechanistic investigation on this topic.

Finally, based on the past results of the research group, from the reactions between *sym*-triaminobenzenes and arenediazonium salts at –30 °C, [23] that provided evidence of their respective Wheland complexes, we decided to perform some reactions between the aryldiazonium salts and the diaminobenzene derivatives, to verify if could be possible to detect the Wheland intermediate (**W3**) reported in Scheme 14.



W3

Schema 14 Formation of the **W3** intermediate from the reaction between 1,3-diaminobenzene derivatives and arenediazonium salts.

The reactions were carried out mixing equimolar amount of different electrophile/nucleophile combinations directly in the NMR spectroscopy tube and in different experimental conditions, both in acetonitrile at -30°C and also in dichloromethane at -85°C; in all cases only signals ascribed to the substitution product in saline form was obtained, as a confirmation, again, that the substitution product is a stronger base respect to the diaminobenzene derivative. The above discussed indicates that the Wheland intermediate from the azo coupling reaction, is stable enough to be detected and characterized, only in presence of three strong electron donating groups on the aromatic ring of the nucleophilic species, as in the case of triaminobenzene derivatives. Probably for the diaminobenzene derivatives, the presence of only two dialkylamino groups on the aromatic ring is not enough to stabilize the positive charge of the Wheland intermediate, making this species unstable and difficult to be detected.

1.3.3 Conclusions

The reactions between different *para* substituted benzenediazonium salts and 1,3-diaminobenzene derivatives, performed under mild conditions, gave regioselectively new substitution products, in high yields.

The azo coupling reaction with the 1,3-dimethoxybenzene needed more time and gave the final products in lower yields, with respect to the reactions involving the diaminobenzene derivatives.

The observed unreactivity at room temperature, for the combination between the less electrophilic diazonium salt **2c** and the dimethoxy derivative **14d**, is an experimental confirmation of the reported predictions in the literature.[37]

The electrophile/nucleophile combinations performed directly in the NMR spectroscopy tube with variable temperature experiments, under different experimental conditions, did not gave evidence for the cationic intermediate (Wheland) of the azocopulation reaction involving diaminobenzene derivatives, likely because the intermediate is not enough stable to be detected and immediately evolves towards the substitution product in saline form.

Moreover, for the first time the protonation reaction also for the diaminobenzene derivatives was carried out; in particular the combination of the 1,3-di(pyrrolidinyl)benzene and tetrafluoroboric acid gave evidence of a Wheland intermediate involving a diaminobenzene derivative. These findings deserve a more detailed investigation that will be made in the future.

1.3.4 Experimental section

The ^{1}H and ^{13}C NMR spectra were recorded with a Varian Inova 300, Varian Mercury 400 and Varian Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H NMR) and 100.56, or 150.80 MHz (for ^{13}C NMR), respectively. *J* values are given in Hz. Signal multiplicities were established by DEPT experiments. The ^{19}F NMR spectra were recorded with a Varian Inova 300 and Varian Mercury 400 operating respectively at 282.3 e 376.3 MHz in $CDCl_3$. Chemical shifts were referenced to the solvent (δ =7.26 and 77.0 ppm), for ^{1}H and ^{13}C NMR, respectively, in $CDCl_3$),

ESI-MS spectra were recorded with a WATERS 2Q 4000 instrument. Chromatographic purifications were carried out on silica gel (0.037-0.063 mm, Merck) columns at medium pressure. Thin layer chromatography (TLC) was performed on silica gel 60 on PET foils (Fluka Analytical). Melting points were measured on a Stuart SMP3 apparatus and are uncorrected. Solvents and reagents were commercial materials (Aldrich or Fluka) if not

specified. 1,3-bis(*N*,*N*-dialkylamino)benzene derivatives **14a-c**, were prepared from 1,3-dichlorobenzene (Sigma-Aldrich) with a modification of the reported literature[32,34] methods.

General procedure for the synthesis of compounds 14a:

In a three-necked flask, under nitrogen flow, 0.85 mL of dichlorobenzene ($7.45x10^{-3}$ mol) with 5.9 mL ($8x10^{-2}$ mol) of piperidine, were dissolved in 50 mL of anhydrous THF. Then 30 mL of phenyllithium ($5.7x10^{-2}$ mol) was added dropwise to the reaction mixture. After 24 h, the reaction mixture was allowed to cool to room temperature and was quenched with water. The aqueous phase was extracted three times with diethyl ether and the combined organic phases were dried over magnesium sulfate, and the solvent removed under *vacuo*. The resulting crude products were purified by silica gel column. The 1,3-di(piperidinyl)benzene **14a** was obtained in 56% yield and its spectroscopic data are in agreement with those reported in literature.[34]

General procedure for the synthesis of compounds 14b,c:

Both syntheses require the same procedure and the only difference is the starting amine, that is morpholine (in case of **14b**) or pyrrolidine (in case of **14c**).

In a pressure vessel, 1.37 mL (0.011 mol) of dicholorobenzene and 0.07 mol of the amino derivative, were dissolved in 10 mL of toluene; after addition of 5.4 g of KO*t*-Bu, the pressure vessel was sealed and heated at 160°C. After 4 days, the reaction mixture was allowed to cool to room temperature and was quenched with water. The aqueous phase was extracted three times with dichloromethane and the combined organic phases were dried over magnesium sulfate, and the solvent removed under vacuo. The resulting crude products were purified by silica gel column. 1,3-di(morpholinyl)benzene **14b**[34] and 1,3-di(pyrrolidinyl)benzene **14c**[32], were obtained in 22% and 68% yields, respectively, and their spectroscopic data are in agreement with those reported in the literature.

Reactions between 14a-d and 2a-c. General Procedure:

To a magnetically stirred solution of the nucleophile (0.1 mmol of **14a-d**) dissolved in CH_3CN (5 mL), was added at room temperature the electrophile (0.1 mmol of **2a-c**).

The reactions were monitored by TLC, using different eluents and by ^{1}H-NMR and ^{19}F-NMR analysis of the crude reaction mixtures.

The obtained products were purified by column chromatography on silica gel (FC) and were characterized by usual spectroscopic methods; chemico physical data are reported as follows.

1,1'-(4-((4-Nitrophenyl)diazenyl)-1,3-phenylene)dipiperidine (15): violet solid, 98% yield, m.p. 151.2 °C dec. **^{1}H NMR** (400 MHz, $CDCl_3$, 25°C) δ (ppm): 8.30 (d, *J* = 8.90 Hz, 2H), 7.90 (d, *J* = 8.81 Hz, 2H), 7.80 (d, *J* = 9.36 Hz, 1H), 6.50 (d, *J* = 9.02 Hz, 1H), 6.37 (s, 1H), 3.45-3.38 (m, 4H), 3.31 (t, *J* = 4.65 Hz, 4H), 1.83 (q, *J* = 4.74 Hz, 4H), 1.74-1.63 (m, 8H, three overlapped signals). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 157.3, 155.5 (two overlapped signals), 146.7, 136.6, 124.8, 122.4, 118.8, 108.2, 102.4, 54.5, 48.7, 26.4, 25.5, 24.4, 24.3. **ESI MS (ES^+) m/z:** 394 $[M+H]^+$, 416 $[M+Na]^+$.

1,1'-(4-((4-bromophenyl)diazenyl)-1,3-phenylene)dipiperidine (16): orange/red solid, 97% yield, m.p. 122.4-125.7 °C. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 7.72 (d, *J*= 6.42 Hz, 3H, two overlapped signals), 7.58 (d, *J*= 8.13 Hz, 2H), 6.52 (s, 1H), 6.43 (s, 1H), 3.35 (s, 4H), 3.24 (s, 4H), 1.81 (s, 4H), 1.73-1.67 (m, 4H), 1.67-1.59 (m, 4H).

^{13}C NMR (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 154.8, 154.0, 152.3, 136.5, 132.1, 123.8, 122.8, 118.2, 108.5, 103.6 (CH), 54.6, 49.1, 26.4, 25.5, 24.3, 22.7. **ESI MS (ES^+) m/z:** 427, 429 $[M+H]^+$, 449, 451 $[M+Na]^+$.

1,1'-(4-((4-methoxyphenyl)diazenyl)-1,3-phenylene)dipiperidine (17): orange solid, 78% yield. **^{1}H NMR** (300 MHz, $CDCl_3$, 25°C) δ (ppm): 7.86 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.58-6.45 (m, 2H,two overlapped signals), 3.87 (s, 3H), 3.30 (br. s, 4H), 3.21 (br. s, 4H), 1.88-1.77 (m, 4H), 1.77-1.55 (m, 8H).**^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 160.8, 154.3, 153.0, 147.8, 136.9, 124.0, 117.8, 114.1, 108.7, 104.3, 55.5, 54.6, 49.4, 26.4, 25.6 (two overlapped signals), 24.3. **ESI MS (ES^+) m/z:** 379 $[M+H]^+$, 401 $[M+Na]^+$.

4,4'-(4-((4-nitrophenyl)diazenyl)-1,3-phenylene)dimorpholine (18): dark violet solid, 97% yield, m.p. > 209.3 °C dec. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 8.33 (d, *J* = 8.95 Hz, 2H), 7.89 (d, *J* = 8.91 Hz, 2H), 7.84 (s, 1H), 6.57 (d, *J* = 9.59 Hz, 1H), 6.39 (s, 1H), 3.96 (s, 4H), 3.87 (t; *J* = 5.05, 4H), 3.41-3.32 (m, 8H, two overlapped saignals).

^{13}C NMR (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 156.8, 155.3, 153.8, 147.4, 137.4, 124.8, 122.6, 118.9, 108.3, 102.5, 67.1, 66.5, 53.4, 47.5. **ESI MS (ES^+) m/z:** 398 $[M+H]^+$, 420 $[M+Na]^+$.

4,4'-(4-((4-bromophenyl)diazenyl)-1,3phenylene)dimorpholine (19): orange/red solid, 96% yield, m.p. 142.4-147.5°C. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 7.76 (d, *J* = 9.07 Hz, 1H), 7.69 (d, *J* = 8.92 Hz, 2H), 7.59 (d, *J* = 8.49 Hz, 2H), 6.56 (d, *J* = 8.82 Hz, 1H), 6.43 (s, 1H), 3.95 (s, 4H), 3.87 (t, *J* = 5.05, 4H), 3.35-3.25 (m, 8H, two overlapped signals).**^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 154.4, 152.5, 151.9, 137.2, 132.3,

123.8, 123.7, 118.5, 108.6, 103.2, 67.1, 66.6, 53.4, 47.9. **ESI MS (ES^+) m/z:** 431, 433 $[M+H]^+$, 453, 455 $[M+Na]^+$.

4,4'-(4-((4-methoxyphenyl)diazenyl)-1,3-phenylene)dimorpholine (20): orange solid, 78% yield, m.p. 136.4-138.8 °C. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 7.82 (d, *J* = 8.81 Hz, 2H), 7.71 (d, *J* = 8.89 Hz, 1H), 6.99 (d, *J* = 8.74 Hz, 2H), 6.57 (d, *J* = 8.05 Hz, 1H), 6.47 (s,1H), 3.95 (t, *J* = 4.44, 4H), 3.88-3.86 (m, 7H, two overlapped signals), 3.30-3.26 (m, 8H, two overlapped signals). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 161.1, 153.7, 151.6, 147.6, 137.7, 124.1, 118.1, 114.2, 108.8, 103.6, 67.2, 66.7, 55.5, 53.4, 48.3. **ESI MS (ES^+) m/z:** 383 $[M+H]^+$, 405 $[M+Na]^+$.

1,1'-(4-((4-nitrophenyl)diazenyl)-1,3-phenylene)dipyrrolidine (21): green petroleum solid, 95% yield, m.p. 197.7 °C dec. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 8.24 (d, *J* = 8.26 Hz, 2H), 7.99 (s, 1H), 7.69 (s, 2H), 6.14 (s, 1H), 5.63 (s, 1H), 3.72 (s, 4H), 3.43 (s, 4H), 2.09-2.01 (m, 8H, two overlapped signals). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 158.4, 152.1, 150.5, 145.0, 134.5, 124.8, 121.4, 119.0, 105.5, 94.5, 52.6, 47.8, 25.9, 25.4. **ESI MS (ES^+) m/z:** 366 $[M+H]^+$, 388 $[M+Na]^+$.

1,1'-(4-((4-bromophenyl)diazenyl)-1,3-phenylene)dipyrrolidine (22): orange/red solid, 77% yield, m.p. 189.4- 192.4 °C. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 7.92 (s, 1H), 7.57 (d, *J* = 8.54 Hz, 2H), 7.52 (d, *J* = 8.57 Hz, 2H), 6.10 (s, 1H), 5.7 (s, 1H), 3.69 (t, *J*= 6.35 Hz, 4H), 3.39 (t, *J*= 6.25 Hz, 4H), 2.04-1.98 (m, 8H). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 153.0, 151.2, 149.5, 133.0, 131.8, 123.1, 120.4, 118.5, 104.0, 95.1, 52.5, 47.7, 25.9, 25.4. **ESI MS (ES^+) m/z:** 399, 401 $[M+H]^+$, 421, 423 $[M+Na]^+$.

1,1'-(4-((4-methoxyphenyl)diazenyl)-1,3-phenylene)dipyrrolidine (23): orange/red solid, 73% yield, m.p. 158.2-161.4 °C. **^{1}H NMR** (600 MHz, $CDCl_3$, 25°C) δ (ppm): 7.90 (s, 1H), 7.71 (d, *J* = 8.72 Hz, 2H), 6.96 (d, *J* = 9.08 Hz, 2H), 6.09 (s, 1H), 5.76 (s, 1H), 3.85 (s, 3H), 3.69 (t, *J* = 6.59 Hz, 4H), 3.38 (t, *J* = 6.43 Hz, 4H), 2.03-1.98 (m, 8H).

^{13}C NMR (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 159.2, 150.6, 149.0, 148.3, 132.8, 123.0, 118.3, 114.0, 103.4, 95.5, 55.4, 52.4, 47.6, 25.9, 25.4. **ESI MS (ES^+) m/z:** 351 $[M+H]^+$.

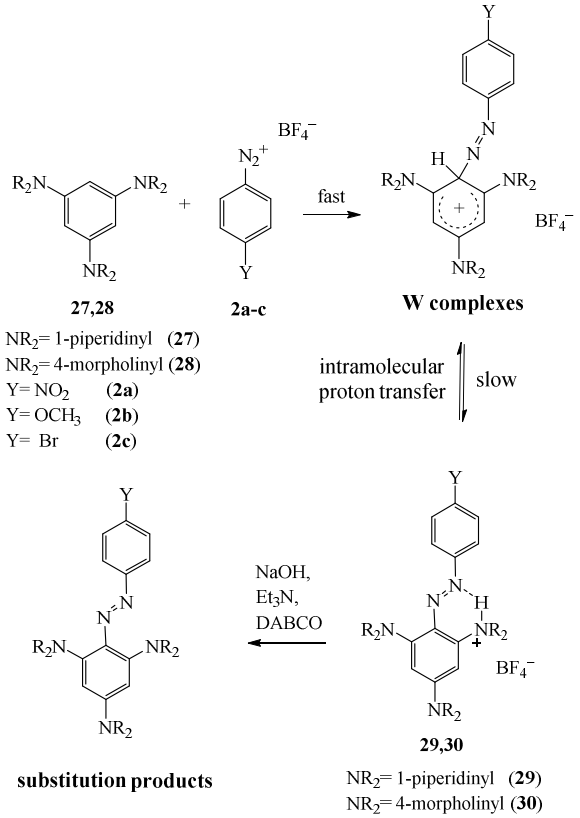
1-(2,4-dimethoxyphenyl)-2-(4-nitrophenyl)diazene (24): orange/red solid, 77% yield, m.p. 192.4 °C. dec. **^{1}H NMR** (400 MHz, CD_3CN, 25°C) δ (ppm): 8.37 (d, *J* = 9.05 Hz, 2H), 7.94 (d, *J* = 9.14 Hz, 2H), 7.78 (d, *J* = 9.18 Hz, 1H), 6.76 (d, *J* = 2.35 Hz, 1H), 6.65 (dd, J_1 = 9.09 Hz, J_2 = 2.53 Hz, 1H), 4.03 (s, 3H), 3.93 (s, 3H). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 165.2, 159.9, 156.5, 147.9, 136.9, 124.7, 123.1, 118.3, 106.2, 98.9, 56.4, 55.7. **ESI MS (ES^+) m/z:** 288 $[M+H]^+$, 310 $[M+Na]^+$.

1-(4-bromophenyl)-2-(2,4-dimethoxyphenyl)diazene (25): yellow solid, 26% yield, m.p. 81.2-84.4 °C. **^{1}H NMR** (400 MHz, $CDCl_3$, 25°C) δ (ppm): 7.78 (d, *J* = 9.09 Hz, 1H), 7.75 (d, *J* = 8.96 Hz, 2H) 7.60 (d, *J* = 8.75 Hz, 2H), 6.59 (d, *J* = 2.42 Hz, 1H), 6.55 (dd, J_1 = 8.83 Hz, J_2 = 2.42 Hz, 1H), 4.02 (s, 3H), 3.89 (s, 3H). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25°C) δ (ppm): 164.1, 159.0, 151.9, 136.7, 132.2, 124.2, 124.1, 118.2, 105.8, 99.0, 56.4, 55.7. **ESI MS (ES^+) m/z:** 321 $[M+H]^+$, 323 $[M+H]^+$, 343 $[M+Na]^+$, 345 $[M+Na]^+$, 359 $[M+K]^+$, 361 $[M+K]^+$.

1.4 NEW BENZIMIDAZOLE DERIVATIVES BY RING CLOSURE OF AZOCOMPOUNDS DERIVED FROM 1,3,5-TRIS(DIALKYLAMINO)BENZENES AND ARYLDIAZONIUM SALTS

1.4.1 Introduction

In the past, the coupling between arenediazonium tetrafluoroborate salts **2** and 1,3,5-tris(dialkylamino)benzenes **27** and **28**, allowed the research group to obtain and characterize the first Wheland intermediates of the azo-coupling reaction[23] (Scheme 15).



Scheme 15. Reactions between 1,3,5-tris(*N*,*N*-dialkylamino)benzenes and arenediazonium salts.

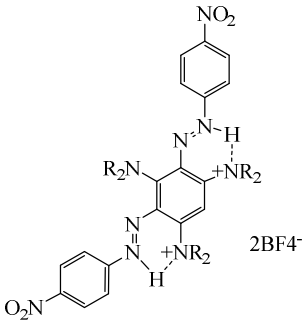
The reactions were carried out directly in the NMR spectroscopy tube, in CD_3CN solution containing an equimolar amount of reagents and a spectrum consistent with the Wheland intermediate was recorded. The σ–complexes resulted to be stable enough to be detected and characterized and they spontaneously produced, in high yields, their salts (**29** and **30**), as reported in Scheme 15.

Owing to the relative stability of the Wheland intermediates, a kinetic study of the separate reaction steps was carried outand gave evidence of the reversibility of the azo-coupling reaction,[25] also confirmed by experiments showing that **W** complexes can undergo the

exchange of the electrophilic part, the less powerful electrophile being replaced by the more powerful one.[24]

The obtained results showed that, opposite to the conventional mechanism of the aromatic substitution reactions, the proton expulsion to obtain the final product (rearomatization process), is the rate-determining step of the reaction and that the two steps are reversible processes.[24,25]

During that study, an interesting behaviour was observed performing the reaction between the 4-nitrobenzenediazonium salt **2a** and compound **27** that gave the double attack product **31**, reported in Figure 8.



31

Figure 8. Double attack product from the reaction between **27** and the 4-nitrobenzenediazonium tetrafluoroborate **2a**.

The new di-cationic species was obtained only when an electron-withdrawing group (*i.e.* NO_2), was present on the diazonium salt.[24]

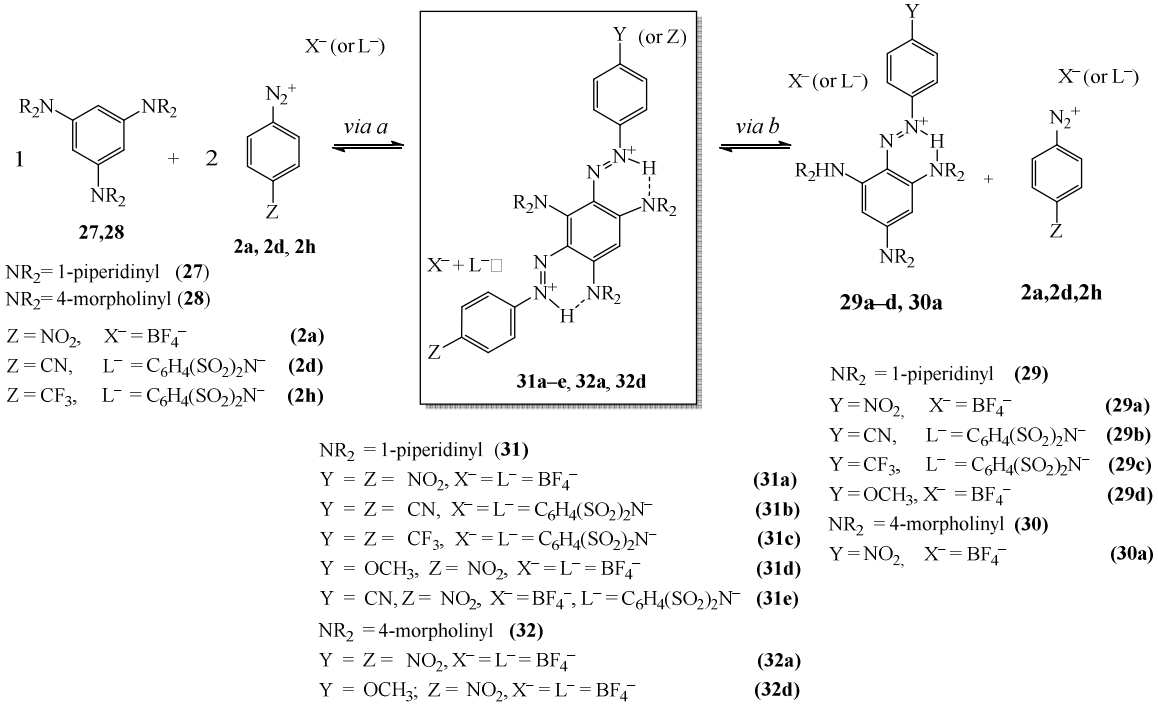
After this result, we decided to perform a more detailed investigation on the reactions between benzenediazonium salts bearing electron withdrawing groups in position 4, and triaminobenzene derivatives. From this study new benzimidazole derivatives containing the *N*-piperidinyl or *N*-morpholinyl moiety as fused ring, were obtained; it was an interesting result because benzimidazoles are versatile compounds used in agro-alimentary, pharmaceutical, textile, and cosmetic industries,[41,42] and their synthesis covers a lot of literature reports.[43–45]

Different examples of synthesis of benzimidazoles from azo compounds have been reported so far: Price reported[46] a cyclization reaction of azo compounds, in the presence of $CoCl_2$, to obtain benzimidazole derivatives. A similar reaction, by using acids as catalyst, was reported by Meth-Cohn and Suschitzky.[47]

Herein I will discuss the observed ring-closure reaction to new benzimidazoles from the azo-coupling of benzenediazonium salts and *sym*-triaminobenzene derivatives.

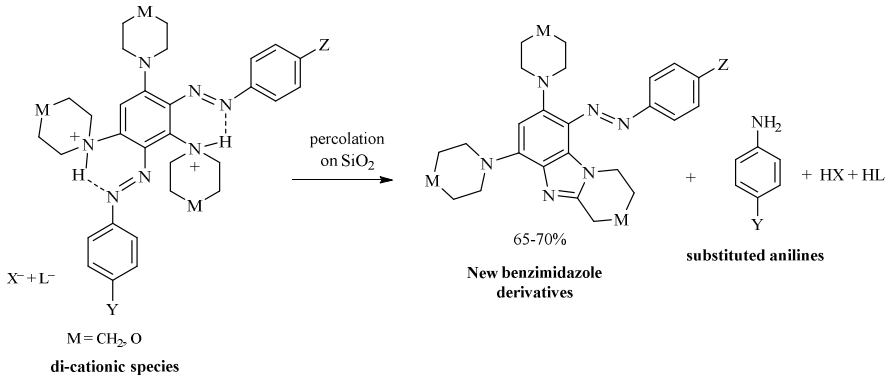
1.4.2. Results and Discussion

sym-triaminobenzenes **27** or **28** (Scheme 16) and diazonium salts **2a**, **2d** and **2h**, bearing electron-withdrawing groups (namely 4-NO_2, 4-CN, and 4-CF_3, respectively) in 1:2 relative molar ratio, quickly afford the di-cationic species **31a-c** and **32a,d**, according to Scheme 16 (*via a*). These species usually precipitated from the reaction mixture and were isolated as coral-red solids. Compounds **31a-c** and **32a,d** can be also obtained from the reaction between the mono-adduct **29a-d** or **30a** and a further amount of aryldiazonium salt (Scheme 16, *via b*).



Scheme 16. Formation of diprotonated species by reaction between triaminobenzenes **27** and **28** and diazonium salts **2**.

Attempts to obtain the corresponding free bases of the di-cationic species (with simple workup or by solubilization in usual organic solvents), produced relevant amounts of the substituted anilines **35a-c** and of compounds **33a-c** and **34a** (Scheme 17) which are new benzimidazole derivatives.



Scheme 17. Formation of new benzimidazole derivatives from di-cationic species **31** and **32**.

Compounds **33a-c** and **34a** were also isolated by percolation of compounds **31a-c** and **32a** on silica gel column and their structure were confirmed by usual spectroscopic methods.

Obviously, to obtain the benzimidazoles **33** and **34**, it is necessary the cleavage of the N=N double bond of the di-cationic species (**31** and **32**) and the subsequent formation of a C=N new double bond involving one of the α carbon atoms of the cyclic amino substituents of the starting di-cationic species **31** or **32**.

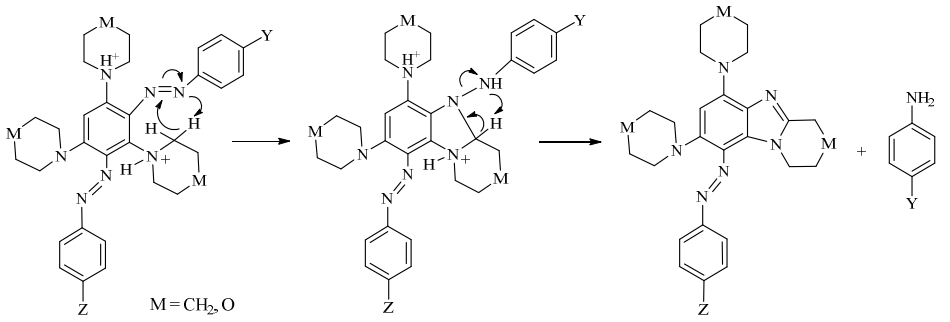
The observed cyclization to obtain benzimidazoles from azo-compounds, with an aromatic ring bears a cyclic amine and an azo group in adjacent position, reminds a process in which the "tert-amino effect" is operative.[39,47-50]

Meth-Cohn and Suschizky coined the term in 1972[47] to generalize cyclization reactions of some tertiary anilines with double bonds in *ortho*-position.

The cyclization proceeds with formation of a new bond to afford a five or six membered fused-ring system and represents a convenient method for the synthesis of a number of nitrogen-containing heterocycles otherwise difficult to obtain. The first instance of this cyclization was reported in 1895[51] when 1,2-dimethylbenzimidazole was unexpectely obtained by prolonged reflux of *o*-aminodimethylaniline in acetic anhydride.

The formation of benzimidazole derivatives from azo compounds has been reported in a few cases[46,47,52] starting from *N*,*N*-dialkylamino *ortho*-substituted azobenzenes: also in these cases the *tert*-amino effect operates.

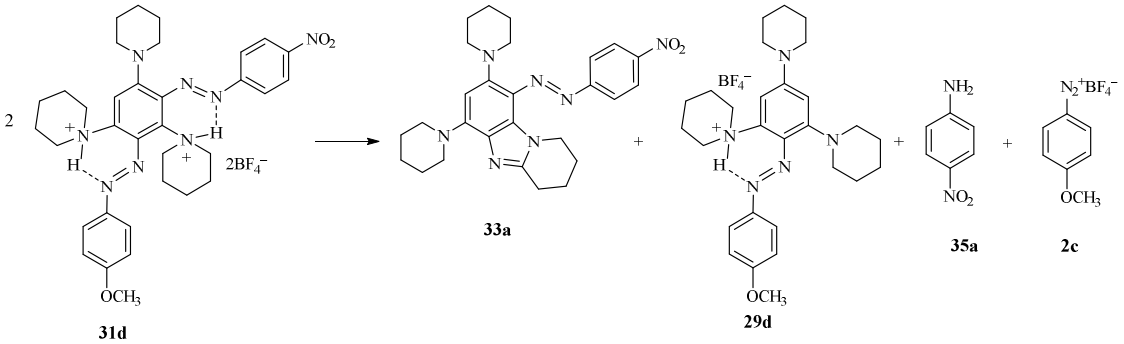
In this context, formation of benzimidazoles from azoderivatives **31** and **32** represents a further example of this cyclization, and a possible reaction pathway involving or proton transfers or internal (intramolecular) salification is depicted in Scheme 18; the cleavage of the N=N double bond is enhanced by the presence of both ammonium ions proximal to the involved diazo group.



Scheme 18. Proposed mechanism for the formation of benzimidazoles **33** and **34**.

Meth-Cohn reported the mechanism of the cyclisation of *N*-(*o*-acylaminophenyl)pyrrolidine by peroxy-acid catalysis, involving the formation of N-oxide species.[53] In our case, the acid catalysis acts favouring the C-H bond cleavage to form the new C-N bond.

In the case of the mixed di-cationic species **31d**, **31e**, and **32d** the behaviour of the reaction is complicated by the presence, in the reaction mixture, of different compounds, as indicated in Schemes 19 and 21.



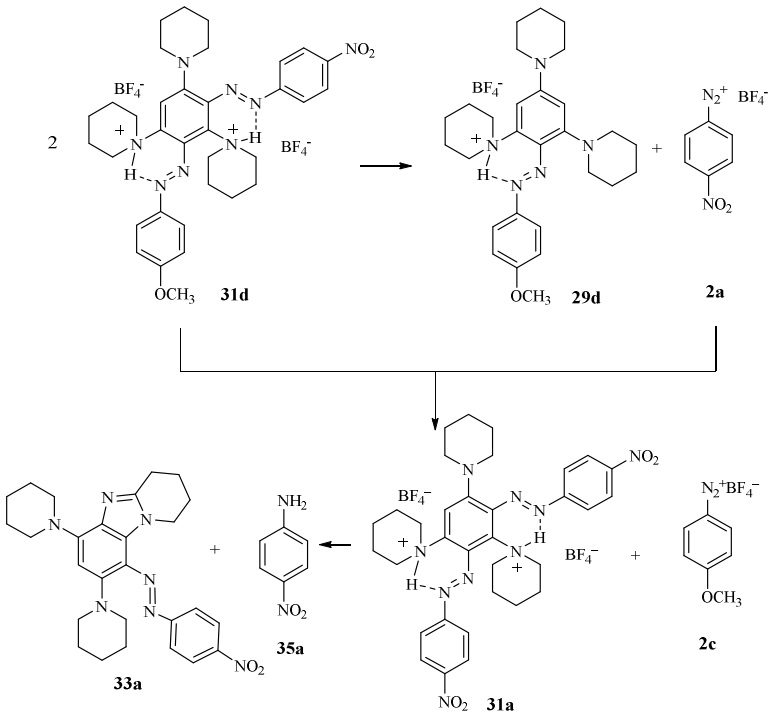
Scheme 19. Evolution of compound **31d**, bearing two groups of different electronic ability, and products observed in the reaction mixture.

Scheme 19 concerns the effect of two groups of different electronic ability on the starting compound **31d**; a strong electron-withdrawing group (NO_2) and a strong electron-releasing group (OCH_3); the reaction product **33a** contains the azo moiety bearing the nitro group.

The ^{1}H NMR spectrum of the crude reaction mixture showed presence of **33a** in yield not exceeding 50% and the remaining percentage includes the mono-cationic species **29d**, together with *p*-nitroaniline **35a**, and also compound **2c**.

The reaction in Scheme 19 can be considered as an indirect evidence of the reversibility of the azo-coupling reaction[25,39]; in the present case the obtained benzimidazole bears the electron-withdrawing group.

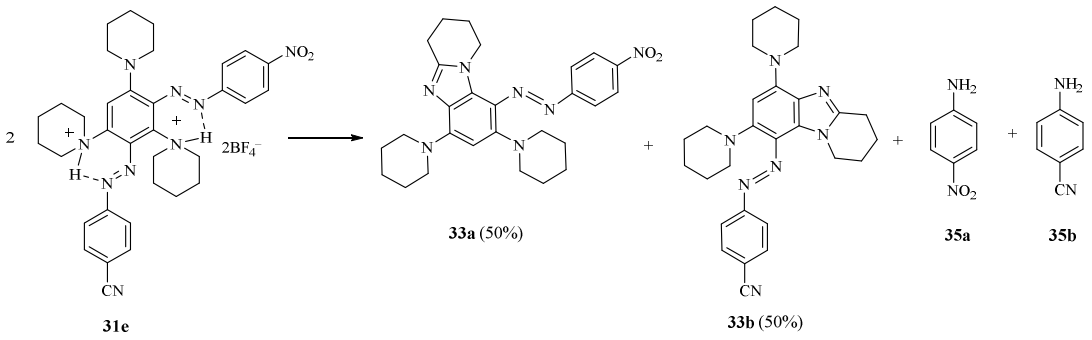
Scheme 20 shows a reasonable mechanistic pathway for the reaction in Scheme 19, consistent with previously reported observations.[25,39]



Scheme 20. Proposed pathway for the reaction shown in Scheme 19.

As a result of the reversibility of the azo-coupling reaction, the *p*-nitrobenzendiazonium salt **2a** is expelled from **31d** and then reacts with a second molecule of **31d** to replace its *p*-methoxybenzenediazo moiety, thus producing **2c** and **31a**, which is the precursor of **33a**.

If two different electron-withdrawing groups are both bound to the benzenediazonium salt moiety, as in the case of compound **31e**, in the reaction mixture both imidazole derivatives **33a** and **33b** were present in 1:1 relative amount together with the respective released substituted anilines **35a** and **35b**, as reported in Scheme 21.



Scheme 21. Evolution of compound **31e**, bearing two different electron-withdrawing groups.

The previous discussion highlights the importance of an electron-withdrawing group, both in the leaving aniline and in the remaining diazo moiety, to obtain benzimidazole derivatives.

An electron-withdrawing group on the leaving aniline favours the N-N bond cleavage, supporting the departure of the substituted aniline.

In conclusion, this means that the presence of two electron-withdrawing groups on both azo moieties (**Z** and **Y** in Scheme 18) it is crucial to obtain the ring closure reaction.

1.4.3 Conclusions

The reactions between equimolar amount of triaminobenzene derivatives **27** or **28** and *p*-substituted benzenediazonium salts, bearing substituents with different electronic effects, gave the salts of the diazo compound deriving from the attack of the neutral carbon atom of the nucleophile to the electrophile.

If additional amount of the same (or a different) benzenediazonium salt is added to the former, a di-cationic species can be obtained and recovered by filtration from the crude reaction mixture; this behaviour was also observed when the reactions were carried out in a 2:1 relative molar ratio between the nucleophile and the electrophilic species.

When the di-cationic species bears electron-withdrawing groups on the diazonium moiety, new benzimidazole derivatives can be isolated after workup or percolation on silica gel column.

The formation of the new benzimidazole derivatives it is a confirmation of the reversibility of the azo coupling reaction, owing to the ability of the more reactive electrophilic diazonium salt (bearing electron-withdrawing group, such as nitro group) to replace the less powerful electrophilic diazonium salt, bearing electron donor groups (*e.g.* *p*-methoxy group).

Part of this chapter is reproduced with permission from "The Journal of Organic Chemistry". Further data can be found in the paper " E. Del Vecchio, C. Boga, L. Forlani, S. Tozzi, G. Micheletti, S. Cino, *J. Org. Chem.*, **2015**, *80*, 2216-2222".

1.4.4 Experimental section

The ^{1}H and ^{13}C NMR spectra were recorded with a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H NMR) and 100.56, or 150.80 MHz (for ^{13}C NMR), respectively. *J* values are given in hertz (Hz). Signal multiplicities were established by DEPT experiments. Chemical shifts were referenced to the solvent [(δ =7.26 and 77.0 ppm for $CDCl_3$), (δ =2.0 and 118.20 ppm for CD_3CN), (δ =4.3 and 57.3 ppm for CD_3NO_2) for ^{1}H and ^{13}C NMR, respectively]. Chromatographic purifications were carried out on silica gel or aluminum oxide (activated, basic, Brockmann I, standard grade ca. 150 mesh) columns at medium pressure. MS spectra were recorded with a MAT 95 XP instrument. 1,3,5-tris(dialkylamino)benzenes **27** and **28** were prepared as described previously by the research group.[23] The arenediazonium tetrafluoroborate salts **2a** and **2c** were commercially available, 4-cyanobenzenediazonium benzo[d][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide (**2d)** and 4-(trifluoromethyl)benzenediazonium benzo[d][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide (**2h)** were prepared as reported in ref.21. Compounds **29a**, **29d**, **30a**, **30d**, **31a**, **31d**, **32a**, and **32d** were prepared as described previously[23,24] and their spectral data agree with those previously reported.

General procedure for the synthesis of compounds 29 and 30:

1,3,5-Tris(dialkylamino)benzene was dissolved in CH_3CN (2 mL) and cooled to -30 °C; then the arenediazonium salt was added, in equimolar amount. Immediately after mixing, the solution became yellow and was stirred for 20 min; in this interval the color turned to red. TLC analysis (eluent: light petroleum/diethyl ether, 50:50) showed the disappearance of the starting 1,3,5-tris(dialkylamino)benzene. After removal of the solvent *in vacuo*, the crude product was dissolved in CH_2Cl_2 (2 mL) and adding Et_2O precipitated compounds **29** and **30**. The products were isolated as dark-red solids in 80-90% yield and, except **29b** and **29c**, crystallized from CH_2Cl_2 and *n*-hexane. Chemico-physical data for compounds **29a**, **29d**, **30a** and **30d** agree with those previously reported.[23]

1-(2-((4-cyanophenyl)diazenyl)-3,5-di(piperidin-1-yl)phenyl)piperidin-1-iumbenzo[d][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide (**29b**): red solid, 90% yield. **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 1.40-2.00 (m, 18 H), 2.90-3.05 (m, 2 H), 3.40-3.50 (m, 2 H), 3.61 (m, 4 H), 3.81 (m, 4 H), 5.72 (d, 1 H, *J* = 2 Hz), 6.18 (d, 1 H, *J* = 2Hz), 7.34 (d, 2 H, *J* = 8.8 Hz), 7.50-7.58 (m, 2 H), 7.60 (d, 2 H, *J* = 8.4 Hz), 7.70-7.77 (m, 2 H), 11.94 (bs, 1 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25 °C) δ (ppm): 23.4, 23.9, 24.1, 25.7, 26.2, 26.4, 50.3, 51.4, 52.0, 91.2, 98.9, 106.3, 115.2, 119.0, 121.0, 128.7, 131.8, 133.8, 142.6, 145.6, 151.6, 159.3, 159.7.

1-(2-((4-trifluoromethylphenyl)diazenyl)-3,5-di(piperidin-1-yl)phenyl)piperidin-1-ium benzo[d][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide (**29c**): dark-red solid, 45% yield, m.p. 167-168 °C. **^{1}H NMR** ($CDCl_3$, 300 MHz, 25 °C) δ (ppm): 1.50-2.00 (m, 18 H), 2.93-3.07 (m, 2 H), 3.36.3.46 (m, 2 H), 3.57-3-67 (m, 4 H), 3.76-3.89 (m, 4 H), 5.75 (d, 1 H, *J* = 2.40 Hz), 6.23 (d, 1 H, *J* = 2.40 Hz), 7.34 (br.d, 2 H, *J* = 8.48 Hz), 7.54 (dd, *J* = 6.13 Hz, *J* = 3.20 Hz, 2 H), 7.34 (br.d, 2 H, *J* = 8.48 Hz), 7.76 (dd, *J* = 6.13 Hz, *J* = 3.20 Hz, 2 H), 12.06 (s, 1 H). **^{13}C NMR** ($CDCl_3$, 75.5 MHz, 25 °C) δ (ppm): 23.4, 23.9, 24.1, 25.8, 26.2, 26.4, 50.2, 51.5, 52.1, 91.4, 98.9, 114.8, 120.9, 127.6, 127.0, 142.8, 144.8, 151.7, 159.1, 159.8.

General procedure for the synthesis of compounds 31 and 32:

To a magnetically stirred solution (0.092 mmol in 2 mL) of **27** (or **28**) in acetonitrile, cooled at -30 °C, the arenediazonium salt **2** (0.184 mmol) was added. Immediately after mixing, the color of the mixture solution became yellow. After 20 min a coral-red solid precipitated. After filtration compound **31** (or **32**, tile-red solid) was isolated as coral red solid in 80-90% yield. Compounds **31** and **32** can be obtained also by addition of an equimolar amount of diazonium salt **2** to a cooled (-30 °C) solution in acetonitrile of compound **29** or **30**, respectively. Compound **31c** did not precipitated and was not isolated but the reaction mixture obtained after addition of 2 equiv of **30c** to 1 equiv of **27** was subjected to column chromatography to give benzimidazole derivative **33c**. Chemico-physical data for compounds **31a** and **32a** agree with those previously reported.[40]

1,1'-{2,4-Bis[(4-cyanophenyl)diazenyl]-5-piperidin-1-yl-1,3-phenylene}dipiperidinium di(benzo[d][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide) (31b): 80% yield. **^{1}H NMR** (CD_3NO_2, 600 MHz, -30 °C) δ (ppm): 1.55-2.50 (m, 18 H), 3.45-4.70 (m, 12 H), 6.45 (br s, 1 H), 7.55 (d, 4 H, *J* = 8.1 Hz), 7.70-7.77 (m, 12 H), 10.31 (br s, 2 H). **^{13}C NMR** (CD_3NO_2,

100.56 MHz, -30 °C) δ (ppm): 18.4, 18.9, 20.9 (2C), 22.4, 23.0, 45.6, 49.3, 54.9, 89.4, 103.4, 111.9, 114.7, 116.6, 121.4, 124.1, 128.6, 129.6, 137.3, 140.4, 150.9, 157.5.

Preparation of Compounds 31d, **31e**, and **32d**. To a solution of salt **29b** (or **29d**, or **30a**) (0.074 mmol in 2 mL of CH_3CN), cooled at -30 °C, was added 0.0176 g (0.074 mmol) of 4-nitrobenzenediazonium tetrafluoborate (**2a**). Immediately the solution became yellow. After magnetic stirring for 20 min the color turned orange-red. After removal of the solvent in vacuo, the crude product **31e** was characterized by ^{1}H and ^{13}C NMR and subjected to column chromatography without further purification. Compounds **31d** and **32d** were dissolved in 2 mL of CH_2Cl_2 and precipitated (80-90%) by adding Et_2O then crystallized from CH_2Cl_2 and *n*-hexane. Chemico-physical data for compounds **31d** and **32d** agree with those previously reported.[43]

1,1'-{4-[(4-Cyanophenyl)diazenyl]-2-[(4-nitrophenyl)diazenyl]-5-piperidin-1-yl-1,3-phenylene}dipiperidinium tetrafluoroborate (benzo[d][1,3,2]dithiazol-2-ide 1,1,3,3-tetraoxide) (31e): orange solid, 60% yield. **^{1}H NMR** (CD_3NO_2 600 MHz, -28 °C) δ (ppm): 1.55-2.45 (m, 18 H), 3.00-4.70 (m, 12 H), 6.45 (s, 1 H), 7.53-7.64 (m, 4 H), 7.70-7.76 (m, 6 H), 8.23 (br.d, 2 H, *J* = 7.9 Hz), 10.2 (bs, 1 H), 10.3 (bs, 1 H) ppm. **^{13}C NMR** (CD_3NO_2, ref at 62.95 ppm, 150 MHz, -28°C) δ (ppm): 24.3, 24.4, 26.4, 28.1, 28.6 (2C), 51.2, 55.0, 60.6, 93.5, 117.2, 117.5, 119.4, 120.1, 122.1, 126.8, 127.6, 134.0, 135.2, 143.0, 145.6, 145.8, 147.5, 156.4, 163.1.

General procedure for the synthesis of compounds 33a-c and 34a:

Compound **27** or **28** (0.092 mmol) was dissolved in acetonitrile (2 mL). The solution was cooled at -30 °C then the arenediazonium salt **2** (0.184 mmol) was added. Immediately the solution became yellow and after magnetic stirring for 20 min the color turned orange-red. After removal of the solvent, the crude residue was treated with water, extracted with dichloromethane (3 x 1 mL) and subjected to chromatography on silica gel (diethyl ether/light petroleum or ethyl acetate-hexane: 7/3). It is possible to isolate compounds **33** and **34** also by percolation of **31** and **32** on silica gel column. Compounds **33** and **34**, dark-purple in color, were unstable to the usual crystallization techniques. Compounds **35** are also recovered and their spectral data agree with those of authentic commercial samples.

9-((4-nitrophenyl)diazenyl)-6,8-di(piperidin-1-yl)-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-*a*]pyridine (33a): 76% yield. **^{1}H NMR** ($CDCl_3$, 600 MHz, 25 °C) δ (ppm): 1.20-2.10 (m, 18 H), 3.00-3.20 (m, 6 H), 3.88-4.01 (m, 4 H), 4.42-4.52 (m, 2 H), 6.13 (s, 1 H), 7.73 (d, 2 H, *J* = 9.4 Hz), 8.28 (d, 2 H, *J* = 9.4 Hz) ppm. **^{13}C NMR** ($CDCl_3$, 75.5MHz, 25 °C)

δ (ppm): 20.4, 23.8, 24.3, 24.7, 26.0 (2C), 26.1, 48.4, 50.4, 54.1, 97.2, 121.1, 124.9, 127.2, 127.9, 136.9, 144.4, 145.2, 147.7, 148.0, 159.2 ppm. MS (EI, 70 eV): *m/z* (%): 487(0.2, M^+), 349 (100), 266 (52), 138 (26). HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for [M+H] $C_{27}H_{34}N_7O_2$, 488.2774; found, 488.2774.

4-((6,8-Di(piperidin-1-yl)-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-*a*]pyridin-9-yl)diazenyl)benzonitrile (33b): 79% yield. **^{1}H NMR** ($CDCl_3$, 600 MHz, -30 °C) δ (ppm): 1.50-2.40 (m, 18 H), 3.04-3.13 (m, 6 H), 3.80-3.90 (m, 4 H), 4.41-4.48 (m, 2 H), 6.16 (s, 1 H), 7.70 (d, 2 H, *J* = 8.44), 7.74 (d, 2 H, *J* = 8.44) ppm. **^{13}C NMR** ($CDCl_3$, 150 MHz, -25 °C) δ (ppm): 20.1, 22.8, 23.6, 24.1, 24.5, 25.8, 26.0, 48.3, 50.3, 53.8, 97.8, 108.6, 119.9, 121.7, 126.5, 128.7, 133.1, 135.7, 142.0, 147.5, 148.8, 159.2 ppm. MS (EI, 70 eV): m/z (%): 467 (3, M^+), 350 (100), 266 (37), 175 v(11), 118 (32). HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for [M+H] $C_{28}H_{34}N_7$, 468.28757; found, 468.2876.

6,8-Di(piperidin-1-yl)-9-((4-(trifluoromethyl)phenyl)diazenyl)-1,2,3,4-tetrahydrobenzo [4,5]imidazo[1,2-a]pyridine (33c): 38% yield. **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 1.50-2.20 (m, 18 H), 3.03-3.23 (m, 6 H), 3.78-3.90 (m, 4 H), 4.40-4.52 (m, 2 H), 6.22 (s, 1 H), 7.68 (d, 2 H, *J* = 8.30), 7.79 (d, 2 H, *J* = 8.30) ppm. **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25 °C) δ (ppm): 20.4, 23.8, 24.3, 24.7, 25.9, 26.1, 26.2, 48.3, 50.5, 54.4, 98.2, 121.4, 124.4 (q, J_{CF} = 127.0 Hz), 126.0 (q, J_{CF} = 3.8 Hz), 126.9, 128.4 (q, J_{CF} = 32.5 Hz), 129.5, 136.2, 143.1, 146.8, 148.2, 157.0 ppm. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for [M+H] $C_{28}H_{34}F_3N_6$, 511.27971; found, 511.2797.

7,9-dimorpholino-6-((4-nitrophenyl)diazenyl)-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c] [1,4]oxazine (34a): 70% yield. **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 3.19-3.24 (m, 4 H), 3.88-4.01 (m, 12 H), 4.13 (t, *J* = 5.25 Hz, 2 H), 4.54 (t, *J* = 5.25 Hz, 2 H), 5.02 (s, 2 H), 6.17 (s, 1 H), 7.74 (d, 2 H, *J* = 9.10), 8.33 (d, 2 H, *J* = 9.10) ppm. **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25 °C) δ (ppm): 47.6, 49.2, 53.2, 64.5, 65.8, 66.8, 67.0, 97.2, 121.5, 125.0, 127.3, 129.4, 136.2, 143.1, 145.0, 144.0, 147.2, 158.3 ppm. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for [M+H] $C_{24}H_{28}N_7O_5$, 494.21519; found, 494.2152.

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CHAPTER 2

S_EAr/S_NAr reactions between aromatic and heteroaromatic neutral substrates

Benzofurazan and benzofuroxan derivatives are an important class of heterocyclic compounds that possess interesting properties for different applications in many theoretical and applied fields. In particular they exhibit a broad spectrum of biological activity including antibacterial, antifungal, antileukemic, acaricide and others.[1-5]

Both heterocyclic derivatives found also application as dyes, fluorescent biosensors and in the field of the high energy materials.[6]

So it should be really interesting the synthesis of new heterocycles containing this organic scaffold.

In this Chapter I report results concerning the reactions between **DNBF** and 7-chloro-4,6-dinitrobenzofuroxan with different nucleophilic species. The aim of this study was to synthesize new substitution products for different applications and when possible to detect new intermediates of the aromatic substitution reaction. Moreover, in the last part of this Chapter, also findings on the reactivity of some isomeric chloronitrobenzofurazanes towards 1,3-bis(*N*,*N*-dialkylamino)benzene derivatives will be reported.

2.1 REACTIONS BETWEEN 4,6-DINITROBENZOFUROXAN DERIVATIVES AND TRISUBSTITUTED ARENES

2.1.1 Introduction

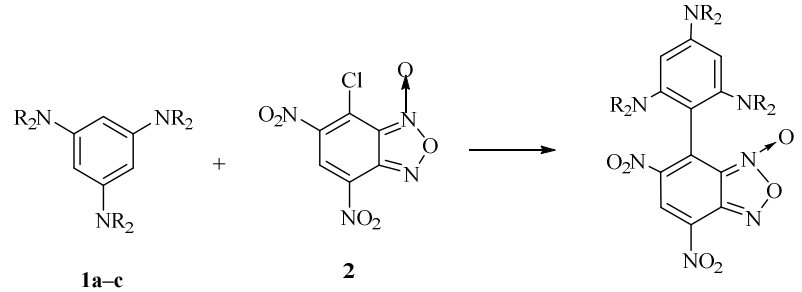
In the Introduction of this thesis I introduced 4,6-dinitrobenzofuroxan (**DNBF**), as a strong electrophile or "superelectrophile";[7,8] in the past its combination with different nucleophilic species, including triaminobenzene derivatives gave stable or relatively stable σ–complexes of the aromatic substitution reaction.[9-12]

7-Chloro-4,6-dinitrobenzofuroxan is also an interesting electrophilic species, and it is known that it reacts with a variety of weak or very weak nucleophiles as water, alcohols, amines,[13,14] and even with the poorly nucleophilic 2,4,6-trinitroaniline,[15,16] giving interesting compounds for different applications. In this study we decided to investigate the

combinations between the above introduced benzofuroxan derivatives and different trisubstituted benzene derivatives and the obtained results will be reported and discussed.

2.1.2 Results and Discussion

First, I have considered the reactions between triaminobenzene derivatives **1a-c** and 7-chloro-4,6-dinitrobenzofuroxan (**2**), reported in Scheme1.



1a–c **2** **3a-c**

Cl-DNBF

NR_2 = piperidinyl (**1a**)
NR_2 = morpholinyl (**1b**)
NR_2 = pyrrolidinyl (**1c**)

NR_2 = piperidinyl (**3a**) 85%
NR_2 = morpholinyl (**3b**) 85%
NR_2 = pyrrolidinyl (**3c**)

Scheme 1. Reactions between 1,3,5-triaminobenzene derivatives and 7-chloro-4,6-dinitrobenzofuroxan.

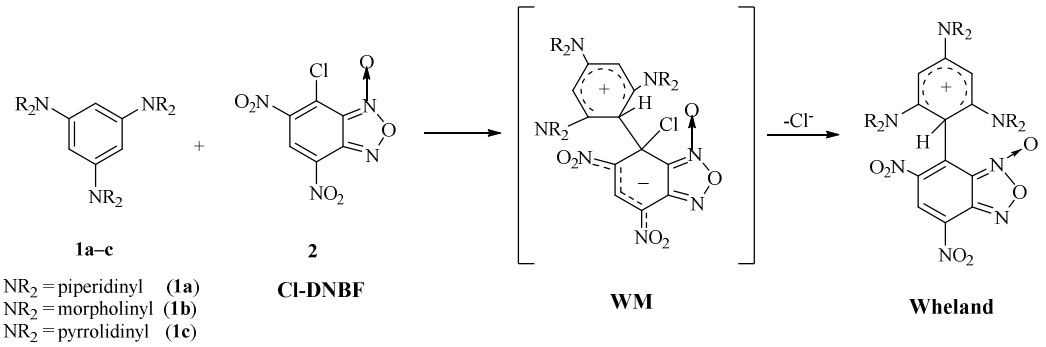
The reactions were carried out mixing equimolar amount of reagents, in chloroform, at room temperature and in presence of a base to neutralize the formation of hydrochloric acid during the reaction progress. In particular, when the reactions were carried out in presence of $NaHCO_3$, the products **3a** and **3b** were isolated after purification on silica gel column in 85% yield. Instead, when basic Al_2O_3 was added to the reaction mixture, the new substitution products **3a** and **3b** were obtained in lower yields respect to those obtained using sodium bicarbonate as the base.

In the case of the reaction between **1c** and **2**, it was no possible to isolate the final product **3c**, due to the presence of numerous compounds in the reaction mixture, probably as a consequence of the very high reactivity of the pyrrolidinyl derivative. Compounds **3a** and 3**b** were characterized by usual spectroscopic methods

In the past the coupling between **1a-c** and 4,6-dinitrobenzofuroxan (**DNBF**) allowed the research group to obtain the first Wheland-Meisenheimer complexes of both the electrophilic and nucleophilic aromatic substitution reactions,[9,17] that were detected and characterized by NMR at low temperature.

Based on these results, the reaction between **1b** and **2** was also performed directly in the NMR spectroscopy tube, mixing equimolar amount of reagents at -75°C in CD_2Cl_2, with the aim to check whether it was possible to observe the intermediates of this aromatic substitution reaction. Even if we were conscious that, in the case of 7-chloro-4,6-

dinitrobenzofuroxan the presence of the chlorine atom, as good leaving group, makes the possibility to detect the **WM** intermediate (**WM** in Scheme 2) a very hard goal, our intent was to try to obtain evidence, at least of the Wheland intermediate (shown in Scheme 2) thanks to the ability of the amino groups on the moiety deriving from the nucleophile, to stabilize the positive charge on this intermediate (Scheme 2).



Scheme 2. Possible intermediates (**WM** and Wheland) from the reactions between **1a-c** and **2**.

In spite of our expectation, we observed only the signals of the salt of compound **3b**, which spectral data were in agreement with the structure bearing the proton bound to the nitrogen atom of the morpholinyl substituent situated in *para* position respect to the attack position of the electrophile (**3bH** in Figure 1).

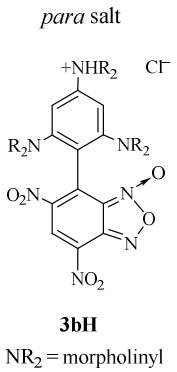
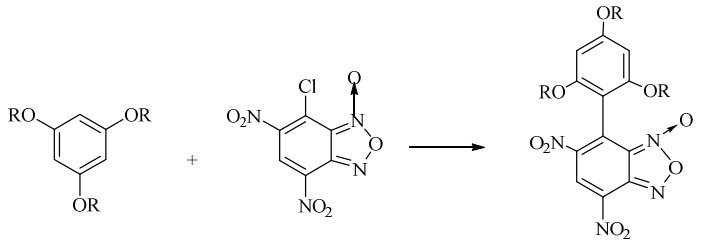


Figure 1. Salt derived from the N-protonation of compound **3b**

The formation of the salt **3bH** was also obtained perfoming the reaction in equimolar amount of reagents, without a base, at room temperature, using greater amount of reagents with respect to the reaction carried out in the NMR tube, in order to characterize the salt also by ^{13}C-NMR spectroscopy.

To extend the study, we decided to carry out also the reactions between 1,3,5-trimethoxybenzene (**4a**) or 1,3,5-trihydroxybenzene (**4b**), and the electrophilic species Cl-DNBF (**2**) (Scheme 3) and 4,6-dinitrobenzofuroxan (**6**) (Scheme 4).

The reactions with Cl-DNBF (**2**) were carried out mixing equimolar amount of reagents, in acetonitrile at 25°C, and under these experimental conditions the new substitution products **5a,b** were obtained (Scheme 3) in good yields, after purification on silica gel column.



4a-b

R = CH_3 (**4a**)
R = H (**4b**)

2

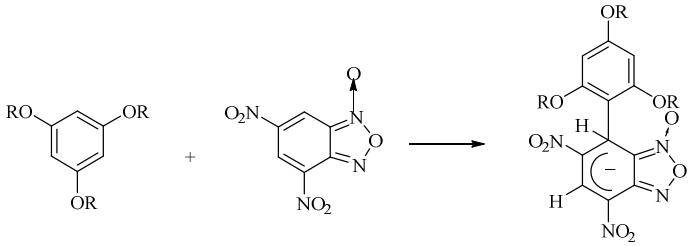
Cl-DNBF

5a-b

R = CH_3 (**5a**) 70%
R = H (**5b**) 54%

Scheme 3. Reactions between **4a** and **4b** and 7-chloro-4,6-dinitrobenzofuroxan.

Considering that **WM** intermediates involving trihydroxy or trimethoxybenzene have never been reported, in contrast to what we obtained from the reaction between **DNBF** and triaminobenzene derivatives, we decided to perform the reactions between **4a,b** and **DNBF** (**6**) directly in the NMR spectroscopy tube, at low temperature (-30°C in CD_3CN), in order to see whether new σ-intermediates were detectable. In both cases, stable Meisenheimer complexes (**M1** and **M2** in Scheme 4), were detected and fully characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT and *g*-HSQC experiments.



4a–c

R = CH_3 (**4a**)
R = H (**4b**)

6

DNBF

Meisenheimer complex

R = CH_3 (**M1**)
R = H (**M2**)

Scheme 4. Meisenheimer complexes from the reactions between **4a,b** and **DNBF**.

No evidence of the Wheland-Meisenheimer complexes from these reactions was obtained under the above experimental conditions, and this can be explained considering that both nucleophilic species, the methoxy- (**4a**) and the hydroxy- (**4b**) derivatives, are less able (compared to the dialkylamino substituents) to stabilize the positive charge on the nucleophilic moiety, in a hypothetical Wheland-Meisenheimer complex.

On the other hand, the Meisenheimer intermediates **M1** and **M2** resulted stable thanks to the ability of the **DNBF** moiety to stabilize the negative charge of this kind of intermediate, mainly because of the presence of the nitro groups on its ring, and owing the presence in the C-7, of a bad leaving group as the hydride ion.

Furthermore, an interesting behaviour was observed for **M1**; in fact, after three days in CD_3CN solution, its evolution in the substitution product **5a**, derived from the departure of the hydride ion from **M1**, was observed. The ^{1}H-NMR spectrum showed the disappearance of the signals belonging to **M1** and appearance of those ascribable to the substitution product **5a**. It should be noted that the formation of **M1** and **M2** σ–adducts, in DMSO solution, was previously reported in the literature,[18] with a partial characterization, and in that case, the authors described formation of **5a** in 50% yield after time (not defined) but only when DMSO was the reaction solvent. In the current case, **M1** and **M2** adducts were obtained in CD_3CN solution and the evolution of **M1** into **5a** was almost complete after about three days, while no presence of **5a** in DMSO-d_6 solution was observed after about 12 days.

Moreover, during this investigation, a coalescence phenomenon was observed in the ^{1}H-NMR spectra at low temperature of each anionic intermediate, involving the hydrogen atoms belonging to the nucleophilic moiety, which appeared not equivalent at low temperature and became equivalent increasing the temperature (in case of **M1**, also methyl groups were involved in the phenomenon).

This phenomenon was explained as a consequence of a constricted rotation around the C-C bond between the nucleophilic and the electrophilic moiety at low temperature, that is not present at higher temperatures, when the molecule possess a free rotation around this bond; the free activation energy for the rotation process was calculated for compound **M1** and the value is 13.2 ± 0.2 Kcal/mol (Figure 2).

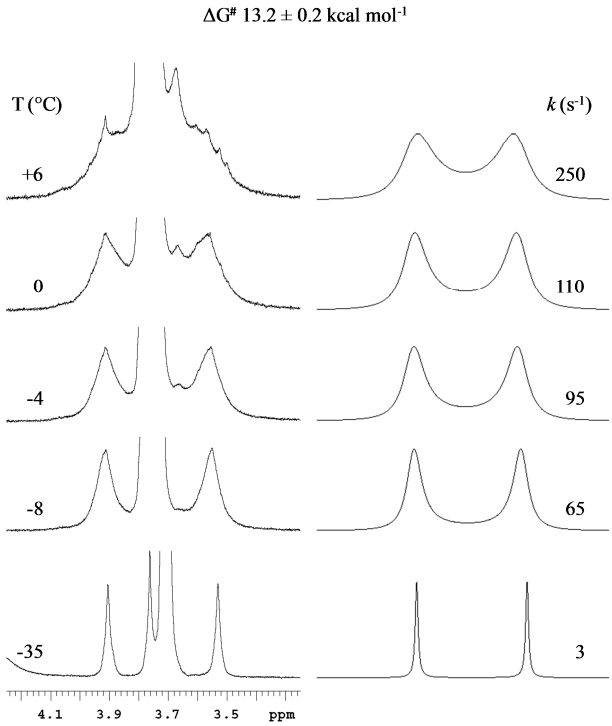


Figure 2. Variable temperature ^{1}H NMR spectra in $CDCl_3$ and dynamic-NMR simulations for proton signals of **M1**.

Since many benzofuroxan derivatives are known to possess biological activity as NO donor (see next paragraph), compound **5a** was used to evaluate its eventual biological effect: preliminar studies showed that it is toxic towards bacteria of the genus *Vibrio* in concentrations up to $1x10^{-6}$ M and for *Escherichia coli* in concentrations up to $1x10^{-5}$M. This compound generates superoxide and NO in bacterial cells and affects Quorum Sensing System Type 1 (biofilm formation by microorganisms, including pathogenic) at all concentrations tested. Damage to DNA and proteins was not detected

2.1.3 Conclusions

New substitution products, potentially interesting for different applications, were obtained in good yield from the reactions between different trisubstituted arenes and 7-chloro-4,6-dinitrobenzofuroxan.

When 4,6-dinitrobenzofuroxan was coupled with 1,3,5-trimethoxy- or 1,3,5-trihydroxy-benzene, directly in the NMR spectroscopy tube, stable Meisenheimer complexes were formed but no evidence of the Wheland-Meisenheimer intermediates were obtained due to the lower ability of both the involved nucleophiles, to stabilize a positive charge with

respect to the triaminobenzene derivatives. A peculiar behaviour was observed in the case of **M1** that spontaneously evolved in the substitution product **5a**, by an unexpected expulsion of a hydride ion.

Finally, a preliminar study on the biological activity of the synthesized compounds was carried out by russian coworkers, at the Research Institute of Biology, of the Russian Academy of Science (in Rostov-on-Don).

2.1.4 Experimental section

The ^{1}H and ^{13}C NMR spectra were recorded with a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H NMR) and 100.56, or 150.80 MHz (for ^{13}C NMR), respectively. *J* values are given in hertz (Hz). Signal multiplicities were established by DEPT experiments. Chemical shifts were referenced to the solvent [(δ=5.32 and 53.8 ppm for CD_2Cl_2), (δ=1.96 and 118.2 ppm for CD_3CN), and (δ=7.26 and 77.0 ppm for $CDCl_3$) for ^{1}H and ^{13}C NMR, respectively]. ESI-MS spectra were recorded with a WATERS 2Q 4000 instrument. Chromatographic purifications were carried out on silica gel (0.037-0.063 mm, Merck) columns at medium pressure. Thin layer chromatography (TLC) was performed on silica gel 60 F254 coated aluminum foils (Fluka). Melting points were measured on a Stuart SMP3 apparatus and are uncorrected. Solvents were commercial materials (Aldrich or Fluka) if not specified. 1,3,5-tris(dialkylamino)benzenes **1a-c** were prepared as described previously by the research group[19] and benzofuroxan derivatives **2** and **6**, were synthesized and purified as described in ref.14 and in ref.20, respectively.

General procedure for the synthesis of compounds 3a,b:

To a magnetically stirred solution of **1a-c** ($6x10^{-5}$ mol) dissolved in $CDCl_3$ (10 mL), in presence of 1.3 eq of sodium bicarbonate, was added an equimolar amount of 7-chloro-4,6-dinitrobenzofuroxan (**2**), at room temperature. TLC and ^{1}H-NMR analysis were used to monitor the progress of the reaction.

The final products **3a,b** were purified by chromatographic column on silica gel (FC), using different eluents. All the products were characterized by usual spectroscopic methods and their chemico-physical data are reported as follows.

4,6-dinitro-7-(2,4,6-tripiperidin-1-ylphenyl)-2,1,3-benzoxadiazole 1-oxide (3a): dark green solid, 85% yield, m.p. > 180 °C dec. **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C) δ (ppm): 8.88 (s,1H), 6.35(s, 2H), 3.32 (t, *J* = 4.86 Hz, 4H), 2.74-2.52 (m, 8H), 1.78-1.60 (m, 4H), 1.41-1.18 (m, 14 H).**^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25 °C) δ (ppm): 155.58, 155.56, 145.1, 141.7, 134.2, 132.5, 128.6, 115.0, 107.9, 102.0, 54.4, 48.9, 26.5, 25.8, 24.4, 24.2. **ESI MS (ES^+) m/z:** 552 $[M+H]^+$, 574 $[M+Na]^+$, 590 $[M+K]^+$.

4,6-dinitro-7-(2,4,6-trimorpholin-4-ylphenyl)-2,1,3-benzoxadiazole 1-oxide (3b): dark green solid, 85% yield, m.p. > 180 °C dec. **^{1}H NMR** ($CDCl_3$, 400 MHz, 25 °C): δ (ppm): 8.88 (s,1H), 6.45 (s, 2H), 3.89 (t, *J* = 4.6 Hz, 4H), 3.48-3.36 (m, 8H), 3.34 (t, *J* = 4.9 Hz, 4H), 2.80-2.63(m, 8H); **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25 °C) δ (ppm): 154.4, 153.9, 144.5, 142.6, 134.0, 132.3, 127.8, 127.4, 114.4, 103.2, 67.0, 66.4, 53.0, 48.5. **ESI MS (ES^+) m/z:** 558 $[M+H]^+$, 580 $[M+Na]^+$, 596 $[M+K]^+$.

General procedure for the synthesis of the salt 3bH:

To a magnetically stirred solution of **1b** ($7.7x10^{-5}$ mol) dissolved in CH_2Cl_2 (6 mL), was added an equimolar amount of 7-chloro-4,6-dinitrobenzofuroxan (**2**), at room temperature. Immediately after mixing, the color of the solution became dark green. After one night, a green solid precipitated. After filtration, compound **3bH** was isolated as a dark green solid and its chemico-physical data are reported as follows.

7-(2,6-dimorpholino-4-(morpholino-4-ium)phenyl)-4,6-dinitrobenzo[c][1,2,5]oxadiazo le1-oxide (3bH): green solid. **^{1}H NMR** (CD_3CN, 400 MHz, 25 °C) δ (ppm): 8.85 (s,1H), 7.02 (s, 2H), 4.03 (t, *J* = 4.8 Hz, 4H), 3.51 (t, *J* = 4.8 Hz, 4H), 3.40-3.32 (m, 8H), 2.77-2.66(m, 8H). **^{13}C NMR** (CD_3CN, 100.56 MHz, 25 °C) δ (ppm): 154.7, 146.1, 144.2, 136.0, 131.4, 129.8, 129.2, 127.3, 115.3, 107.1, 67.4, 65.8, 53.4, 52.1.

Variable temperature experiment for the reaction between 1b and Cl-DNBF (2):

0.038 mmol of Cl-DNBF (**2**), was dissolved in CD_2Cl_2 (1 mL) and introduced in a NMR spectroscopy tube, that was inserted in the NMR probe. When the probe temperature reached -75°C, an equimolar amount of 1,3,5-trimorpholinylbenzene (**1b**) was added to the solution, that became dark green, and the ^{1}H-NMR spectrum of the resulting solution was quickly recorded. The system was monitored after various times and at different temperatures until 25°C was reached. The recorded spectra showed the presence of the *para*-salt of compound **3b**, named **3bH** and the obtained chemico-physical data resulted in

agreement with those obtained from the above reported preparative procedure to synthesize this salt.

General procedure for the synthesis of compounds 5a,b:

To a magnetically stirred solution of **4a** or **4b** ($2x10^{-4}$ mol) dissolved in CD_3CN (5 mL and 10 mL, respectively), was added an equimolar amount of 7-chloro-4,6-dinitrobenzofuroxan (**2**), at room temperature. TLC with different eluents and ^{1}H-NMR analysis were used to monitor the reaction progress.

Compound **5a** was purified by chromatographic column on silica gel (FC), using as eluent diethyl ether/light petroleum (7:3). In the case of compound **5b**, it was purified by crystallization from diethyl ether and light petroleum. All the products were characterized by usual spectroscopic methods. Chemico-physical data are reported as follows.

4,6-dinitro-7-(2,4,6-trimethoxyphenyl)-2,1,3-benzoxadiazole 1-oxide (5a): red solid, 70% yield. **^{1}H-NMR** (CD_3CN, 300 MHz, 25 ^{o}C) δ (ppm): 8.79 (s, 1H), 6.34 (s, 2H), 3.92 (s, 3H), 3.73 (s, 6H). **^{13}C-NMR** (CD_3CN, 150.80 MHz, 25 ^{o}C): 166.1, 159.9, 146.0, 129.21, 129.0, 116.3, 97.9, 92.0, 65.08, 65.07, 56.8, 56.5. **ESI MS (ES^+) m/z:** 415 $[M+Na]^+$.

2-(5,7-dinitro-3-oxido-2,1,3-benzoxadiazol-4-yl)benzene-1,3,5-triol (5b): red solid, 54% yield. **^{1}H-NMR** (CD_3CN, 300 MHz, 25 ^{o}C) δ (ppm): 8.77 (s, 1H), 6.01 (s, 1H). **^{13}C-NMR** (CD_3CN, 100.56 MHz, 25 ^{o}C): 162.7, 157.6, 146.0, 144.3, 135.8, 129.8, 129.1, 116.4, 95.9, 95.7. **ESI MS (ES^+) m/z:** 373 $[M+H]^+$, 389 $[M+Na]^+$. **ESI MS (ES^-) m/z:** 349 $[M-H]^-$.

Study of the formation of σ–complexes M1 and M2 by ^{1}H-NMR spectroscopy: 4,6-dinitrobenzofuroxan (**6**) ($4.4x10^{-5}$ mol) was dissolved in CD_3CN and the solution was cooled at -35°C. This solution was added to a solution of compound **4a** in the case of **M1** or **4b** in the case of **M2** (in 1:1 molar ratio), in CD_3CN, directly prepared in the NMR spectroscopy tube at -35°C. The ^{1}H-NMR spectra were recorded at 5-10°C intervals, from -35°C to room temperature. The systems were monitored until no further change coul be detected in the recorded spectra. Herein are reported NMR data for both complexes.

5,7-dinitro-4-(2,4,6-trimethoxyphenyl)-4,5-dihydro-2,1,3-benzoxadiazol-5-ide 3-oxide (M1): red solution,**^{1}H NMR** (DMSO-d_6, 400 MHz, 25 ^{o}C) δ (ppm): 8.59 (s, 1H), 6.16 (s, 2H), 5.78 (s, 1H), 3.72 (s, 6H), 3.68 (s, 3H). **^{13}C NMR** (DMSO-d_6, 150.80 MHz, 25 ^{o}C) δ (ppm): 160.3, 149.7, 130.9, 127.2, 114.1, 110.3, 105.0 , 92.8, 91.4, 56.1, 55.2, 28.7.

5,7-dinitro-4-(2,4,6-trihydroxyphenyl)-4,5-dihydro-2,1,3-benzoxadiazol-5-ide 3-oxide (M2): red/orange solution**, ^{1}H NMR** (DMSO-d_6, 400 MHz, 25 ^{o}C) δ (ppm): 8.57(s, 1H), 5.68(s, 1H), 5.65(s, 2H). **^{1}H NMR** (CD_3CN, 400 MHz, -35 ^{o}C) δ (ppm): 8.2(s, 1H), 6.9(br.s, 3H), 5.9(s, 1H), 5.78(s, 1H), 5.76(s, 1H). **^{13}C-NMR** (CD_3CN, 100.56 MHz, -35 ^{o}C) δ (ppm): 158.7, 157.7, 157.2, 147.4, 141.9, 126.5, 123.7, 114.2, 113.5, 97.1, 94.5, 94.2, 29.3.

2.2 NOVEL STRUCTURAL HYBRIDS FROM THE REACTION BETWEEN BENZOFUROXAN AND BENZOTHIAZOLE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY.

2.2.1 Introduction

In this paragraph I will discuss a study involving benzofuroxan derivatives as the electrophilic species and benzothiazole derivatives as the nucleophilic species. From these reactions new and interesting hybrid heterocycles were obtained; some of these compounds showed also biological activity.

As reported in the previous paragraph, the benzofuroxan derivatives are an important class of heterocyclic compounds with interesting properties in many teoretical and applied fields;[1–4] in particular this organic scaffold is able to release nitric oxide (NO) molecules under physiological conditions[21,22] and in medicinal and biological fields this is an important property, because NO is considered the biologically important form of the endothelium-derived relaxing factor (EDRF), which endogenous formation plays an essential role in many bioregulatory systems, such as smooth muscle relaxation, platelet inhibition, neurotransmission and immune stimulation.[23] Due to the instability of aqueous solutions of NO, the interest to find compounds that are able to generate NO *in situ* (NO donors or NO releasing agents) is increasing. Benzofuroxan derivatives display typical NO-dependent activities both *in vitro* and *in vivo*, and the possibility of modulating NO release by changing the substituent on the ring makes them versatile tools in designing NO donor/drug hybrids.[24]

So, the combination of a benzofuroxanyl moiety with another biologically active substructure in a single molecule has recently received particular attention.

Also the benzothiazole scaffold posses interesting properties and it is mostly used in a variety of pharmacologically active synthetic and natural compounds exhibiting antimicrobial,[25-30] anticancer,[31-33] antihelmintic,[34] and anti-diabetic[35] activity. They are widely found in bioorganic and medicinal chemistry with application in drug discovery.[36]

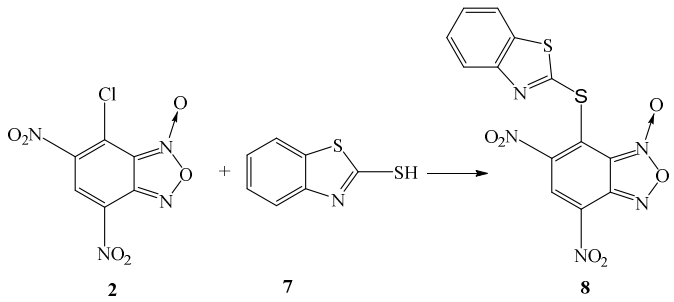
Based on the above considerations it would be of interest to synthesize novel structural hybrids containing both heterocyclic ring systems, benzofuroxan, able to release NO, and benzothiazole, a nucleus still receiving considerable attention in the drug field due to the biological effects[37] related to its structure.

2.2.2 Results and Discussion

In this study we used 2-thiobenzothiazole (**7**) and a series of 2-aminobenzothiazole derivatives (**12**) and their behaviour toward 7-chloro-4,6-dinitrobenzofuroxan (**2**, Cl-DNBF) was investigated and it will be discuss separately, as follows.

- *Reactions between Cl-DNBF (**2**) and 2-mercaptobenzothiazole (**7**)*

The reaction between Cl-DNBF and 2-mercaptobenzothiazole (**7**) was carried out mixing equimolar amounts of **2** and **7** in acetonitrile (Scheme 5), in presence of basic alumina; this reaction resulted complete after 2h at room temperature, and the product **8** was isolated in 86% yield.



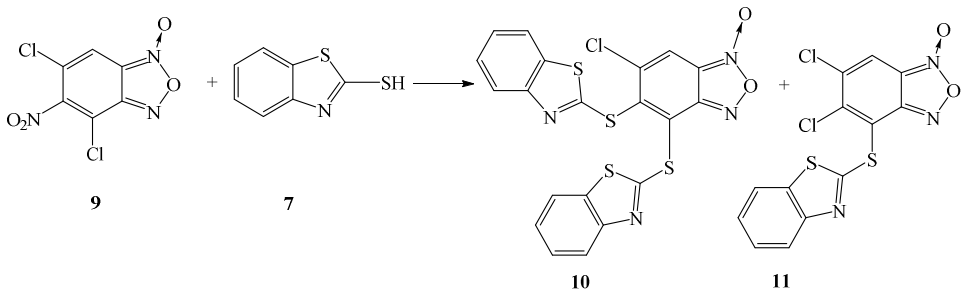
Scheme 5. Reaction between 7-chloro-4,6-dinitrobenzofuroxan and 2-mercaptobenzothiazole.

The observed high reactivity was expected on the basis of the following factors: i) the well known nucleophilic power of the sulphur nucleophiles; ii) the low aromaticity of the neutral heteroaromatic 10π-system (**2**); iii) the good leaving group ability of the chloride ion.

Based on the obtained result, we decided to perform the reaction of **7** with a less electrophilic reagent, namely 4,6-dichloro-5-nitrobenzofuroxan (**9**). Recently, it has been shown that reactions of **9** with aliphatic and aromatic amines is going along with the substitution of chlorine atom in the fourth position of the carbocyclic ring of the benzofuroxan derivative.[38,39] The optimal condition to increase the yield and pureness of the final product, was the use of DMSO as reaction solvent.[40] The nitro-group and the chlorine atom in the 6 position were inactive under any conditions.

In contrast to these findings, the reaction of the benzofuroxan derivative **9** with 2-mercaptobenzothiazole (**7**) gave a totally unexpected result. When compounds **9** and **7** were mixed in solvents such as chloroform, acetonitrile, and acetone, the reaction did not occur.

Only the reaction in the more polar dimethyl sulfoxide at 80-90 °C leads to formation of a mixture of two products (Scheme 6).



Scheme 6. Reaction between 4,6-dichloro-5-nitrobenzofuroxan and 2-mercaptobenzothiazole.

On the basis of spectroscopic analyses and, for compound **11**, X-ray diffraction analysis (Figure 3), we have established the structure of the reaction products. Compound **10** derives from a double nucleophilic attack with the displacement of the chlorine atom in the fourth position of the carbocyclic ring and of the nitro group in position 5 (this latter remembers the displacement of a nitro group by mercaptide ions in dipolar aprotic solvents[41]).

The formation of compound **11** is very unusual, in this case the replacement of the nitro group by chlorine might be explained by a mechanism involving radical species[42] or by reaction of compound **10** and chloride.[43,44]

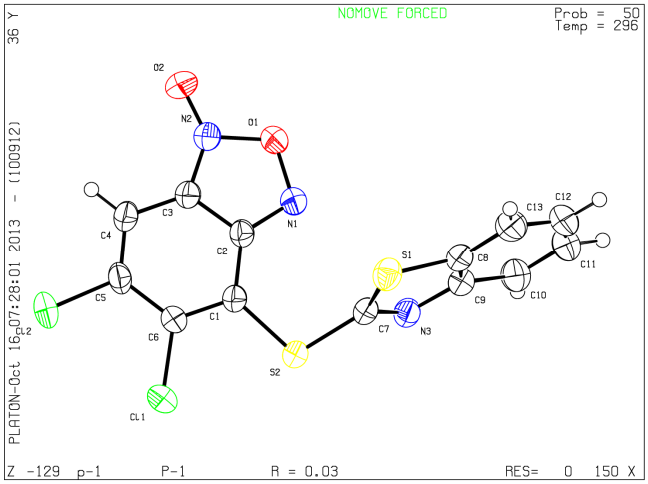
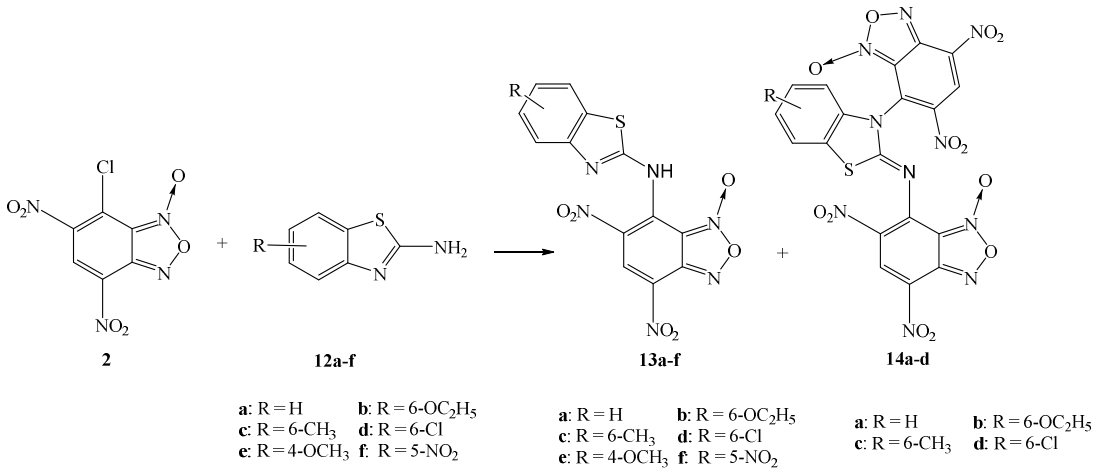


Figure 3. The ORTEP drawing of compound **11** at 50% ellipsoid probability.

- *Reactions between Cl-DNBF and 2-aminobenzothiazole derivatives*

Afterward, changing the electrophile/nucleophile combinations, we performed the reactions between Cl-DNBF (**2)** and the series of 2-aminobenzothiazoles **12a-f** (Scheme 7).



Scheme 7. Reaction between **2** and aminobenzothiazole derivatives **12a-f**.

From the reaction between **2** and 2-aminobenzothiazole derivatives **12a-d**, a mixture of mono-adducts **13a-d** and di-adducts **14a-d**, were obtained, while using the derivatives **12e** and **12f** only the mono-adducts were recovered.

Our supposition is that for compound **12e**, the second attack doesn't occur due to the steric hindrance of the methoxy substituent in position 4 on the aromatic ring; instead in the case of the 5-nitro derivative **12f**, the presence of the nitro group might deactivate the second attack of the electrophile.

Regarding the structure of the mono-adduct, it is important to note that, due to the ambident nitrogen reactivity of 2-aminobenzothiazoles and their possibility of existence in different forms, structure **A** (and its tautomeric form) and **B** might be formed by reaction with **2**, with the electrophile linked to the exo- or endo-cyclic nitrogen atom of the aminobenzothiazole derivative, respectively (Figure 4).

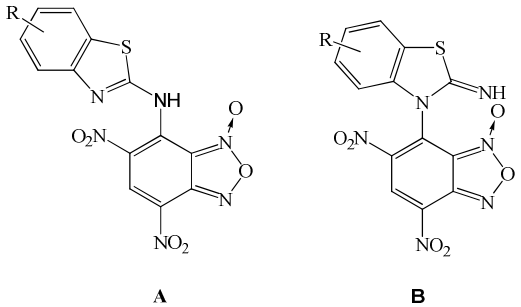
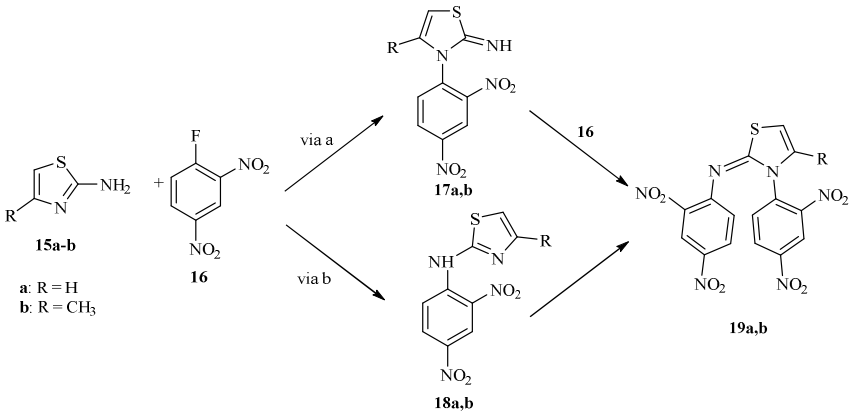


Figure 4. Possible structures for mono-adducts **13** formed between compounds **2** and **12**.

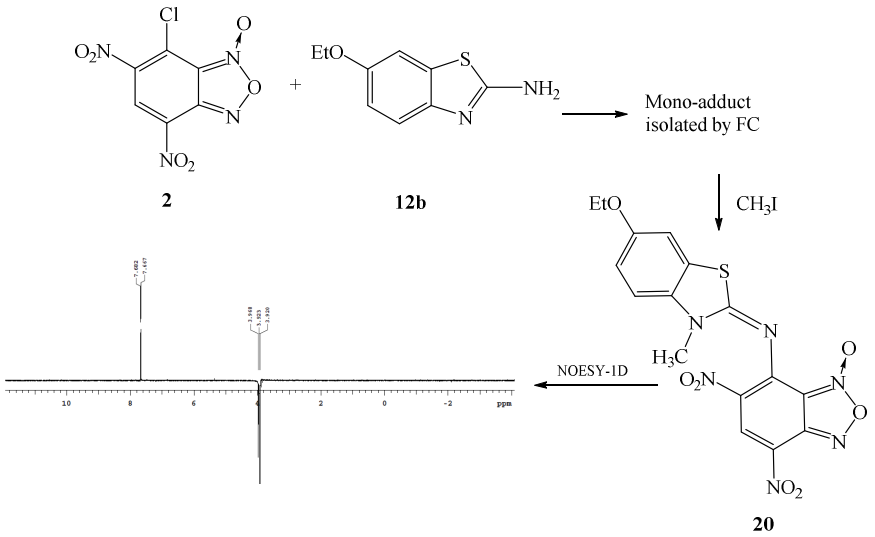
It has been reported[45] that 2-aminothiazole (**15a**) and 4-methyl-2-aminothiazole (**15b**) act as bidentate nucleophiles toward 2,4-dinitrofluorobenzene (**16**) in dimethyl sulfoxide (Scheme 8). In particular, in the absence of steric hindrance, the endo aza nitrogen of 2-aminothiazole is the preferred reactive site in the nucleophilic aromatic substitution of 2,4-dinitrofluorobenzene (**16**, via a) while when the approach of the electrophile from the aza center is sterically hindered as in case of compound **15b**, the reaction takes place first at the amino nitrogen to give **18b** (via b). Because the second and much faster reaction occurs at the imino nitrogen of the monosubstituted product **17a**, the diadduct **19a** is obtained as the major product.



Scheme 8. Reported reaction between 2-aminothiazoles and 2,4-dinitrofluorobenzene.[45]

Recently, it has been also reported that 2-aminobenzothiazole reacts with 2-((4-chloro-6-methylpyrimidin-2-ylthio)methyl)benzothiazole at the exocyclic amino group[25] while with glycidyl phenyl ether the reaction proceeds at both exo-and endocyclic nitrogen atoms, giving a diadduct.[46]

As a result of our investigations we have found that the interaction between benzofuroxan **2** and 2-aminobenzothiazole derivatives **12** gave a mixture of mono- (**13**) and di-adducts (**14**). However, since all attempts to crystallize some mono-adducts failed, to gain further indications about the structure of compounds **13**, we prepared the methyl derivative of the mono-adduct derived from the reaction between **2** and **12b** (Scheme 9) and NOESY-1D experiments were carried out on it.



Scheme 9. Methylation of the monoadduct **12b** and spectrum obtained from NOESY-1D experiment.

The obtained results agreed with structure **20**, thus indicating that the benzofuroxan moiety on compounds **13** is bound to the exocyclic amino nitrogen atom.

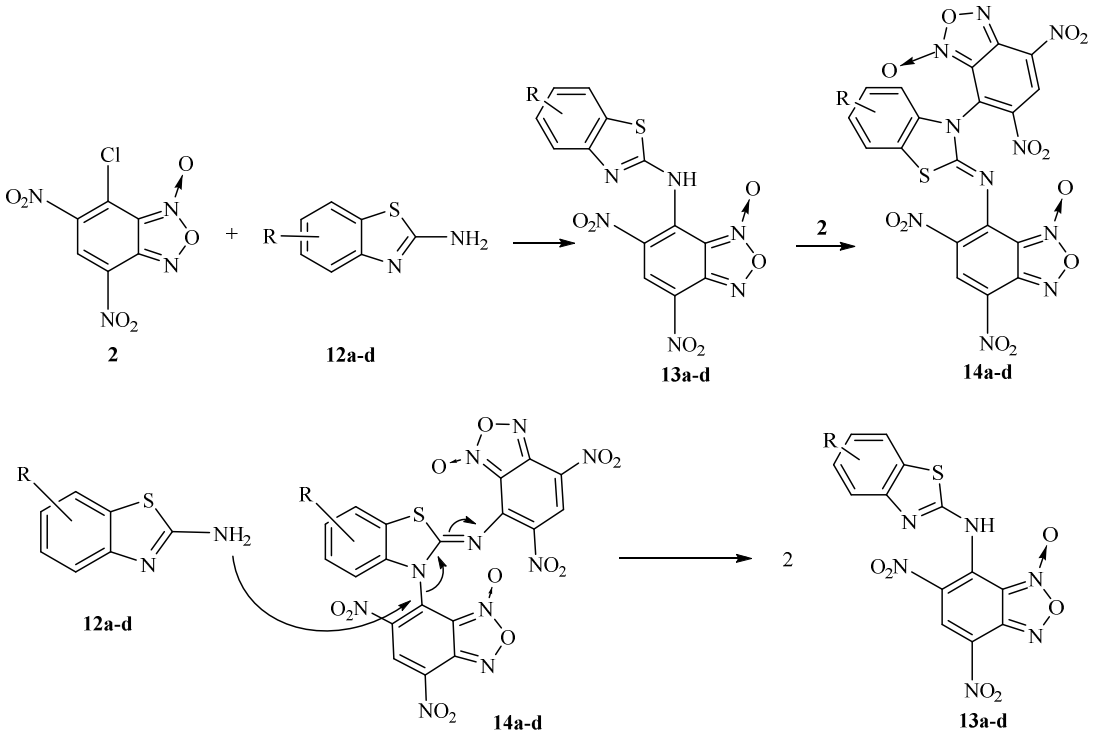
Even if, on the basis of the above cited literature findings, the formation of the di-adduct **14** was not completely unexpected, we decided to investigate more in detail the reaction course. The reactions between **2** and **12a–e** were carried out directly in the NMR spectroscopy tube in acetone-d_6 at 25 °C and their progress was monitored over time. In Table 1 are reported the results obtained from this NMR study.

Table 1 Relative percentage of products[a] **13** and **14** dependent on the reaction time for the reaction between **2** and **12a-e** in 1:2 and in 1:4[b] molar ratio.

Reaction time →		4 h	24 h	5 days	14 days	21 days
R ↓ Product						
H	**13a**	29[c] (50)[c]	34 (86)	60 (100)	92	99
	14a	71 (50)	66 (14)	40 (0)	8	1
6-OC_2H_5	**13b**	35 (71)	55 (97)	72	91	99
	14b	65 (29)	45 (3)	28	9	1
6-CH_3	**13c**	10[c,d] (68)	43 (84)	73 (100)	88	100
	14c	90 (32)	57 (16)	27 (0)	12	-
6-Cl	**13d**	34 (35)[c]	19 (67)	62 (100)	99	[f]
	14d	66 (65)	81 (33)	38 (0)	1	[f]
4-OCH_3	**13e**	100[e]	100	[f]	[f]	[f]
	14e	-	-	[f]	[f]	[f]

[a] Calculated from the ^{1}H NMR spectrum recorded in acetone-d_6. [b] In brackets. [c] After this time the spectrum showed a singlet probably belonging to a benzofuroxanic species, that disappeared with time. [d] The spectrum showed presence of ~6% of **2.** [e] In this case the spectrum showed presence of **2** and **13e** in 25/75 relative ratio. [f] Not measured.

Data of Table 1 for the reactions carried out using a 1:2 molar ratio between **2** and **12** show that in the first reaction times (4 h) the diadducts **14a-d** are formed in greater amount with respect to the respective monoadducts **13a-d**. As time passes, a gradual shift of the **13a-d**/**14a-d** relative ratio towards the monoadduct **13a-d** was observed, until to reach complete formation of this latter after about two weeks. This behavior suggests the occurrence, in the first reaction time, of a behaviour similar to that already observed and above cited for the reaction between 2-aminothiazole and 2,4-dinitrofluorobenzene. In present case, after formation of the mono adduct, a second fast attack of **2** might occur thus giving the diadduct **14**. Then, the presence of further amount of 2-aminobenzothiazole derivative in the reaction mixture might induce formation of mono-adduct through the pathway proposed in Scheme 10. This hypothesis is supported by the fact that, when the reaction is carried out with a 4:1 relative molar ratio between the benzothiazole derivative **12a-d** and the benzofuroxan **2**, the monoadducts **13a-d** were present as major products since the first reaction days and the relative **13**/**14** ratio became almost quantitative in favor of the first after a few days (compare the relative **13**/**14** ratios with those in brackets in Table 1).



Scheme 10. Proposed pathway to explain the observed time-dependence of the ratio between products **13a-d** and **14a-d**.

Moreover, the pathway proposed and depicted in Scheme 10 was supported also by the observation that acetone-d_6 solution of the diadduct **14d**, monitored by ^{1}H NMR spectroscopy for a week, resulted unchanged (as well as after 40 days); after this time, **12d**

was added to this solution, and the mono-adduct **13d** was present in 13% yield after one weak and in about 33% yield after about 40 days.

Taking into account the bioactivity of many benzofuroxan and benzothiazole derivatives, we also decided to evaluate the biological effect of the obtained compounds on natural strain *Vibrio* genus and different bacterial lux-biosensors. The biological studies were carried out at the Research Institute of Biology, of the Russian Academy of Science (in Rostov-on-Don) by our russian coworkers.

Among all the benzofuroxanes containing the 2-aminobenzothiazole fragment, only compound **13e** showed the average level of toxicity for a bacterial cell in concentrations up to 10^{-7}M and only concerning *V. aquamarinus* VKPM B-11245. For other investigated benzofuroxans, the noticeable bacteriotoxic effect at concentration lower than 10^{-3} - 10^{-4} M is revealed neither for a *vibrio*, nor for a constitutive biosensor on the basis of *E. coli* MG1655.

Introduction of mercaptobenzothiazole fragment instead of the aminobenzothiazole fragment leads to considerable strengthening of biological activity.

As shown in Figure 5, the benzofuroxan derivative **8** is highly toxic for *V. aquamarinus* VKPM B-11245 in the concentration range: $1x10^{-3}$ M – $1x10^{-6}$ M.

For *E. coli* MG1655 (pXen7), the substance is toxic in the concentration of $1x10^{-5}$ M and highly toxic in the concentration of $1x10^{-4}$ M and higher. Sensitivity of *V. aquamarinus* VKPM B-11245 to the studied substance was higher that is likely to be connected with more expressed sensitivity of this strain to toxic influences.

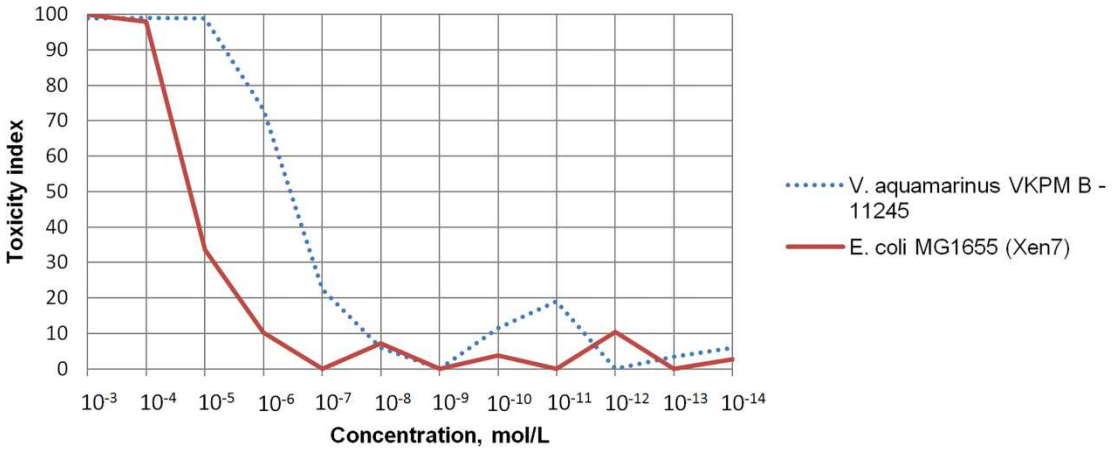


Figure 5. Toxicity index of compound **8** registered for natural and gene engineered strains.

For researching possible mechanisms of the compound **8** influence on a bacterial cell, a number of experiments were carried out with genetically engineered luminescent biosensors of *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pColD-lux), *E. coli* MG1655 (pGrpE-lux), *E. coli* MG1655 (pIbpA-

lux) and *E. coli* MG1655 (pVFR1-lux) that allowed to reveal certain influence on bacterial cell homeostasis.

From the obtained data we can affirm that during the interaction of compound **8** with bacterial cells there is no noticeable increase of peroxide compound level, damage of DNA and proteins.

Whereas, a significant effect of superoxide-anion radical or NO level increase is registered in a bacterial cell in concentration of $1x10^{-4}$ M. and a weak effect in concentration of $1x10^{-3}$ M.

The most significant of the observed biological effects of **8** is expressed by 1st type Quorum Sensing system activation.

The compounds influencing the formation of bacterial biofilms, deserve more carefull research because for many pathogenic microorganisms an obligatory stage of infectious process development is biofilm formation.

2.2.3 Conclusions

The ability of benzofuroxan derivatives to release nitric oxide (NO) under physiological conditions and the bioactivity of many benzothiazole derivatives have inspired this research focused on the synthesis of novel structural hybrids bearing these two heterocyclic moieties and on the evaluation of their antibacterial activity. The new compounds have been synthesized through electrophile/nucleophile combination of nitrobenzofuroxan derivatives and 2-mercapto- or 2-aminobenzothiazole derivatives. The reaction between 4,6-dichloro-5-nitrobenzofuroxan and 2-mercaptobenzothiazole gave two products, one derived from a double nucleophilic attack with the displacement of both, the chlorine atom and the nitro group of the benzofuroxan reagent, and the second one implying an unexpected replacement of the nitro group by chlorine.

From the reaction between 7-chloro-4,6-dinitrobenzofuroxan and different 2-aminobenzothiazole derivatives two products have been isolated, one bearing the benzofuroxan moiety linked to the exocyclic amino nitrogen of the nucleophile, and the second derived from the attack of two molecules of the electrophile to both the nitrogen atoms of the benzothiazole reagent. The reaction was monitored directly in the NMR spectroscopy tube and this experiment revealed that the relative ratio of the two products is time-dependent thus suggesting the possibility to tune the reaction depending on the product of interest.

The biological effect of the new hybrids on the natural strain *Vibrio* genus and different bacterial lux-biosensors was studied.

Compound **13e** displayed bacteriotoxic properties towards *Vibrio* in the concentration up to 10^{-7} M; whereas, compound **8** displayed not only the bacteriotoxic effect but it also activated the 1st type Quorum Sensing system effectively.

Part of this paragraph is reproduced with permission from "European Journal of Medicinal Chemistry". Further experimental data, included characterization data of the related products here described, can be found in the paper "E. Chugunova, C. Boga, I. Sazykin, S. Cino, G. Micheletti, A. Mazzanti, M. Sazykina, A. Burilov, L. Khmelevtsova, N. Kostina, *Eur. J. Med. Chem*. **2015**, *93*, 349–359".

2.2.4 Experimental section

The ^{1}H- and ^{13}C-NMR spectra were recorded with a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H-NMR) and 100.56, or 150.80 MHz (for ^{13}C-NMR), respectively. Signal multiplicities were established by DEPT experiments. Chemical shifts were measured in δ (ppm) with reference to the solvent (δ= 1.96 ppm and 118.20 ppm for CD_3CN; δ = 2.05 ppm and 29.84 ppm for $(CD_3)_2CO$; δ= 7.26 ppm and 77.00 ppm for $CDCl_3$, for ^{1}H- and ^{13}C-NMR, respectively). *J* values are given in Hz. Electron spray ionization mass spectra (ESI-MS) were recorded with a WATERS 2Q 4000 instrument. Elementary analyses were performed on a Carlo Erba Model EA-1108 elemental analyser. Chromatographic purifications (FC) were carried out on glass columns packed with silica gel (Merck grade 9385, 230–400 mesh particle size, 60 Å pore size) at medium pressure. Thin layer chromatography (TLC) was performed on silica gel 60 F254 coated aluminum foils (Fluka). Aluminum oxide used was activated, basic, Brockmann I, standard grade ca. 150 meshes. Melting points were measured on a Büchi 535 apparatus and are uncorrected; compounds **13** and **14** are red-brown solids that decompose in the melting tube above about 200 °C. 2-Mercaptobenzothiazole (**7)** and 2-aminobenzothiazoles **12a-f** were purchased from Sigma Aldrich (Milan, Italy). Benzofuroxans **2** and **9** were prepared using the methods reported in the literature.[14,47] Genetically engineered biosensor strains of *E. coli* MG1655 (pXen7), *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pColD-lux*), E. coli* MG1655 (pGrpE-lux), *E. coli* MG1655 (pIbpA-lux), *E. coli* MG1655 (pVFR1-lux) have been kindly furnished by Manukhov I.V., Federal State Unitary Enterprise "GosNIIGenetika". All chemical preparations for biological assays were of analytical purity: zinc sulfate (Aquatest, Russia),

Dioxydin (Sigma-Aldrich), paraquat (Sigma-Aldrich), hydrogen peroxide (Ferrain, Russia), MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, Sigma-Aldrich), ethanol (NeoSources Inc.), 3-oxohexanoyl-homoserine lactone (Sigma-Aldrich). Biological essays were carried out as described in tha above cited paper.

Copies of ^{1}H- and ^{13}C-NMR spectra for compounds **8**, **10**, **11**, **13a-e**, **14a-d**, and **20** and other tabulated data are reported in Supporting Information of the above cited paper from which this study was extracted.[48]

General procedure for the synthesis of compounds 8:

To a magnetically stirred solution of 7-chloro-4,6-dinitrobenzofuroxan **2** (0.020 g, 0.077 mmol) dissolved in $CHCl_3$ (10 mL) was added an equimolar amount of 1,3-benzothiazole-2-thiol **7** (0.013 g, 0.077 mmol) and 0.08 g of basic aluminium oxide, at room temperature. Immediately after mixing the solution turned from pale yellow to red. The solution was stirred for 1 h and the progress of the reaction was monitored by TLC (eluent: dichloromethane) and ^{1}H-NMR analysis. After filtration and removal of the solvent in vacuum, product **8** was washed with a little amount of Et_2O then *n*-hexane was added and compound **8** precipitated as dark red solid. The purification by FC (eluent: dichloromethane) gave **8** in lower yield probably because of its partial decomposition on silica gel.

7-(1,3-benzothiazol-2-ylthio)-4,6-dinitro-2,1,3-benzoxadiazole 1-oxide (8): dark red solid, 86% yield. **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C) δ (ppm): 7.41-7.47 (m, 2H), 7.69-7.71 (m, 1H), 7.86-7.89 (m, 1H), 8.98 (m, 1H); **^{13}C NMR** (150.80 MHz, ($CDCl_3$, 25 °C) δ (ppm): 115.8, 121.6, 122.7, 126.3, 126.7, 127.0, 130.5, 135.6, 136.1, 144.2, 145.6, 152.1, 158.9. Anal. calcd for $C_{13}H_5N_5O_6S_2$: C 39.90, H 1.29, N 17.90; found: C 40.00, H 1.30, N 17.94. **ESI MS (ES^+) m/z:** 414 $[M+Na]^+$.

Reaction between 4,6-dichloro-5-nitrobenzofuroxan (9) and 1,3-benzothiazole-2-thiol (7):

To a solution of 4,6-dichloro-5-nitrobenzofuroxan **9** (0.125 g, 0.0005 mol) in 5 mL of DMSO at room temperature was added a solution of 2-mercaptobenzothiazole (**7**, 0.166 g, 0.001 mol) in 5 mL. The reaction mixture was heated at 80-90 °C for 5-6 h (the reaction was monitored by TLC). After verification of the completion of the reaction by TLC, distilled water was added to the crude reaction mixture and a yellow solid precipitated. It was filtered off, washed with water and dried under vacuum (0.06 mm Hg) at 40 °C until to constant

weight. The mixture of products **10** and **11** was separated by column chromatography, using ethyl acetate as eluent. The same results were obtained using an equimolar ratio of the reagents. All the products were fully characterized by usual spectroscopic methods.

Chemico-physical data are reported as follows.

4,5-bis(benzo[d]thiazol-2-ylthio)-6-chlorobenzo[c][1,2,5]oxadiazole 1-oxide (10): yellow oil, 45% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C), δ (ppm): 7.32-7.37 (m, 2H), 7.41-7.45 (m, 2H), 7.64 (s, 1H), 7.74-7.78 (m, 2H), 7.84-7.86 (m, 2H); **^{13}C NMR** (100.56 MHz, $CDCl_3$, 25 °C), δ (ppm): 113.7, 114.2, 116.7, 121.1, 121.2, 122.5, 122.9, 125.2, 125.6, 125.8, 126.5, 132.7, 133.0, 135.8, 136.5, 138.5, 152.8, 152.9, 153.0, 160.2. **ESI MS (ES^+) m/z:** 523,525 $[M+Na]^+$.

4-(benzo[d]thiazol-2-ylthio)-5,6-dichlorobenzo[c][1,2,5]oxadiazole 1-oxide (11): Yellow solid, 52% yield; m.p. 199–201 °C (CH_2Cl_2/n-hexane). **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C), δ (ppm): 7.38 (t, *J* = 7.78 Hz, 1H), 7.46 (t, *J* = 7.78 Hz, 1H), 7.65 (s, 1H), 7.57 (dm, *J* = 8.06 Hz, 1H), 7.88 (br.d, *J* = 8.01 Hz, 1H); **^{13}C NMR** (100.56 MHz, $CDCl_3$, 25 °C), δ (ppm): 113.2, 116.2, 118.3, 121.3, 123.2, 125.5, 126.0, 126.8, 136.6, 149.2, 152.8, 153.9, 157.4. Anal. calcd for $C_{13}H_5Cl_2N_3O_2S_2$: C 42.17, H 1.36, N 11.35; found: C 42.19, H 1.36, N 11.34. **ESI MS (ES^+) m/z:** 392, 394 $[M+Na]^+$. Crystal data for **11** are deposited in CCDC 1028845.

General procedure for the synthesis of compounds 13a-f and 14a-d

To a solution of 4,6-dinitro-7-chlorobenzofuroxan **2** (0.025 g, 0.0001 mol) in 5 mL of acetonitrile or chloroform at room temperature was added a solution of 2-aminobenzothiazole **12** (0.0002 mol) in 5 mL of acetonitrile or chloroform. The reaction mixture was stirred for 2-24 h; the reaction products and their relative yields depend from the reaction time, with the increase of time amount of mono-substituted product increase (see Table 1). The reaction was carried out also with a 1:4 molar amount of **2**:**12**, and the results obtained are reported in Table 1. After removal of the solvent under reduced pressure, the products were separated by column chromatography, using ethyl acetate as eluent.

7-(benzo[d]thiazol-2-ylamino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (13a): ^{1}H NMR (400 MHz, CD_3CN, 25 °C), δ (ppm): 7.23 (td, *J* = 8.41 Hz, *J* = 1.2 Hz, 1H), 7.36 (td, *J* = 8.41 Hz, *J* = 1.2 Hz, 1H), 7.60 (dd, *J*=8.2 Hz, *J*=0.6 Hz, 1H), 7.81 (dd, *J*=8.0 Hz, *J* = 0.78 Hz, 1H), 8.89 (s, 1H); **^{13}C NMR** (100.56 MHz, DMSO-d_6, 25 °C), δ (ppm): 112.0, 115.8, 120.6, 121.5, 123.1, 125.7, 125.8, 133.7, 134.0, 134.1, 142.0, 147.5, 150.8. Anal.

calcd for $C_{13}H_6N_6O_6S$: C 41.72, H 1.62, N 22.45; found: C 41.89, H 1.63, N 22.52. **ESI MS (ES^+) m/z:** 373 $[M-H]^-$.

7-((6-ethoxybenzo[d]thiazol-2-yl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole1-oxide (13b): ^{1}H NMR (400 MHz, acetone-d_6, 25 °C), δ (ppm): 1.38 (t, *J* = 6.95 Hz, 3H), 4.09 (q, *J* = 6.95 Hz, 2H), 6.92 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 8.92 (s, 1H); **^{13}C NMR** (100.56 MHz, acetone-d_6, 25 °C), δ (ppm): 15.2, 64.6, 106.1, 112.8, 114.6, 115.5, 122.2, 134.8, 136.5, 142.7, 146.6, 148.8, 156.5, 169.3. Anal. calcd for $C_{15}H_{10}N_6O_7S$: C 43.07, H 2.41, N 20.09; found: C 43.24, H 2.42, N 20.07. **ESI MS (ES^+) m/z:** 417 $[M-H]^-$.

7-((6-methylbenzo[d]thiazol-2-yl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (13c): ^{1}H NMR (400 MHz, acetone-d_6, 25 °C), δ (ppm): 2.39 (s, 3H, CH_3), 7.13 (dd, *J* = 8.35, *J* = 1.97 Hz, 1H), 7.45 (d, *J* = 8.35 Hz, 1H), 7.60-7.57 (m, 1H), 8.93 (s, 1H); **^{13}C NMR** (100.56 MHz, acetone-d_6, 25 °C), δ (ppm): 21.4, 112.8, 121.4, 121.6, 121.8, 127.4, 127.6, 133.5, 134.8, 135.5, 142.9, 148.7, 150.5, 170.6. Anal. calcd for $C_{14}H_8N_6O_6S$: C 43.30, H 2.08, N 21.64; found: C 43.50, H 2.09, N 21.60. **ESI MS (ES^-) m/z:** 387 $[M-H]^-$.

7-((6-chlorobenzo[d]thiazol-2-yl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (13d): **^{1}H NMR** (400 MHz, acetone-d_6, 25 °C), δ (ppm): 7.30 (dd, *J* = 8.68 Hz, *J* = 1.74 Hz, 1H), 7.52 (dd, *J* = 8.68 Hz, *J* = 1.74 Hz, 1H), 7.83-7.88 (d, *J* = 1.74 Hz, 1H), 8.93 (s, 1H); **^{13}C NMR** (100.56 MHz, $(CD_3)_2CO$, 25 °C) δ (ppm): 112.8, 116.8, 121.5, 122.7, 126.6, 126.8, 128.3, 134.9, 137.2, 143.2, 148.6, 151.9, 172.3. Anal. calcd for $C_{13}H_5ClN_6O_6S$: C 38.20, H 1.23, N 20.56; found: C 38.21, H 1.23, N 20.55. **ESI MS (ES^-) m/z:** 407, 409 $[M-H]^-$.

7-((4-methoxybenzo[d]thiazol-2-yl)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole1-oxide (13e): ^{1}H NMR (400 MHz, acetone-d_6, 25 °C), δ (ppm): 3.92 (s, 3H), 6.90 (d, *J* = 8.20 Hz, 1H), 7.15 (t, *J* = 8.20 Hz, 1H), 7.38 (d, *J* =8.20 Hz, 1H), 8.93 (s, 1H); **^{13}C NMR** (100.56 MHz, acetone-d_6, 25 °C), δ (ppm): 56.6, 108.7, 112.8, 114.5, 117.1, 124.8, 126.4, 135.1, 136.2, 142.0, 143.4, 148.7, 152.7, 170.7. Anal. calcd for $C_{14}H_8N_6O_7S$: C 41.59, H 1.99, N 20.79; found: C 41.73, H 2.01, N 20.78. **ESI MS (ES^-) m/z:** 403 $[M-H]^-$.

4,6-dinitro-7-((5-nitrobenzo[d]thiazol-2-yl)amino)benzo[c][1,2,5]oxadiazole 1-oxide (13f): ^{1}H NMR (400 MHz, acetone-d_6, 25 °C), δ (ppm): 7.41 (dd, *J* = 8.75, *J* = 2.33 Hz, 1H), 7.81 (d, *J* = 2.33 Hz, 1H), 7.85 (d, *J* = 8.75 Hz, 1H), 8.95 (s, 1H, H-7); **^{13}C NMR** (100.56 MHz, acetone-d_6, 25 °C), δ (ppm): 111.2, 112.7, 116.3, 117.0, 124.6, 127.1, 129.9,

135.6, 141.8, 146.2, 149.0, 153.4. Anal. calcd for $C_{13}H_5N_7O_8S$: C 37.24, H 1.20, N 23.38; found: C 37.27, H 1.21, N 23.36. **ESI MS (ES^-) m/z:** 418 $[M-H]^-$.

7-((3-(4,6-dinitro-1-oxidobenzo[c][1,2,5]oxadiazol-7-yl)benzo[d]thiazol-2(3H)-ylidene) amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (14a): Brown oil. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C), δ (ppm): 6.85-6.87 (m, 1H), 7.43-7.46 (m, 2H), 7.64-7.66 (m, 1H), 9.06 (s, 1H), 9.13 (s, 1H); **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 113.0, 113.1, 115.0, 123.3, 124.5, 125.2, 126.7, 128.7, 129.1, 130.2, 131.2, 132.2, 137.3, 139.0, 141.1, 144.6, 146.1, 146.7, 162.1. Anal. calcd for $C_{19}H_6N_{10}O_{12}S$: C 38.14, H 1.01, N 23.41; found: C 38.12, H 1.00, N 23.38. **ESI MS (ES^+) m/z:** 599 $[M+H]^+$, 621 $[M+Na]^+$.

7-((3-(4,6-dinitro-1-oxidobenzo[c][1,2,5]oxadiazol-7-yl)-6-ethoxybenzo[d]thiazol-2(3H)-ylidene)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (14b): ^{1}H NMR: (400 MHz, acetone-d_6, 25 °C), δ (ppm): 1.38 (t, *J* = 6.77 Hz, 3H), 4.12 (q, *J* = 6.77 Hz, 2H), 7.04 (dd, *J* = 8.8 Hz, *J* = 1.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.53 (d, *J* = 1.8 Hz, 1H), 9.00 (s, 1H), 9.25 (s, 1H); ^{13}C NMR (100.56 MHz, acetone-d_6, 25 °C), δ (ppm): 15.0, 65.1, 109.5, 114.3, 115.1, 116.7, 125.0, 125.5, 128.9, 129.0, 130.0, 131.2, 131.4, 132.0, 139.1, 141.6, 144.9, 146.3, 146.9, 158.3, 162.7. Anal. calcd for $C_{21}H_{10}N_{10}O_{13}S$: C 39.26, H 1.57, N 21.80; found: C 39.41, H 1.58, N 21.77. **ESI MS (ES^+) m/z:** 665 $[M+Na]^+$.

7-((3-(4,6-dinitro-1-oxidobenzo[c][1,2,5]oxadiazol-7-yl)-6-methylbenzo[d]thiazol-2(3H) -ylidene)amino)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (14c): ^{1}H NMR (400 MHz, acetone-d_6, 25 °C), δ (ppm): 2.43 (s, 3H), 7.26-7.33 (m, 2H, H-4), 7.72-7.74 (m, 1H), 9.02 (s, 1H), 9.25 (s, 1H); **^{13}C NMR** (100.56 MHz, acetone-d_6, 25 °C), δ (ppm): 21.1, 113.1, 115.0, 123.7, 124.5, 125.5, 128.9, 129.9, 130.3, 131.3, 132.2, 135.5, 137.1, 139.1, 141.5, 144.9, 146.3, 146.9, 162.7, 164.5. Anal. calcd for $C_{20}H_8N_{10}O_{12}S$: C 39.22, H 1.32, N 22.87; found: C 39.20, H 1.34, N 22.82. **ESI MS (ES^+) m/z:** 613 $[M+H]^+$, 635 $[M+Na]^+$, 651 $[M+K]^+$.

7-(6-chloro-2-((4,6-dinitro-1-oxidobenzo[c][1,2,5]oxadiazol-7-yl)imino)benzo[d]triaz olo-3(2H)-yl)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (14d): ^{1}H NMR (400 MHz, acetone-d_6, 25 °C), δ (ppm): 7.42 (d, *J*=8.8 Hz, 1H),7.52 (dd, *J* = 8.8 Hz, *J* = 1.7 Hz, 1H), 8.03 (d, 1H, *J* = 1.7 Hz), 9.02 (s, 1H), 9.25 (s, 1H); **^{13}C NMR** (100.56 MHz, acetone-d_6, 25 °C) δ (ppm): 113.1, 114.6, 115.1, 124.4, 125.0, 125.4, 128.9, 129.2, 130.8, 131.1, 131.5, 132.6, 136.6, 139.3, 141.0, 145.1, 146.3, 146.8, 162.0. Anal. calcd for $C_{19}H_5ClN_{10}O_{12}S$: C 36.06, H 0.80, N 22.13; found: C 36.20, H 0.80, N 22.09. **ESI MS (ES^+) m/z:** 655,657 $[M+Na]^+$.

General procedure for the synthesis of compound 20.

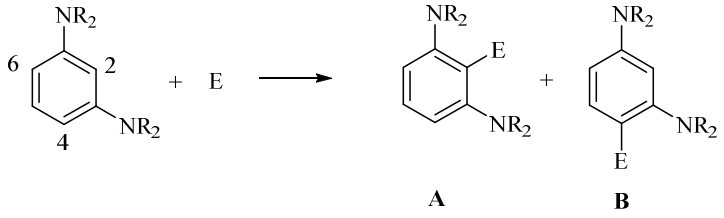
To a solution (15 mg, 0.036 mmol) of the mono-adduct derived from the reaction between **2** and **12b,** dissolved in 3 mL of anhydrous THF, 150 μL (2,4 mmol) of methyl iodide was added. The reaction mixture was heated under reflux in nitrogen atmosphere for 24 hours. The solvent was removed and flash chromatography on silica gel (eluent: ethyl acetate) of the residue gave compound **20.**

7-((6-ethoxy-3-methylbenzo[d]thiazol-2(3H)-ylidene)amino)-4,6-dinitrobenzo[c][1,2,5] oxadiazole 1-oxide (20): dark violet solid, 64% yield, m.p.: 187.5-188.7 °C. **^{1}H NMR** (600 MHz, acetone-d_6, 25 °C): δ (ppm): 1.39 (t, *J* = 6.8 Hz, 3H), 3.92 (s, 3H), 4.13 (q, *J* = 6.8 Hz, 2H), 7.21 (dd, *J* = 8.9 Hz, *J* = 2.5 Hz, 1H), 7.47 (d, *J* = 2.5 Hz, 1H), 7.67 (d, *J* = 8.9 Hz, 1H), 9.06 (s, 1H); **^{13}C NMR** (150.80 MHz, acetone-d_6, 25 °C), δ (ppm): 14.9, 33.0, 65.0, 108.6, 113.3, 115.0, 117.0, 125.5, 125.7, 128.3, 133.0, 133.9, 144.5, 147.6, 158.0, 166.3. NMR experiment carried out by irradiating methyl signal showed NOE effect with the H-4 proton of the benzothiazole moiety, indicating that compound **20** bears the benzofuroxan moiety bound to the 2-aminobenzothiazole exocyclic nitrogen atom (see Scheme 9).

2.3 REACTIONS OF CHLORO-NITRO-BENZOFURAZAN- AND BENZOFUROXAN-DERIVATIVES WITH 1,3-BIS(*N*,*N*-DIALKYLAMINO)BENZENE DERIVATIVES

2.3.1 Introduction

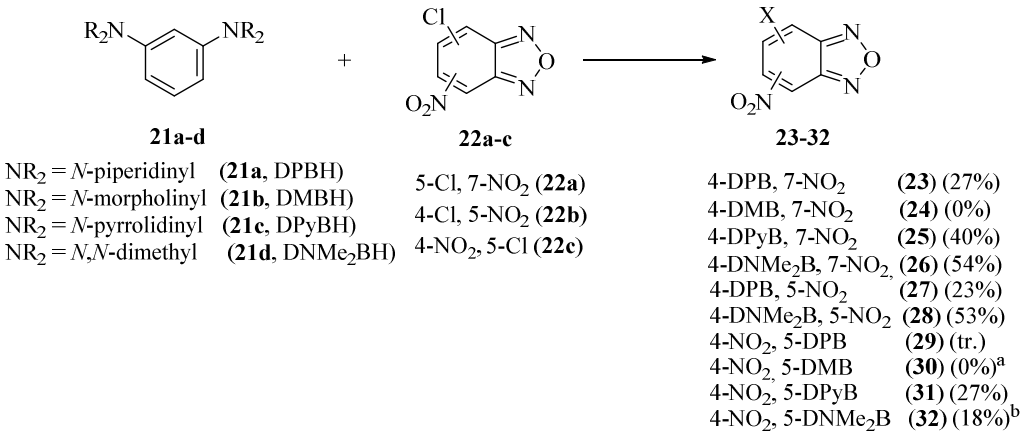
In this paragraph the results obtained from the reactions between benzofurazan or benzofuroxan derivatives and diaminobenzenes as nucleophilic species will be presented and discussed. These nucleophilic species have been very poorly studied,[49,50] so our interest was devoted to the investigation of their reactivity with different benzofurazan derivatives and with 7-chloro-4,6-dinitrobenzofuroxan. Moreover, it has to be considered that all the synthesized substitution products, from the reactions with both electrophilic species, are new conjugated systems with an electron rich and an electron poor moiety on the same molecule and this peculiarity makes these products good candidates for different applications (*e.g.* solar cells,[51] optoelectronic devices,[52] and chromogenic materials[53]).
As reported in Chapter 1 of this thesis, in which the reactions between 1,3-disubstituted arenes and benzenediazonium salts are reported, also in this case, two different products could be obtained; one with the electrophile in *ortho* position to both the amino groups (position 2, **A** in Scheme 11) and the other with the electrophile in *ortho* respect to one substituent and in *para* respect to the other one (position 4 or 6, **B** in Scheme 11).



Scheme 11. Possible products from the reaction involving diaminobenzene derivatives

2.3.2 Results and Discussion

The reactions between diaminobenzene derivatives **21a-d** and benzofurazan derivatives **22a-c** gave the substitution products **23-32**, in different yields as reported in Scheme 12; all the reactions were carried out in equimolar amount of reagents, in acetonitrile, at room temperature.



Scheme 12. Coupling reactions between the nucleophiles **21a-d** and the electrophiles **22a-c**.

[a] The reaction was also carried out at 80°C but no conversion was obtained. [b] in presence of basic alumina the yield was 60%.

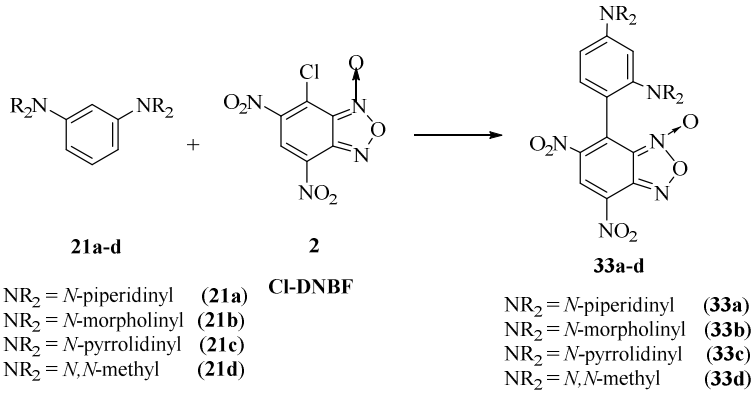
In all the performed reactions, except in the case of compound **21b**, only the substitution product derived from the attack of the electrophilic species in position 4 of the nucleophile, was obtained as the **B** form in Scheme 11.

In the case of 1,3-dimorpholinylbenzene (**21b**) no product was obtained, neither in the reported experimental conditions nor under reflux or in presence of a base (basic alumina or triethylamine).

As introduced in Chapter 1, given that the nucleophilicity values of **21a-d** are not yet known, the nitrogen nucleophilicity values, reported in the literature, for the secondary amines, in acetonitrile, might be useful to draw some considerations. The values in decreasing order, are: pyrrolidine 18.64,[54] dimethylamine 17.96,[55] piperidine 17.35,[54] morpholine 15.65.[54]

These data showed that the morpholine is the lower nucleophilic species among the involved amines and probably this is reflected in the absence of reaction between the dimorpholinyl derivative **21b** and the benzofurazan derivatives **22a-c**.

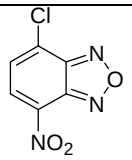
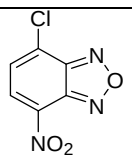
The reactions between diaminobenzene derivatives **21a-d** and 7-chloro-4,6-dinitrobenzofuroxan (**2**) gave the substitution products **33a-d** as reported in Scheme 13.

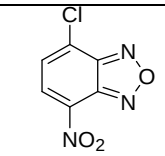
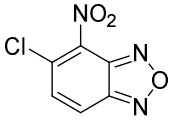
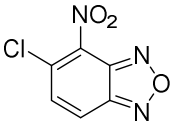
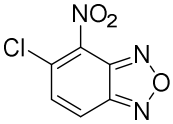
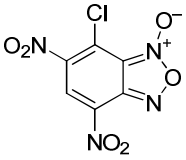
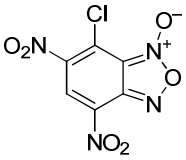
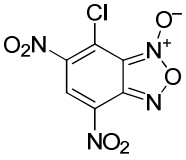
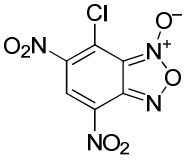


Scheme 13. Reactions between 7-chloro-4,6-dinitrobenzofuroxan (**2**) and 1,3-diaminobenzene derivatives **21a-d**.

In this case, thanks to the stronger electrophilic power of 7-chloro-4,6-dinitrobenzofuroxan (**2**), due to the presence of a further nitro group on the carbocyclic ring, also the substitution product **33b**, derived from the reaction with the morpholinyl derivative **21b**, was obtained.
Having in hands the substitution products and their spectral data, with the aim to investigate on the reactivity of the considered nucleophiles and electrophiles, we decided to perform the reactions between **21a-d** and the electrophilic species **2, 22a** and **22c**, directly in the NMR spectroscopy tube and to monitor the reaction outcome by ^{1}H-NMR spectroscopy. The reactions were carried out by mixing equimolar amount of reagents and the obtained results are collected in Table 2.

Table 2. Electrophile/nucleophile combinations monitoring via ^{1}H-NMR spectroscopy[a]

Reac.	**Electrophile**	**Nucleophile**	**Solv.**	Time → / Conv.%[b] / Product	**10 min**	**2h**	**24h**	**48h**	**72h**
1	 **(22a)**	DPBH **(21a)**	$CDCl_3$	**23**	-	4	21	26	26[c]
2			CD_3CN		9	12	48	50	52[d]
3	 **(22a)**	$DNMe_2BH$ **(21d)**	$CDCl_3$	**26**	4	21	40	40	n.c-
4			CD_3CN		25	65	73	76	n.c-

5	 **(22a)**	DPyBH (**21c**)	CD_3CN	**25**	42	53	53	56	n.c.
6	 **(22c)**	DPBH (**21a**)	CD_3CN	**29**	15	40	55	n.c.-	60
7	 **(22c)**	$DNMe_2BH$ **(21d)**	CD_3CN	**32**	16	40	55	-n.c.	55
8	 **(22c)**	DPyBH (**21c**)	CD_3CN	**31**	35	55	63	70	n.c.
9	 **(2)**	DPBH **(21a)**	CD_3CN	**33a**	73	100	/-	/	/
10	 **(2)**	DMBH **(21b)**	CD_3CN	**33b**	25	87	95	100	-/
11	 **(2)**	$DNMe_2BH$ **(21d)**	CD_3CN	**33d**	7	12	87	100	/
12	 **(2)**	DPyBH (**21c**)	CD_3CN	**33c**	100[e]	/	/	/	/

[a] Reactions carried out in equimolar amount of reagents. [b] Relative % conversion, calculated with respect to the signals ascribed to the unreacted electrophile in the ^{1}H-NMR spectrum. [c] 24 h after having added triethylamine the conversion reached 55%. [d] 24 h after having added triethylamine the conversion reached 100%. [e] In the ^{1}H-NMR spectrum are present also others unidentifined products.

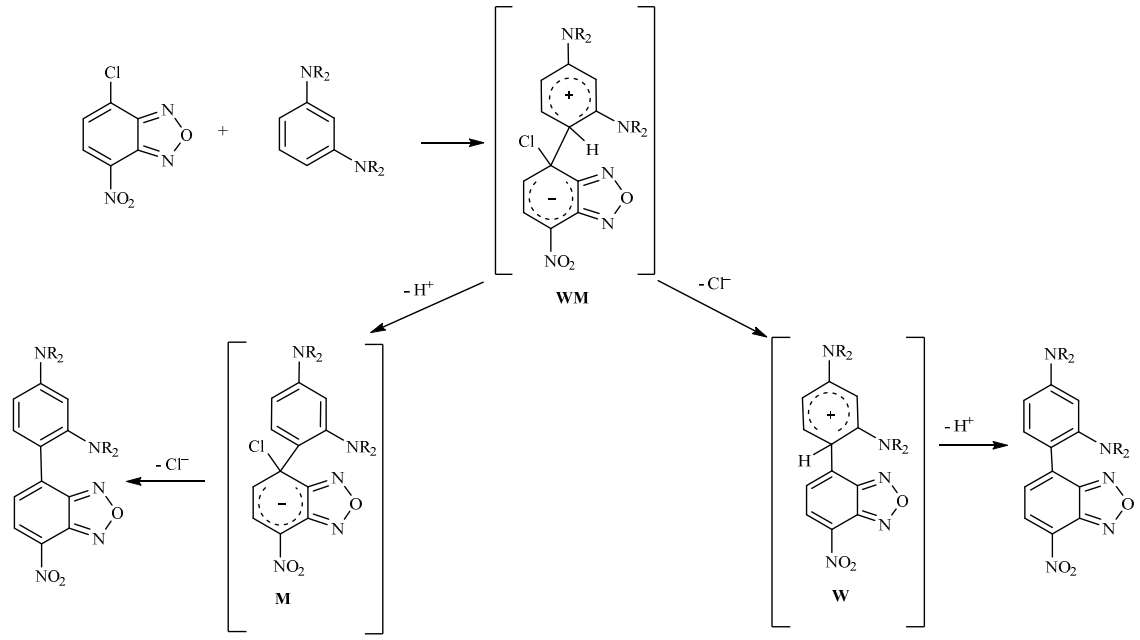
The data in Table 2 show that in the case of compound **22a** with the nucleophiles **21a** and **21d**, the reaction was performed in two different solvents (reactions 1-4) to investigate the effect of the solvent on the reagents conversion. The results showed an increasing of the conversion, when the reactions were performed in CD_3CN with respect to $CDCl_3$; based on these results, the subsequent reactions were carried out in deuterated acetonitrile.

It is interesting to note that, as obtained in the case of the reactions between 1,3-diaminobenzene derivatives and aryldiazonium salts, even if the reactions were carried out with equimolar amount of reagents, the final products were obtained in yields above 50% (except for reactions 1 and 3 carried out in chloroform), thus indicating that the produced hydrochloric acid in the reaction mixture doesn't react with the nucleophilic reagents, hindering the reaction progress, but that, likely, the proton expelled during the rearomatization process salifies a nitrogen atom of the coupling product, as observed in a previous study involving triaminobenzene and benzofurazan derivatives.[56] In the case of the reaction between **22a** and **21a**, since after 72 hours the conversion didn't increased, 5 equivalents of triethylamine was added to the reaction mixture to enhance the reaction progress; after 24 hours 55% (in $CDCl_3$) or 100% (in CD_3CN) yields, were obtained.

Comparing the data obtained from the reactions between **22a** and **2** with **21a** (reactions 2 and 9) and **21c** (reactions 5 and 12) in acetonitrile, a drastic increase of the conversion was observed on going from the nitrobenzofurazan reagent to the dinitrobenzofuroxan one, as expected for the presence of another nitro group on the aromatic ring that enhances the electrophilicity of the reaction center; moreover, when the reaction was carried out between **2** and 1,3-di(morpholinyl)benzene (case 10), opposite to the case involving the nitrobenzofurazan **22a**, the substitution product was obtained quantitatively.

In the case of the reactions involving the 4-chloro-7-nitrobenzofurazan (**22a**) (reactions 2, 4, 5) it can be observed that in the first reaction time, the conversion decreases varying the nucleophile in the order: DPYBH>$DNMe_2$BH>DPBH. Analogous considerations can be made for the reactions between **22c** with **21a**, **21c** and **21d** (reactions 6-8). In the cases of reactions between **2** and **21a-d**, a reactivity order DPYBH>DPBH>DMBH can be observed. Unexpectedly, the reaction with $DNMe_2$BH (reaction 11) gave low conversion that reached 100% after 48 h. This finding might be explained in terms of steric hindrance in case of approaching of the reagents, due to the presence of the dimethylamino substituents and of the nitro group in *ortho* to the reactive center of the electrophile.

The mechanism of the above considered reactions between benzofurazan derivatives and 1,3-diaminobenzene derivatives, involves the formation of different σ–intermediates, as reported in Scheme 14 for the reaction between **22a** and a generic 1,3-diaminobenzene.



Scheme 14. Possible intermediates in the S_EAr/S_NAr reactions between benzofurazan and 1,3-diaminobenzene derivatives.

First, a **WM** complex is formed, but, due to the presence of the chlorine as good leaving group, it is an elusive species, as well as the **M** intermediate. On the contrary, the observation of a **W**-like intermediate cannot be completely ruled out. In present cases NMR investigations at low temperature did no evidence of sigma intermediates.

Recently, a Wheland intermediate like **W** in Scheme 14 has been isolated and characterized from the reaction between **22a** and 1,3,5-tris(*N*-pyrrolidinyl)benzene.[56]

As in the study reported in Chapter 1, with the arenediazonium salts, again the diaminobenzene derivatives resulted not able enough to stabilize the positive charge of the σ–cationic intermediate, with respect to the triaminobenzene derivatives.

2.3.3 Conclusions

In this study the electrophile/nucleophile combination between 1,3-diaminobenzene derivatives and benzofuroxan and benzofurazan derivatives, gave selectively only the substitution product in *ortho* position (the less hindered position) to one of the two substituents on the aromatic ring of the nucleophile.

The obtained data gave new informations about the nucleophilicity power of the poorly studied diaminobenzene derivatives.

All the synthesized substitution products are new conjugated systems with an electron rich and an electron poor moiety on the same molecule and this peculiarity makes these products good candidates for different applications; finally the benzofuroxan derivatives are known to be interesting compound in pharmaceutical field due to their ability as NO donor, so the biological activity of the new synthesized benzofuroxan derivatives might be studied in the future for further applications.

2.3.4 Experimental section

The ^{1}H and ^{13}C NMR spectra were recorded with a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H NMR) and 100.56, or 150.80 MHz (for ^{13}C NMR), respectively. *J* values are given in hertz (Hz). Signal multiplicities were established by DEPT experiments. Chemical shifts were referenced to the solvent [δ =7.26 and 77.0 ppm for $CDCl_3$), (δ =2.0 and 0.3 ppm for CD_3CN), for ^{1}H and ^{13}C NMR, respectively]. ESI-MS spectra were recorded with a WATERS 2Q 4000 instrument. Chromatographic purifications were carried out on silica gel (0.037-0.063 mm, Merck) columns at medium pressure. Thin layer chromatography (TLC) was performed on silica gel 60 F254 coated aluminum foils (Fluka). Melting points were measured on a Stuart SMP3 apparatus and are uncorrected. Solvents and reagents were commercial materials (Aldrich or Fluka) if not specified. 1,3-bis(*N*,*N*-dialkylamino)benzene derivatives **21a-d**, were prepared from 1,3-dichlorobenzen (Sigma-Aldrich) with a modification of the reported literature[57,58] methods.

General procedure for the synthesis of compounds 21a,d:

The procedure to synthesize the nucleophilic species **21a** and **21d**, is the same, except for the starting amine that is piperidine (in case of **21a**) or dimethylamine (in case of **21d**).

In a three-necked flask, under nitrogen flow, 0.85 mL of dichlorobenzene ($7.45x10^{-3}$ mol) with 5.9 mL ($8x10^{-2}$ mol) of the amine (piperidine or dimethylamine), were dissolved in 50 mL of anhydrous THF. Then 30 mL of phenyllithium ($5.7x10^{-2}$ mol) was added dropwise to the reaction mixture. After 24 h, the reaction mixture was allowed to cool to room temperature and was quenched with water. The aqueous phase was extracted three times with diethyl ether and the combined organic phases were dried over magnesium sulfate, and

the solvent removed under vacuo. The resulting crude products were purified by silica gel column.

General procedure for the synthesis of compounds 21b,c:

Also in this case both syntheses require the same procedure and the only difference is the starting amine, that is morpholine (in case of **21b**) or pyrrolidine (in case of **21c**).

In a autoclave, 1.37 mL (0.011 mol) of dichlorobenzene and 0.07 mol ot the amine, were dissolved in 10 mL of toluene; after addition of 5.4 g of KO*t*-Bu, the vessel was sealed and heated at 160°C. After 4 days, the reaction mixture was allowed to cool to room temperature and was quenched with water. The aqueous phase was extracted three times with dichloromethane and the combined organic phases were dried over magnesium sulfate, and the solvent removed under vacuo. The resulting crude products were purified by silica gel column.

Reactions between 21a-d with 22a-c and 2. General Procedure:

To a magnetically stirred solution of the nucleophile (0.1 mmol of **22a-d**) dissolved in CH_3CN (5mL) was added the electrophile (**22a-c** or **2**, 0.1 or 0.2 mmol, respectively), at room temperature. TLC was used to monitor the reactions progress, with different eluents and ^{1}H-NMR analysis. Finally, the products were purified by column chromatography on silica gel (FC), using different eluents.

Some products, in particular the substitution products from the pyrrolidinyl derivatives, were obtained in low yields, for their partial decomposition on the chromatographic column. All the products were characterized by usual spectroscopic methods and their chemico-physical data are reported as follows.

4-(2,4-di(piperidin-1-yl)phenyl)-7-nitrobenzo[c][1,2,5]oxadiazole (23): 27% yield, m.p. > 200 ^{o}C dec. **^{1}H NMR** ($CDCl_3$, 600 MHz, 25°C) δ (ppm): 8,52 (d, *J* = 8,1Hz, 1 H); 8,26 (br.s, 1 H); 7.66 (d, *J* = 8.1 Hz, 1 H); 6.64 (br.s, 2 H); 3.35 (br.s, 4 H); 2.85 (br.s, 4 H); 1.85-1.60 (m, 6 H); 1.46 (br.s, 6 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C) δ (ppm): 154.5, 154.0, 150.1, 143.5, 139.5, 133.7, 131.3, 125.8, 116.8, 109.0, 105.8, 53.4, 49.1, 25.9, 25.5, 23.9. **ESI MS (ES$^+$) m/z:** 408 $[M+H]^+$, 430 $[M+Na]^+$, 446 $[M+K]^+$.

4-(2,4-di(pyrrolidin-1-yl)phenyl)-7-nitrobenzo[c][1,2,5]oxadiazole (25): 40% yield, m.p. > 280 ^{o}C dec. **^{1}H NMR** ($CDCl_3$, 600 MHz, 25°C) δ (ppm): 8.49 (d, *J* = 8.2 Hz, 1 H); 7.72 (d, *J* = 8.9 Hz, 1 H); 7.25 (d, *J* = 8.3 Hz, 1 H); 6.27 (dd, J_1 = 9.0 Hz, J_2 = 2.2 Hz, 1 H), 6.10 (s, 1 H), 3.41 (t, *J* = 6.7 Hz, 4 H), 3,06 (t, *J* = 6.7 Hz, 4 H), 2.10-2.03 (m, 4 H), 1.89-1.81 (m, 4 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C) δ (ppm): 150.4, 150.1, 149.9,

143.6, 140.5, 134.7, 132.0, 130.9, 124.1, 111.3, 104.7, 97.3, 52.2, 48.0, 25.7, 25.4. **ESI MS (ES^+) m/z:** 380 $[M+H]^+$, 402 $[M+Na]^+$, 418 $[M+K]^+$.

***N^1,N^1,N^3,N^3*-tetramethyl-4-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)benzene-1,3diamine (26):** 54% yield, m.p. > 280 °C dec. **^{1}H NMR** ($CDCl_3$, 300 MHz, 25°C): δ (ppm): 8.51 (d, *J* = 8.0 Hz, 1 H), 8.01 (d, *J* = 7.6 Hz, 1 H), 7.73 (d, *J* = 8.6 Hz, 1 H), 6.49 (d, *J* = 8.6 Hz, 1 H), 6.43 (s, 1 H), 3.09 (s, 6 H), 2.68 (s, 6 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 153.6, 152.2, 150.1, 143.7, 139.6, 134.4, 132.5, 131.8, 125.2, 114.9, 106.6, 102.6, 43.9, 40.7. **ESI MS (ES^+) m/z:** 328 $[M+H]^+$, 350 $[M+Na]^+$, 366 $[M+K]^+$.

4-(3,5-di(piperidin-1-yl)phenyl)-5-nitrobenzo[c][1,2,5]oxadiazole (27): 23% yield, **^{1}H NMR** ($CDCl_3$, 400 MHz, 25°C): δ (ppm): 7.91 (d, *J* = 9.5 Hz, 1 H), 7.82 (d, *J* = 9.5 Hz, 1 H), 7.42 (d, *J* = 8.4 Hz, 1 H), 6.85-6.54 (m, 2 H), 3.31 (br.s. 4 H), 2.70-2.53 (m, 4 H), 1.90-1.59 (m, 8 H), 1.35 (m, 4H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 153.9, 150.7, 149.2, 146.3, 131.7, 128.0, 126.7, 114.5, 109.7, 107.4, 53.6, 49.4, 25.8, 25.5, 24.8. **ESI MS (ES^+) m/z**: 408 $[M+H]^+$, 430 $[M+Na]^+$, 466 $[M+K]^+$.

***N^1,N^1,N^3,N^3*-tetramethyl-4-(5-nitrobenzo[c][1,2,5]oxadiazol-4-yl)benzene-1,3diamine (28):** 53% yield, m.p. > 155 °C dec. **^{1}H NMR** ($CDCl_3$, 300 MHz, 25°C): δ (ppm): 7.81 (d, *J* = 9.4 Hz, 1 H), 7.77 (d, *J* = 9.4 Hz, 1 H), 7.46 (d, *J* = 9.0 Hz, 1 H), 6.52 (d, *J* = 9.0 Hz, 1 H), 6.41 (br.s, 1 H), 3.06 (s, 6 H), 2.45 (br.s, 6 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 153.4, 152.5, 150.6, 149.2, 145.9, 132.1, 128.2, 126.4, 113.9, 112.3, 106.5, 103.1, 42.0, 40.3. **ESI MS (ES^+) m/z:** 328 $[M+H]^+$, 350 $[M+Na]^+$, 366 $[M+K]^+$.

5-(2,4-di(piperidin-1-yl)phenyl)-4-nitrobenzo[c][1,2,5]oxadiazole (29): m.p. > 120 °C dec. **^{1}H NMR** ($CDCl_3$, 600 MHz, 25°C): δ (ppm): 7.96 (d, *J* = 9.0 Hz, 1 H), 7.67 (d, *J* = 9.0, 1 H), 7.10 (br.s, 1 H), 6.63 (br.s, 2 H), 3.30 (br.s, 4 H), 2.84 (br.s, 4 H), 1.73 (br.s., 4 H), 1.64 (br.s, 4 H), 1.43 (br.s, 4 H). **^{13}C-NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 154.2, 153.5, 149.0, 144.2, 140.9, 137.2, 130.8, 122.5, 121.3, 118.7, 110.0, 106.1, 53.5, 49.3, 29.7, 25.9 (two signals overlapped), 24.1. **ESI MS (ES^+) m/z:** 408 $[M+H]^+$, 430 $[M+Na]^+$, 446 $[M+K]^+$.

5-(2,4-di(pyrrolidin-1-yl)phenyl)-4-nitrobenzo[c][1,2,5]oxadiazole (30): 27 % yield, m.p. > 115 °C dec. **^{1}H NMR** ($CDCl_3$, 400 MHz, 25°C): δ (ppm): 7.89 (d, *J* = 9.4 Hz, 1 H), 7.62 (d, *J* = 9.4 Hz, 1 H), 7.05 (d, *J* = 8.6 Hz, 1 H), 6.34-6.25 (m; 2 H, two signals overlapped), 3.42 (t, *J* = 6.5 Hz, 4 H), 3.16 (s; 2 H), 3.04 (s, 2 H), 2.08 (t, *J* = 6.48 Hz, 4 H), 1.83 (t, *J* = 6.33 Hz, 4 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 149.4,

148.9, 144.3, 142,2; 136.5, 132.1, 118.9, 105.8, 100.8, 51.7, 50.0, 25.6, 25.2. (selected data). **ESI MS (ES^+) m/z:** 380 $[M+H]^+$, 402 $[M+Na]^+$.

***N^1,N^1,N^3,N^3*-tetramethyl-4-(4-nitrobenzo[c][1,2,5]oxadiazol-5-yl)benzene-1,3-diamine (31):** 18 % yield, m.p. > 130 °C dec. **^{1}H NMR** ($CDCl_3$, 600 MHz, 25°C): δ (ppm): 7.94 (d, *J* = 9.4 Hz, 1 H), 7.64 (d, *J* = 9.4 Hz, 1 H), 7.11 (d, *J* = 8.6 Hz, 1 H), 6.50 (br.s, 2H, two signals overlapped), 3.07 (s, 6 H), 2.65 (s, 6 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 152.9, 152.1, 149.0, 148.9, 144.3, 140.8, 136.4, 132.8, 131.4, 127.5, 119.1, 107.7, 103.5, 43.3, 41.2. **ESI MS (ES^+) m/z:** 328 $[M+H]^+$, 350 $[M+Na]^+$.

4-(2,4-di(pyrrolidin-1-yl)phenyl)-5-nitrobenzo[c][1,2,5]oxadiazole (33a): 16% yield, m.p. > 280 °C dec. **^{1}H-NMR** ($CDCl_3$, 400 MHz, 25°C): δ (ppm): 8.80 (s, 1 H), 7.66 (d, *J* = 9.7 Hz, 2 H, two signals overlapped), 6.66 (br.s, 1 H), 3.43–3.30 (m, 4 H), 2.91-2.75 (m, 4 H), 1.77 (br.s, 2 H), 1.69 (s, 2 H), 1.49 (br.s, 4 H). **ESI MS (ES^+) m/z:** 469 $[M+H]^+$, 491 $[M+Na]^+$, 507 $[M+K]^+$.

4-(2,4-dimorpholinophenyl)-5,7-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (33b): 80% yield, m.p. > 280 °C dec. **^{1}H NMR** ($CDCl_3$, 300 MHz, 25°C): δ (ppm): 8.77 (s, 1 H), 7.00 (d, *J* = 9.0 Hz, 1 H), 6.67 (dd, J_1 = 8.5 Hz, J_2 = 1.8 Hz, 1 H), 6.62 (d, *J* = 1.85 Hz, 1 H), 3.87 (t, *J* = 4.6 Hz, 4 H), 3.50-3.39 (m, 4 H), 3.35 (t, *J* = 4.6 Hz, 4 H), 2.97-2.78 (m, 4 H). **^{13}C NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 154.3, 153.9, 144.3, 142.1, 134.1, 133.9, 131.1, 127.7, 113.7, 111.8, 110.3, 105.5, 67.0, 66.3, 52.8, 47.8. **ESI MS (ES^+) m/z:** 473 $[M+H]^+$, 495 $[M+Na]^+$, 511 $[M+K]^+$.

7-(2,4-di(pyrrolidin-1-yl)phenyl)-4,6-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide (33c): **^{1}H-NMR** ($CDCl_3$, 400 MHz, 25°C): δ (ppm): 8.85 (s, 1 H), 7.01 (d, *J* = 8.7 Hz, 1 H), 6.75 (d, *J* = 8.7 Hz, 1 H), 6.72 (br.s, 1 H), 3.68-3.59 (m, 4 H), 4.48-3.41 (m, 4 H), 2.17-2.05 (m, 8 H).

4-(2,4-bis(dimethylamino)phenyl)-5,7-dinitrobenzo[c][1,2,5]oxadiazole 1-oxide(33d): 45 % yield and 60% in presence of basic Al_2O_3, m.p. > 280 °C dec **^{1}H-NMR** ($CDCl_3$, 300 MHz, 25°C): δ (ppm): 8.88 (s, 1 H), 7.66 (d, *J* = 8.9 Hz 1 H), 6.56 (dd J_1 = 8.8 Hz, J_2 = 2.1 Hz, 1 H), 6.35 (s, 1 H), 3.14 (s, 6 H), 2.54 (s, 6 H). **^{13}C-NMR** ($CDCl_3$, 100.56 MHz, 25°C): δ (ppm): 154.1, 151.8, 143.2, 142.3, 134.2, 133.5, 131.4, 128.2, 111.3, 107.2, 102.4, 43.2, 40.2.

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CHAPTER 3

New electron-withdrawing/donor architectures from nitrothiophenes and 1,3,5-tris(dialkylamino)benzene derivatives

3.1 INTRODUCTION

Thiophene is an interesting compound from both synthetic and biological points of view.[1] Thanks to its interesting biological activity, such as nematocidal, insecticidal, antibacterial, antifungal, antiviral and antioxidant activity,[2] it is also incorporated in several pharmacologically active compounds.

Thiophene-based compounds have also found widespread use in drug design,[3] biodiagnostics,[4] electronic and optoelectronic devices[5] and conductive and electroluminescent polymers.[6] Also several reviews of various aspects of thiophene coordination and reactivity in transition metal complexes have been reported.[7]

Based on the above reported, thiophene derivatives are clearly interesting heterocycles and in order to obtain new compounds for applications in different fields and to extend our research to new nucleophile/electrophile combinations, we decided to investigate on their reactivity towards 1,3,5-tris(*N*,*N*-dialkylamino)benzenes.

As reported in the previous Chapters the reactions between different heterocycles bearing electron-withdrawing groups (mainly nitro groups) and nucleophiles at neutral carbon atom such as *sym*-triaminobenzene derivatives, gave relatively stable σ-anionic complexes of the aromatic substitution reactions.

Also in nitrothiophene series, several examples of formation of π neutral (with naphthalene) and σ-anionic complexes (with anionic nucleophiles) were reported;[8,9] these π and σ complexes were characterized by different spectroscopic techniques.

In this Chapter I will discuss results obtained performing S_NAr/S_EAr reactions between thiophene derivatives activated by nitro groups, and triaminobenzene derivatives.

In particular we decided to use two nitrothiophene derivatives, the 2-bromo-3,4,5-trinitrothiophene (**1**) and 2,3,4-trinitrothiophene (**2**), reported in Figure 1.

Figure 1. Nitrothiophene derivatives involved in this study

It has been reported[10] that 2-bromo-3,4,5-trinitrothiophene (**1**) reacts with aromatic amines giving, depending on the experimental conditions, either displacement of the nitro group in position 4 or of both nitro group, and the bromine atom. Also thiophenols replace simultaneously these groups whereas benzenesulfinic acid displaces the bromine and the nitro group in position 5. Up to now, only a few papers have appeared so far on the reactivity of **1**[8,10,11,12] while no reactions of trinitrothiophene (**2**) have been reported in the literature except about the formation of a π-complex with naphthalene,[13] and also its chemical properties have been very scarcely[2,13] investigated.

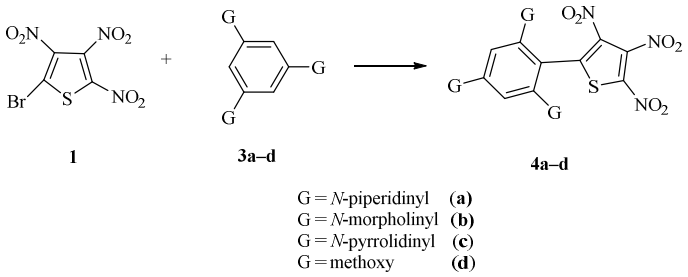
The aim of this study was to investigate on the possibility to detect reaction intermediates also in the combination between **1** or **2** and triaminobenzene derivatives. Obviously, when a powerful leaving group (X= Br) is present on the thiopene ring, the isolation of a σ-complex is a very hard goal. σ-Anionic complexes formation, in such kind of substrates is 'only' an hypothesis, but when X= H, it is expected to isolate moderately stable σ-complexes, because of the low ability of the hydride to act as a leaving group.

Moreover, in planning the present study, we considered that the hypothetical new coupling products from these reactions, might be new and interesting thiophene derivatives, bearing simultaneously an electron-rich and an electron-poor moiety, making them good candidates for application such as solar energy conversion and optoelectronic devices.[14]

3.2 RESULTS AND DISCUSSION

- *Reactions between 2-bromo-3,4,5-trinitrothiophene and tris(N,N-dialkylamino)benzene derivatives*

The reactions between 2-bromo-3,4,5-trinitrothiophene (**1**) and tris(*N*,*N*-dialkylamino)benzene derivatives **3a** and **3b** afforded the product derived from the expected substitution reaction at the carbon bearing the bromine atom (Scheme 1).



Scheme 1. Reactions between 2-bromo-3,4,5-trinitrothiophene (**1**) and nucleophiles **3a-d**.

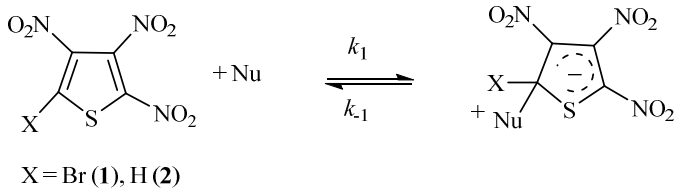
When the reactions were carried out in acetonitrile (reactants in equimolar ratio), and without a base, **4a** and **4b** have been obtained in 61 and 55% yield, respectively. In these conditions, the formed hydrobromic acid can react with **3** giving the relevant salt: the finding that compounds **4a** and **4b** have been obtained in yield higher than 50% can be considered an indication of partial salification of the final product. When the reactions of Scheme 1 were carried out in equimolar ratio of reagents and in the presence of basic alumina to avoid the formation of salts between HBr and the starting nucleophiles (or reaction products), **4a** and **4b** were obtained in 82% and 65% yield, respectively. In contrast, the reaction between **1** and **3c** afforded a complex reaction mixture. To extend this behaviour to other nucleophilic benzenes, we carried out also the reaction between 1,3,5-trimethoxybenzene (**3d**) and **1**: the reaction appeared slower that those with **3a-c** and compound **4d** was obtained in 47% yield.

It is known that the reactions of **1** with anionic or neutral nucleophiles yielded both bromo and nitro substitution. [8,10-12] In the present case, also carrying out the reactions with two or more equivalents of tris(amino)benzene only the product **4** of bromo substitution was isolated: the replacement of bromine atom is surely the main process even if in the reaction mixtures there are, in some cases, low amount of starting materials and traces of unidentified compounds.

- *Reactions between 2,3,4-trinitrothiophene and tris(N,N-dialkylamino)benzene derivatives*

Starting from the consideration that the departure of H^- from a σ-complex is a difficult process and usually it can only return-back to starting materials, as depicted in Scheme 2,

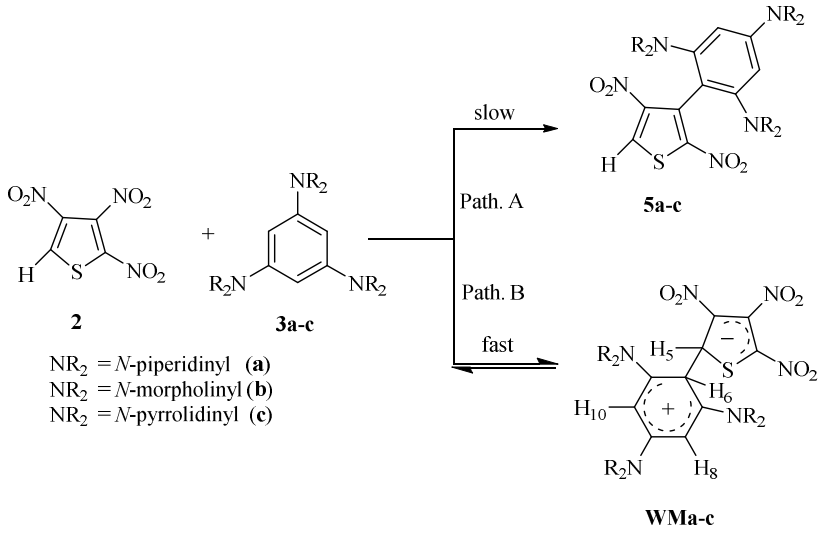
we planned to investigate more in detail on the reactivity of 2,3,4-trinitrothiophene (**2**), towards triaminobenzenes **3a-c**.



Scheme 2. Reaction pathways for the trinitrothiophene derivatives/nucleophile interactions.

The reactions were carried out directly in the NMR spectroscopy tube, in CD_2Cl_2, with variable temperature experiments (from –70°C to +25°C). The recorded ^{1}H NMR spectra of the reaction mixtures obtained by mixing at -70 °C equimolar amounts of **2** and **3a** (or **3c**) showed that this reaction is complicated by the presence of several products.

Among them, **WMa** and **WMc** complexes (Scheme 3) were identified owing the presence, in the ^{1}H NMR spectrum, of four signals with the same integration value in a region typical of diagnostic signals of **WM** complexes.[15]



Scheme 3. Formation of products **5a-c** and **WMa-c**.

In particular, immediately after the mixing of **2** and **3a**, four broad singlets at 5.48, 5.36, 4.98, and 4.95 ppm appeared as reported in Figure 2.

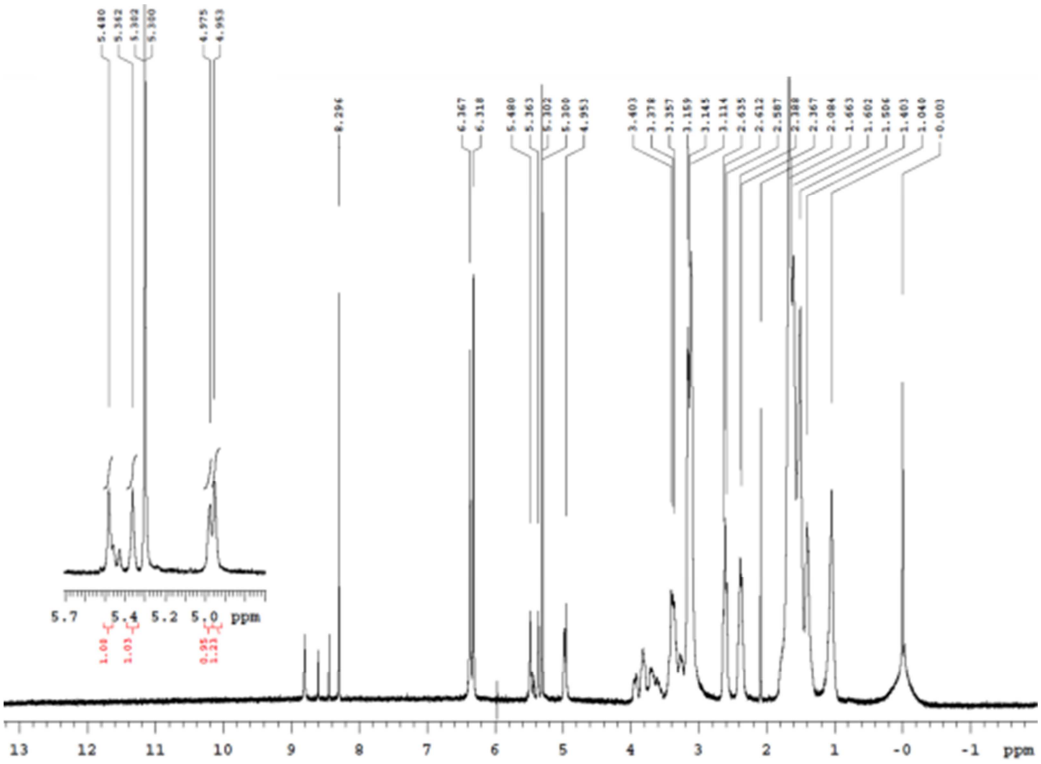


Figure 2. ^{1}H NMR spectrum, in CD_2Cl_2, at -70 °C of the reaction mixture from **2** and **3a**,with expanded view of diagnostic signals belonging to **WMa** (solvent peak at 5.3 ppm).

Direct proton to carbon correlation, obtained at −70 °C showed that the two signals at δ = 4.95 and 4.98 ppm are connected directly to carbon atoms resonating at δ = 55.3 and 39.3 ppm, respectively, a clear evidence for the sp^3 hybridization of these carbon atoms. The two hydrogen atoms which resonate at δ = 5.48 and 5.36 ppm are connected to two carbon atoms at δ = 91.8 and 87.4 ppm: chemical shift values typical for the sp^2-hybridized CH carbon atoms of 1,3,5-triaminobenzene derivatives.[15-17] The two distinct hydrogen (and carbon) signals are due to the presence of an asymmetric carbon center on the thiophene moiety and a "C-2 center" (sp^3 carbon) of the triaminobenzene moiety that makes the two carbon atoms (and the hydrogen atoms bound to them, H-8 and H-10 in Scheme 3) diastereotopic and thus anisochronous signals in both the ^{1}H and ^{13}C NMR spectra appear.

The reaction between **2** and **3c** also evidenced the presence of the zwitterionic intermediate (**WMc**) in the NMR spectrum at −70 °C, whose structure was ascertained by both direct proton to carbon (*g*-HSQC sequence) and proton to proton (*g*-COSY sequence) correlation experiments. When the temperature was slowly increased, signals related to **WMa** and

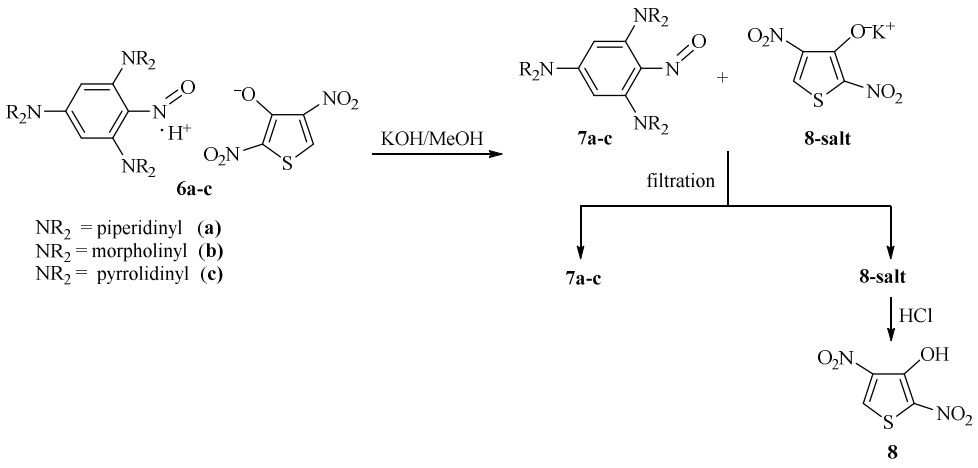
WMc gradually broadened until to disappear at about –30 °C (successive lowering of the temperature did not give return-back to the **WM** signals).

From these experiments we were also able to isolate and identify compounds **5a** and **5c**, among other compounds formed during the mixing of the reagents at –70 °C, whose signals remained almost unchanged until +25 °C.

No evidence of **WMb** was obtained from the reaction carried out in CD_2Cl_2 at –70 °C between **2** and **3b**; only peaks of starting reagents and traces of **5b** were present in the spectrum until about 0 °C whereas at 25 °C the spectrum became more complex and signals of **5b** gradually increased as those of the starting reagents disappeared. Compounds **5a–c** arise from a de-nitro-substitution reaction in position 3 of the thiophene ring and they have been obtained in yield lower than 50%.

After each experiment we noted the presence of a precipitate in the NMR tube. This solid was separated and its ^1H NMR signals matched with those of minor signals observed in the spectra of the reaction mixture recorded at different temperatures; likely, due to its scarce solubility in CD_2Cl_2, this compound seemed to be a minor constituent in the reaction mixture.

This solid resulted to be compounds **6a-c**, as reported in Scheme 4. Structure **6** was confirmed by NMR spectral data and also by isolation and characterization of its neutral constituents **7** and **8** (Scheme 4).



Scheme 4. Isolation of compounds **7a-c** and **8**.

NMR data of the free bases, *i.e.* 1-nitroso-1,3,5-(*N*,*N*-dialkylamino)benzene derivatives **7a-c**, obtained by treatment of **6a-c** with methanolic solution of KOH, agree with literature data,[18] whereas 2,4-dinitrothiophen-3-ol (**8**) obtained by treatment of **8-salt** with HCl solution, has never been reported so far. Moreover, the mixing of equimolar amounts of

compound **7b** and **8** produced ^{1}H NMR signals of the triaminobenzene moiety matching with those of **6b**.

Based on the previous data of the research group[19] about the interaction between triaminobenzenes and proton, there are four main possibilities (**A**-**D** in Figure 3) about the proton position on the cationic part of the salts **6a-c**.

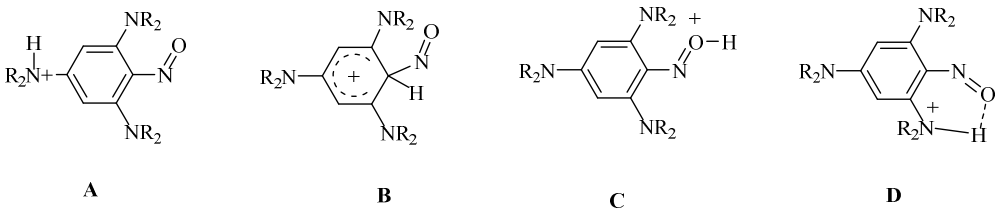


Figure 3. Possible structures for the cationic part of salts **6a-c**.

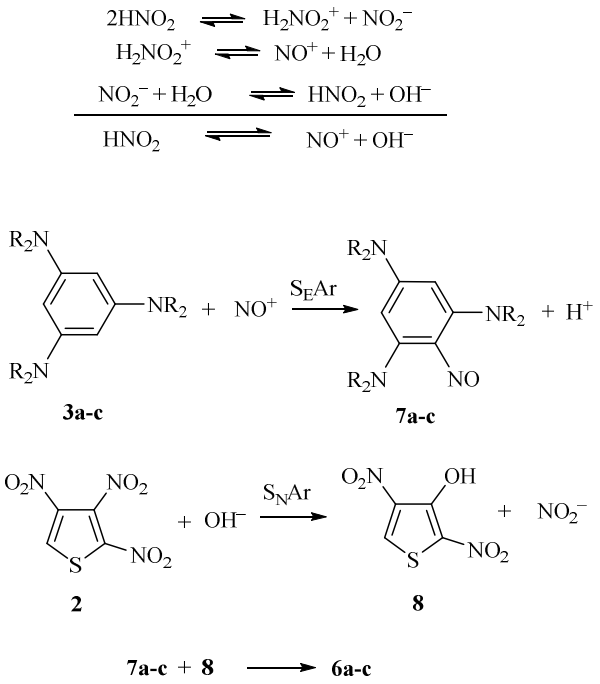
In structure **A** the proton is on a nitrogen atom of the piperidine moiety, instead **B** is a Wheland complex which may be in equilibrium with **A**.[19]

Structure **C** presents the protonated nitroso group, similarly to what indicated by Effenberger[20] in a paper in which compounds **6a–c** were prepared from **3a–c** and N_2O_4.

The ^{1}H NMR spectra recorded for the salts **6a–c**, showed two signals related to protons bound to the aromatic ring, indicating **A** and **C** as the unprobable structures, owing to the symmetry of the two protons of the aromatic ring. In our opinion, structure depicted as **D** in Figure 3, in which the proton bound to the nitrogen atom is involved in a hydrogen bond between the piperidinyl nitrogen and the oxygen atom of the nitroso group, is the more probable structure.

*Proposed reaction pathway for the formation of compounds **6a-c***

It is interesting to observe that compounds **6a-c** have been obtained as the major products; the pathway depicted in Scheme 5 might tentatively explain the unexpected formation of salts **6a-c**.



Scheme 5. Proposed reaction pathway to explain the formation of compounds **6a-c.**

Nitrous acid, derived from the reaction between **2** and **3** to give **5**, can decompose, in absence of water (reactions were carried out in dichloromethane or in acetonitrile) into nitrosonium and hydroxide ions through the self-protonation process depicted in Scheme 5 (up). The two ions thus formed can attack triaminobenzene and trinitrobenzene by S_EAr and S_NAr, respectively.

The reaction produces, besides **7** and **8**, a further amount of nitrous acid that, in turn, can decompose promoting the formation of a further amount of **7** and **8**, as occur in an autocatalytic cycle.

Compounds **7** and **8** can form the salts **6**, as confirmed by adding **7a** to a CD_3CN solution of **8**. The occurrence of these reactions might be the possible reason of both, the low yields found for compound **5a**–**c** and the high yields of the recovered salts **6a**–**c**.

3.3 CONCLUSIONS[21]

In conclusion, in the present study the first examples of reactions between trinitrothiophene derivatives and sym-triaminobenzene derivatives. The structure of the coupling product obtained using 2-bromo-3,4,5-trinitrothiophene (**1**), revealed that only the de-bromination substitution reaction occurs; under our experimental conditions, no evidence of de-nitrosubstitution reactions was obtained.

A very peculiar reactivity was observed from the reactions between 2,3,4-trinitrothiophene (**2**) and triaminobenzenes **3a–c**, that gave the first detection of zwitterionic σ-complexes (**WM**) in thiophene series; these intermediates were obtained by the attack, in a fast step, on the unsubstituted carbon atom (C-5) of the thiophene ring. This attack competes with that on the carbon bearing the nitro group in position 3 of the thiophene ring that produces new compounds bearing the triaminobenzene moiety at C-3; the nitro group departure eliminates the possibility to return back to starting materials while the only possibility for **WM** is the return to starting reagents.

These reactions are also complicated by other processes, one of them is the formation of a salt that, after neutralization, provided 1-nitroso-2,4,6-triaminobenzene derivatives and the hitherto unknown 2,4-dinitrothiophen-3-ol. Moreover, present findings can be considered a new method to synthesize 1-nitroso-2,4,6-triaminobenzenes and, even more interestingly, the C–C couplings herein reported gives access to new highly conjugated structures, bearing both electron-poor and electron-rich moieties, probably interesting substrates for different applications.

3.4 EXPERIMENTAL SECTION

The ^{1}H and ^{13}C NMR spectra were recorded on a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H NMR) and 100.56, or 150.80 MHz (for ^{13}C NMR), respectively. Chemical shifts were measured in δ (ppm) with reference to the solvent [for ^{1}H and ^{13}C NMR, respectively: δ = 5.30 ppm and 54.2 ppm for CD_2Cl_2; δ = 7.26 ppm and 77.0 ppm for $CDCl_3$; δ = 2.50 ppm and 39.50 ppm for $(CD_3)_2SO$; δ = 3.31 ppm and 49.2 ppm for CD_3OD; δ = 1.96 ppm and 118.1 ppm for CD_3CN. *J* values are given in Hz. Signal multiplicities were established by DEPT experiments. The variable–temperature NMR spectra and 2D low-temperature spectra (*g*-COSY and *g*-HSQC) were recorded on a Mercury 400 spectrometer. ESI-MS spectra were recorded with a WATERS 2Q 4000 instrument. Chromatographic purifications were carried out on columns of silica gel (0.037-0.063 mm) or aluminium oxide, activated, basic, Brockmann I, standard grade ca. 150 mesh at medium pressure. 1,3,5-Trimethoxybenzene (**3d**) is commercially available, 1,3,5-tris(*N*,*N*-dialkylamino)benzenes **3a–c** were prepared as described previously,[15] as well as bromotrinitrothiophene (**1**) and trinitrothiophene (**2**).[22] Given that NMR spectra of **1** and **2** have been never reported so far, we report them below (it is noteworthy that ^{13}C NMR spectra show some signals as triplet, likely due to carbon-nitrogen coupling).[23]

2-bromo-3,4,5-trinitrothiophene (1): **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25 °C), δ (ppm): 140.1 (br.s., C), 136.8 (br.s., C), 136.2 (t, J_{C-N} = 15.0 Hz, C).

2,3,4-trinitrothiophene (2): ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C), δ (ppm): 8.57; **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25 °C), δ (ppm): 142.3 (t, J_{C-N} = 13.2 Hz, C), 137.8 (br.s., C), 135.8 (t, J_{C-N} = 14.8 Hz, C), 129.9 (CH).

Preparation of compounds 4a–d. General procedure. 2-Bromo-3,4,5-trinitrothiophene (**1**) (0.030 g, 0.1 mmol) was added to an equimolar amount of 1,3,5-tris(dialkylamino)benzene (**3a**, **3b**, **3c**, or **3d**) dissolved in CH_3CN (5 mL). Immediately after mixing, the colour of the reaction mixture turned to red or blue. The progress of the reaction, magnetically stirred, was monitored by TLC and ^{1}H NMR analysis. The product was purified by flash chromatography on silica gel (petroleum light/Et_2O 8:2 v/v for **4a**, n-hexane/ethyl acetate 4:6 for **4b**). The reactions were carried out also in the presence of basic alumina; that was filtered off after disappearance of starting material on TLC; products were then quickly purified as above described. The yields reported below are referred to the first procedure with equimola amount of reagents.

1,1',1''-[2-(3,4,5-trinitro-2-thienyl)benzene-1,3,5-triyl]tripiperidine (4a): blu-violet solid, 33 mg, 61% yield, m.p.: > 300 °C (dec.). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C), δ (ppm): 6.36 (s, 2 H), 3.32 (t, *J* = 4.78 Hz, 4 H), 2.80-2.66 (m, 8 H), 1.74-1.62 (m, 6 H), 1.62-1.53 (m, 8 H), 1.53-1.43 (m, 4 H). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25 °C), δ (ppm): 154.9 (C), 154.8 (C), 144.6 (C), 137.0 (C), 136.0 (C), 134.4 (C), 107.6 (C), 102.0 (CH), 54.0 (NCH_2), 48.6 (NCH_2), 25.7(NCH_2CH_2), 25.6 (NCH_2CH_2), 24.2 ($NCH_2CH_2CH_2$), 24.1 ($NCH_2CH_2CH_2$). **ESI MS (ES$^+$) m/z:** 545 $[M+H]^+$, 567 $[M+Na]^+$, 583 $[M+K]^+$. Anal. Calcd for $C_{25}H_{32}N_6O_6S$: C, 55.13; H, 5.92; N, 15.43. Found: C, 55.21; H, 5.94; N, 15.45.

4,4',4''-[2-(3,4,5-trinitro-2-thienyl)benzene-1,3,5-triyl]trimorpholine (4b): purple solid, 30.3 mg, 55% yield, m.p.: 200 °C (dec.). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C), δ (ppm): 6.43 (s, 2 H), 3.87 (t, *J* = 4.9 Hz, 4 H), 3.70 (t, *J* = 4.9 Hz, 8 H), 3.31 (t, *J* = 4.9 Hz, 4 H), 2.85-2.79 (m, 8 H). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25 °C), δ (ppm): 154.9 (C), 153.5 (C), 143.1 (C), 137.0 (C), 136.6 (C), 134.8 (C), 108.7 (C), 102.4 (CH), 66.51 (OCH_2), 66.45 (OCH_2), 52.6 (NCH_2), 47.5 (NCH_2). **ESI MS (ES$^+$) m/z:** 573 $[M+Na]^+$, 589 $[M+K]^+$. Anal. Calcd for $C_{22}H_{26}N_6O_9S$: C, 48.00; H, 4.76; N, 15.27. Found: C, 48.12; H, 4.78; N, 15.30.

2,3,4-trinitro-5-(2,4,6-trimethoxyphenyl)thiophene(4d): orange solid, 18.1 mg, 47% yield. **^{1}H NMR** (600 MHz, CD_3CN, 25 °C), δ (ppm): 6.35 (s, 2 H, aromatics), 3.92 (s, 3H, OCH_3), 3.84 (s, 6 H, OCH_3). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C), δ (ppm): 6.18 (s, 2 H, aromatics), 3.89 (s, 3H, OCH_3), 3.81 (s, 6 H, OCH_3). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25

°C,), δ (ppm, selected): 165.0 (C), 158.8 (C), 152.1 (C), 150.3 (C), 140.5 (C), 97.7 (C), 91.0 (CH), 55.9 (OCH_3), 55.7 (OCH_3). **ESI MS (ES^+) m/z:** 386 $[M+H]^+$, 408 $[M+Na]^+$, 428 $[M+K]^+$. Anal. Calcd for $C_{13}H_{11}N_3O_9S$: C, 40.52; H, 2.88; N, 10.91. Found: C, 40.41; H, 2.89; N, 10.88.

Preparation of compounds 5a–c and 6a-c.

Compounds **5a**–**c** and **6a**–**c** were first isolated by chromatography on silica gel column of the final reaction mixture between **2** and **3** (or **4**, **5**) derived from experiments carried out in the NMR spectroscopy tube. Compounds **6a-c** were isolated by filtration from the above reaction mixture. Compounds **5a**–**c** were also obtained carrying out the reaction in a larger scale: to a magnetically stirred solution of 1,3,5-tris(dialkylamino)benzene (0.15 mmol) in CH_2Cl_2 or CH_3CN (5 mL), an equimolar amount of 2,3,4-trinitrothiophene (**2**) was added. Immediately after mixing, the reaction mixture became dark red or violet. The solution was stirred for 1 hour (using **3** or **5**) and 12 hours (for **4**) and the progress of the reaction was monitored by TLC and ^{1}H NMR analysis. During the reaction time a solid was formed and then separated from the reaction mixture by filtration. Compounds **5a-c** (very dark solids) were purified by flash chromatography on silica gel (eluent: dichloromethane/n-hexane, in different ratio depending on the polarity of the differents products) of the concentrated mother liquor. The solid precipitated were compounds **6a-c**; in some cases precipitation was favored by addition of diethyl ether to the reaction mixture. Crude compounds **6a-c** were subjected to treatment for obtaining neutral components (see below).

1,1',1''-[2-(2,4-dinitro-3-thienyl)benzene-1,3,5-triyl]tripiperidine (5a): dark blue solid, 18.7 mg, 25% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C), δ (ppm): 8.22 (s, 1 H, CH thioph), 6.41 (s, 2 H, CH arom.), 3.24 (t, *J* = 5.7 Hz, 4 H, NCH_2), 2.70–2.56 (m, 8 H, NCH_2), 1.78–1.66 (m, 4 H, NCH_2CH_2), 1.66–1.57 (m, 2 H, NCH_2CH_2), 1.42–1.29 (m, 12 H, NCH_2CH_2 and $NCH_2CH_2CH_2$). **^{13}C NMR** (100.56 MHz, $CDCl_3$, 25 °C), δ (ppm): 154.2 (C), 154.0 (C), 146.6 (C), 145.6 (C), 133.5 (C), 127.3 (CH), 111.7 (C), 103.0 (CH), 53.6 (NCH_2), 49.5 (NCH_2), 26.4 (2 sign. overlapped, NCH_2CH_2), 25.9 (CH_2), 24.3 ($NCH_2CH_2CH_2$). **ESI MS (ES^+) m/z:** 500 $[M+H]^+$, 522 $[M+Na]^+$, 538 $[M+K]^+$. Anal. Calcd for $C_{25}H_{33}N_5O_4S$: C, 60.10; H, 6.66; N, 14.02. Found: C, 60.19; H, 6.68; N, 14.05. 'X-ray diffraction analysis of a single crystal of **5a** showed that the triaminobenzene moiety is bound at the C-3 of the thiophene ring but, unfortunately, due to the symmetry of the cell, the resolution of the structure was not satisfactory for the requirements for the deposit in CCDC.

4,4',4''-[2-(2,4-dinitro-3-thienyl)benzene-1,3,5-triyl]trimorpholine (5b): dark purple solid, 21.2 mg, 28% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C), δ (ppm): 8.27 (s, 1 H, CH thioph), 6.46 (s, 2 H, arom), 3.88 (t, *J* = 4.9 Hz, 4 H, OCH_2), 3.53–3.47 (m, 8 H, OCH_2), 3.27 (t, *J* = 4.9 Hz, 4 H, NCH_2), 2.72–2.65 (m, 8 H, NCH_2). **^{13}C NMR** (150.80 MHz, $CDCl_3$, 25 °C), δ (ppm): 153.5 (C), 152.6 (C), 146.5 (C), 146.1 (C), 132.1 (C), 127.5 (CH), 113.0 (C), 103.1 (CH), 67.0 (OCH_2), 66.7 (OCH_2), 52.4 (NCH_2), 48.4 (NCH_2). **ESI MS (ES$^+$) m/z:** 506 $[M+H]^+$, 528 $[M+Na]^+$. Anal. Calcd for $C_{22}H_{27}N_5O_7S$: C, 52.27; H, 5.38; N, 13.85. Found: C, 52.33; H, 5.39; N, 13.81.

1,1',1''-[2-(2,4-dinitro-3-thienyl)benzene-1,3,5-triyl]tripyrrolidine (5c): dark brown solid, 30.2 mg, 44% yield. **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C), δ (ppm): 8.10 (s, 1 H, CH thioph), 5.94 (s, 2 H, arom), 3.34 (t, *J* = 6.6 Hz, 4 H, NCH_2), 2.83–2.78 (m, 4 H, NCH_2), 2.78–2.72 (m, 4 H, NCH_2), 2.01–1.97 (m, 4 H, $NCH_2C\mathit{H}_2$), 1.77–1.65 (m, 8 H, $NCH_2C\mathit{H}_2$); **^{1}H NMR** (400 MHz, CD_2Cl_2, –70 °C) δ (ppm): 8.13 (s, 1 H), 5.76 (s, 2 H), 3.25 (br.t, *J* = 6.11 Hz, 4 H), 2.73–2.55 (m, 8 H), 1.90 (br.t, *J* = 6.11 Hz, 4 H), 1.70–1.53 (m, 8 H);
^{1}H NMR (400 MHz, CD_3CN, 25 °C), δ (ppm): 8.35 (s, 1 H), 5.96 (s, 2 H), 3.33 (t, *J* = 6.7 Hz, 4 H), 2.82–2.75 (m, 4 H), 2.75-2.67 (m, 4 H), 2.05–2.00 (m, 4 H), 1.75–1.63 (m, 8 H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, 25 °C) δ (ppm): 151.6 (C), 150.5 (C), 148.2 (C), 145.9 (C), 136.8 (C), 128.0 (CH), 104.1 (C), 95.6 (CH), 52.1 (NCH_2), 48.3 (NCH_2), 26.3 ($NCH_2\mathit{C}H_2$), 25.7 ($NCH_2\mathit{C}H_2$). **ESI MS (ES$^+$) m/z:** 458 $[M+H]^+$, 480 $[M+Na]^+$, 496 $[M+K]^+$. Anal. Calcd for $C_{22}H_{27}N_5O_4S$: C, 57.75; H, 5.95; N, 15.31. Found: C, 57.72; H, 5.96; N, 15.28.

1-(2-nitroso-3,5-dipiperidin-1-ylphenyl)piperidin-1-ium2,4-dinitrothiophen-3-olate (6a): dark red solid, 49.1 mg, 60% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 8.31 (s, 1 H, thiop), 5.36 (d, *J* = 1.9 Hz, 1 H, arom), 5.24 (d, *J* = 1.9 Hz, 1 H, arom), 3.62–3.56 (m, 4 H, NCH_2), 3.53–3.47 (m, 4 H, NCH_2), 3.37–3.19 (m, 4 H, NCH_2), 1.88–1.56 (m, 18 H, $NCH_2C\mathit{H}_2$ and $NCH_2CH_2C\mathit{H}_2$). **^{13}C-NMR** (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm): 162.0 (C), 160.8 (C), 157.2 (C), 151.0 (C), 141.0 (C), 140.6 (C), 134.6 (CH), 124.5 (C), 87.4 (CH), 86.5 (CH), 51.2 (NCH_2), 50.8 (NCH_2), 49.5 (NCH_2), 26.18 ($NCH_2\mathit{C}H_2$), 25.8 ($NCH_2\mathit{C}H_2$), 25.5 ($NCH_2\mathit{C}H_2$), 24.1 ($NCH_2CH_2\mathit{C}H_2$), 24.0 ($NCH_2CH_2\mathit{C}H_2$), 23.8 ($NCH_2CH_2\mathit{C}H_2$). **ESI MS (ES$^+$) m/z:** 357 $[M]^+$; **ESI MS (ES$^-$) m/z:** 189 $[M-H]^-$.

4-(3,5-dimorpholin-4-yl-2-nitrosophenyl)morpholin-4-ium 2,4-dinitrothiophen-3-olate (6b): dark red solid, 53.8 mg, 65% yield. **^{1}H NMR** (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 8.71 (s, 1 H, thioph), 5.77 (br.s., 1 H, arom), 5.66 (br.s., 1 H, arom), 3.85–3.67 (m, 4 H,

OCH_2), 3.74–3.64 (m, 12 H, OCH_2 and NCH_2), 3.62–3.49 (m, 4 H, NCH_2), 3.44–3.30 (m, 4 H, OCH_2). **^{13}C-NMR** (100.56 MHz, DMSO-d_6 , 25 °C) δ (ppm): 161.6 (C), 160.7 (C), 156.4 (C), 150.8 (C), 141.9 (C), 140.8 (C), 137.1 (CH), 121.1 (C), 89.0 (CH), 87.4 (CH), 66.0 (OCH_2), 65.8 (OCH_2), 49.6 (NCH_2), 48.2 (NCH_2). **ESI MS (ES^+) m/z:** 363 $[M]^+$; **ESI MS (ES^-) m/z:** 189 $[M\text{-}H]^-$.

1-(2-nitroso-3,5-di(pyrrolidin-1-yl)phenyl)pyrrolidin-1-ium 2,4-dinitrothiophen-3-olate (6c): dark red solid, 31.0 mg, 41% yield. **^{1}H NMR** (400 MHz, CD_3CN, 25 °C) δ (ppm): 8.39 (s, 1 H, thioph), 5.00 (d, *J* = 2.3 Hz, 1 H, arom), 4.89 (d, *J* = 2.3 Hz, 1 H, arom), 3.83–3.20 (m, 12 H, NCH_2), 2.10–1.95 (m, 12 H, $NCH_2C\mathit{H}_2$). **^{13}C NMR** (100.56 MHz, CD_3CN , 25 °C) δ (ppm): 163.7 (C), 162.8 (C), 159.2 (C), 154.3 (C), 151.6 (C), 149.8 (C), 144.9 (C), 136.2 (CH), 87.2 (CH), 85.9 (CH), 51.8 (NCH_2), 50.2 (NCH_2), 50.1 (NCH_2), 25.8 ($NCH_2\mathit{C}H_2$), 25.4 ($NCH_2\mathit{C}H_2$), 25.3 ($NCH_2\mathit{C}H_2$). **ESI MS (ES^+) m/z:** 315 $[M]^+$; **ESI MS (ES^-) m/z:** 189 $[M\text{-}H]^-$.

Isolation of compounds 7a–c and 8. General procedure

A $3.9x10^{-2}$ M methanolic/KOH solution was added to an equimolar amount (0.05 mmol) of the salt **6** dissolved in methanol. After about 30 min a red solid precipitated; this solid was collected by filtration and dried. NMR analysis indicated presence of a single product. The solid was treated with an equimolar amount of 0.15 M aqueous hydrochloric acid. After dilution with water and extraction with ethyl acetate, the organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated; chemico-physical data of the residue agreed with structure **8**. The mother liquor remained after treatment of **6** with KOH/CH_3OH was concentrated and the ^{1}H NMR of the residue revealed the presence of a main product that was isolated by chromatography on basic alumina (eluent: dichloromethane/methanol, 9.5/0.5) and was identified as the neutral compound **7**. Mixing equimolar amount of **7b** and **8** in CD_3CN gave signals of **6a**. Moreover, the treatment of compound **7a** (or **7b**) with one equivalent of picric acid produced ^{1}H NMR signals of the triaminobenzene moiety similar to those of **6a** (or **6b**).

Chemico-physical data of compounds **7a–c** were according to those reported in literature. [4,5] Since in the literature NMR data for **7a–c** are partial, below we reports NMR and mass data for them, together with data for compound **8**.

1,1',1''-(2-nitrosobenzene-1,3,5-triyl)tripiperidine (7a):[18,20] red solid, 12.5 mg, 70% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 5.50 (s, 2 H, arom), 3.48–3.42 (m, 4 H, NCH_2), 3.36–3.24 (m, 8 H, NCH_2), 1.81–1.61 (m, 18 H, $NCH_2C\mathit{H}_2$ and $NCH_2CH_2C\mathit{H}_2$). **^{13}C**

NMR (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm): 158.0 (C), 147.7 (C), 103.0 (C), 88.6 (CH), 52.5 (NCH_2), 48.5 (NCH_2), 25.8 (NCH_2CH_2), 25.6 (NCH_2CH_2), 24.5 ($NCH_2CH_2CH_2$), 24.4 ($NCH_2CH_2CH_2$). **ESI MS (ES^+) m/z:** 357 $[M+H]^+$, 379 $[M+Na]^+$.

4,4',4''-(2-nitrosobenzene-1,3,5-triyl)trimorpholine (7b):[18,20] green solid, 17.5 mg, 97% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 5.60 (s, 2 H, arom), 3.94 (t, *J* = 4.4 Hz, 8 H, OCH_2), 3.82 (t, *J* = 4.4 Hz, 4 H, OCH_2), 3.43 (t, *J* = 4.4 Hz, 4 H, NCH_2), 3.25 (t, *J* = 4.4 Hz, 8 H, NCH_2). **^{13}C NMR** (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm), selected: 157.5 (C), 149.0 (C), 89.5 (CH), 66.7 (OCH_2), 66.4 (OCH_2), 52.1 (NCH_2), 47.0 (NCH_2). **ESI MS (ES^+) m/z:** 363 $[M+H]^+$, 385 $[M+Na]^+$, 401 $[M+K]^+$.

1,1',1''-(2-nitrosobenzene-1,3,5-triyl)tripyrrolidine (7c):[18,20] dark red solid,15.2 mg, 97% yield. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 5.02 (d, *J* = 2.1 Hz, 1 H, arom), 4.80 (d, *J* = 2.3 Hz, 1 H, arom), 3.75–3.61 (m, 4 H, NCH_2), 3.44 (t, *J* = 6.6 Hz, 4 H, NCH_2), 3.39–3.20 (m, 4 H, NCH_2), 2.03–1.88 (m, 12 H, NCH_2CH_2); **^{13}C NMR** (100.56 MHz, $CDCl_3$, 25 °C) δ (ppm, selected): 156.1 (C), 155.8 (C), 145.0 (C), 85.0 (CH), 83.6 (CH), 51.7 (br.s., NCH_2), 51.02 (br.s., NCH_2), 48.3 (NCH_2), 25.8 (NCH_2CH_2), 25.6 (NCH_2CH_2), 25.4 (NCH_2CH_2). **ESI MS (ES^+) m/z:** 315 $[M+H]^+$, 337 $[M+Na]^+$.

2,4-Dinitrothiophene-3-ol (8): mustard-color solid, 6.7 mg, 70% yield, m.p.: > 120 °C (dec.). **^{1}H NMR** (400 MHz, CD_3OD, 25 °C) δ (ppm): 8.77 (s, 1 H). **^{13}C NMR** (100.56 MHz, CD_3OD, 25 °C) δ (ppm): 151.4, 138.1, 133.8, 130.0. **ESI MS (ES^-) m/z:** 189 $[M-H]^-$.

Formation and detection of Wheland-Meisenheimer intermediates WMa and WMc.

A solution of 1,3,5-triaminobenzene derivative (**3a** or **3c**, 0.04 mmol) was dissolved in CD_2Cl_2 (1 mL) and introduced in a NMR spectroscopy tube that was inserted in the NMR probe. When the probe temperature reached −70°C, an equimolar amount of 2,3,4-trinitrothiophene (9.5 mg, 0.04 mmol) was added to the solution, that became blue-colored, and the ^{1}H NMR spectrum of the resulting solution was quickly recorded. The system was monitored after various times and at different temperatures until 25 °C. Immediately after the mixing, the spectrum at −70 °C showed the appearance of new signals, some of them ascribed to compound WM, also with the aid of g-COSY and g-HSQC experiments. On raising the temperature, signals belonging to WM gradually broadened then disappeared at about −35 °C for **WMa** and −30 °C for **WMc**; a return-back from previous temperature did not produced re-appearance of signals of WM. In case of reaction of **2** with **3a**, the ^{1}H NMR spectrum recorded at −70 °C immediately after the mixing of the reagents at −70 °C showed presence of compound **5a** in a relative molar ratio 57/43 with **WMa**.

In case of reaction of **2** with **3c**, the ^{1}H NMR spectrum recorded at −70 °C immediately after the mixing of the reagents showed presence of other signals, some of them ascribed to compound **5c** and **6c**. These latter fall in the same region of **WMc** but were distinguishable because the signals of **WMc** broadened and disappeared on raising the temperature while those of **6c** increased on raising the temperature probe and remained stable.

3,4,5-Trinitro-2-(2,4,6-tri(piperidin-1-yl)cyclohexa-2,4-dien-1-ylium-1-yl)-2,3-dihydro thiophen-3-ide (WMa): ^{1}H NMR (400 MHz, CD_2Cl_2, −70 °C) δ (ppm): 5.48 (br.s, 1 H), 5.36 (br.s, 1 H), 4.98 (br.s, 1 H), 4.95 (br.s, 1 H), 4.05–3.51 (m, 4 H), 3.5–3.23 (m, 8 H), 1.85–0.9 (m, 18 H, overl. with those of **5a**). ***g*-HSQC** (CD_2Cl_2, −70 °C): ^{1}H-^{13}C correlations (solvent signal set at 54.47 ppm): 5.48-91.8, 5.36-87.4, 4.95-55.3, 4.98-39.3.

3,4,5-trinitro-2-(2,4,6-tri(pyrrolidin-1-yl)cyclohexa-2,4-dien-1-ylium-1-yl)-2,3-dihydro thiophen-3-ide (WMc): ^{1}H NMR (400 MHz, CD_2Cl_2,−70 °C) δ (ppm): 5.03 (d, *J* = 2.36, 1H), 4.87 (br.s, 1H), 4.78 (br.s, 1H), 4.73 (br.s, 1H), 3.82–3.40 and 2.20–1.50 (signals overl. with those of other compounds); ***g*-COSY** (CD_2Cl_2, −70 °C): ^{1}H-^{1}H correlation: 5.03–4.73; ***g*-HSQC** (CD_2Cl_2, −70 °C): ^{1}H-^{13}C correlations: 5.03-54.9, 4.87-89.4, 4.78-85.8, 4.73-44.6.

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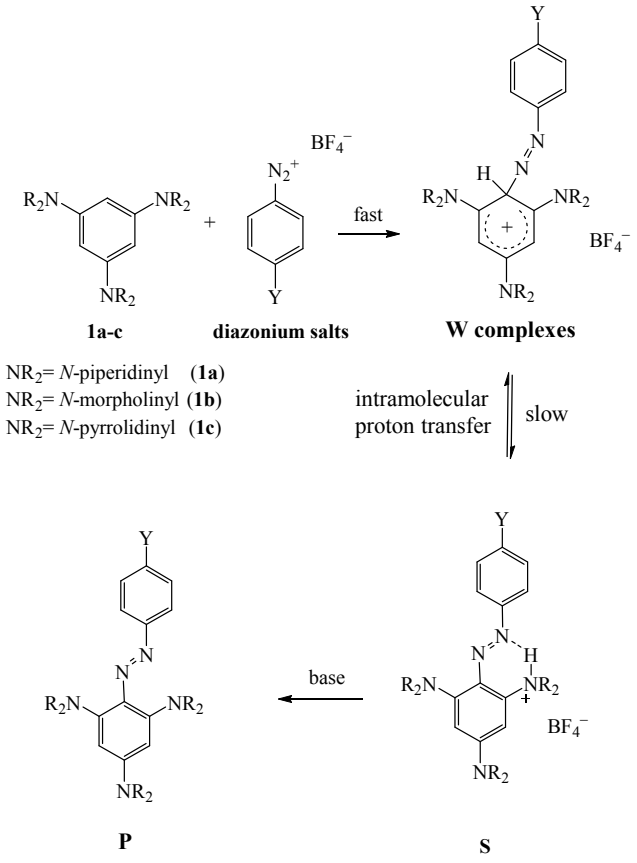
CHAPTER 4

Triaminobenzene derivatives *versus* benzhydrylium ions: further evidence of the reversibility of the σ intermediates formation step in S_EAr/S_NAr reactions

4.1 INTRODUCTION

As reported in Chapter 1, in the past the coupling between triaminobenzene derivatives and charged electrophilic species such as arenediazonium salts, allowed to detect the related σ−cationic intermediate **W** as reported in Scheme 1.[1]



Scheme 1. Reactions between 1,3,5-tris(dialkylamino)benzenes and arenediazonium salts.

This latter slowly evolved to the salt (**S**) and the Wheland intermediate stability permitted to separately study the two steps of this S_EAr reaction and to gain evidence of the reversibility of the whole reaction.[1]

In the frame of our investigation on the di- and triaminobenzene derivatives as nucleophilic species, we decided to perform the reactions between them and others charged carbon electrophiles. The selected electrophilic species were a series of benzhydrylium ions, whose electrophilicity parameters (according to Mayr's electrophilicity scale),[2-5] are known.

During my research period at the Ludwig-Maximilians-University of Munich, I did start a kinetic study on the di- and triaminobenzene derivatives, with the aim to develop a methodology to measure the rate constants for the substitution reactions involving aminobenzene derivatives and finally calculate the nucleophilicity parameters of both di- and triaminobenzene derivatives. Preliminary results have been obtained for diaminobenzene derivatives but work is still in progress on this topic; so, the partial obtained data will not be reported on this chapter.

Herein I will report the results obtained in Bologna through NMR experiments and, for the sake of clarity, I will discuss separately the studies involving diaminobenzene and triaminobenzene derivatives.

4.2 RESULTS AND DISCUSSION

- *Reactions between 1,3,5-tris(N,N-dialkylamino)benzene derivatives and benzhydrylium ions.*

As it can be seen in Figure 1, the electrophilic power calculated by Mayr and coworkers[2-5] of the selected benzhydrylium ions, grows from the bottom to the top; so, **mfa** is the stronger and **dma** is the lower electrophilic species, among those chosen.

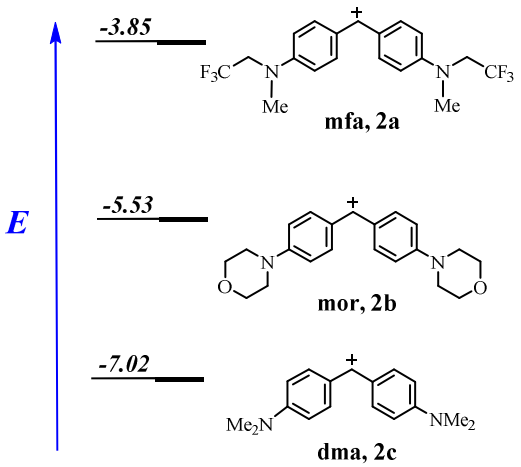
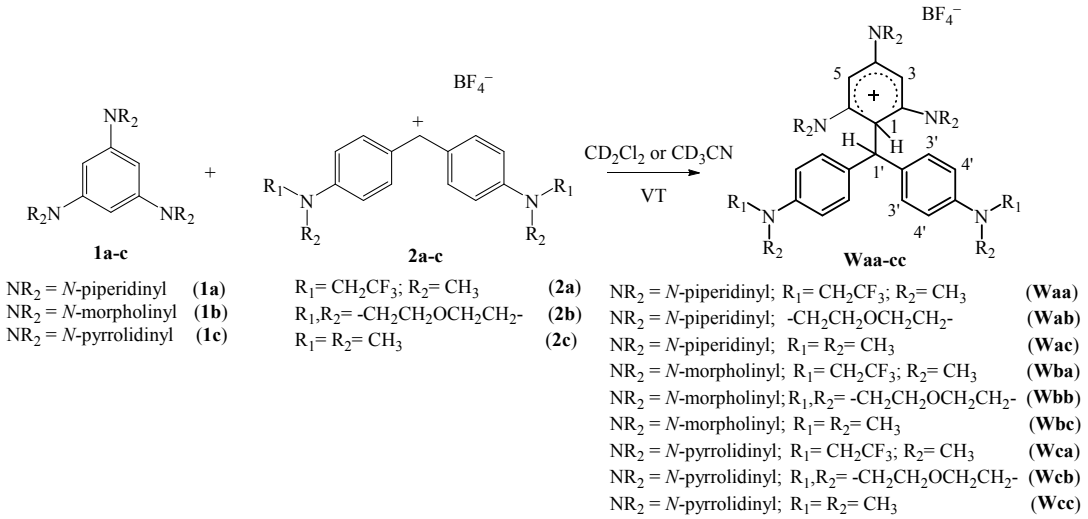


Figure 1. Selected benzhydrylium ions from the Mayr's electrophilicity scale.[2-5]

1,3,5-tris(*N*,*N*-dialkylamino)benzene derivatives **1a-c** were coupled with benzhydrylium tetrafluoroborates **2a-c** as shown in Scheme 2.



Scheme 2. Formation of **Waa-cc** from the reactions between **1a-c** and **2a-c** in the NMR tube, at variable temperatures.

The first studies were carried out at room temperature and we observed that coupling the more nucleophilic species (**1a,c**) with the stronger and medium electrophilic species (**2a** or **2b**, respectively), the ^{1}H-NMR spectrum showed a set of signals ascribable to complexes **Waa**, **Wab**, **Wca** and **Wcb** (Scheme 2). The Wheland intermediate formation was hypothesized for the absence in the ^{1}H-NMR spectra, of the signals of both reagents and owing the presence of two doublets, in the range of 4.0-4.6 ppm, typical region of sp^{3} proton of the Wheland intermediate, integrating each for one proton; one of these doublets was ascribed to H-1 of **W** (Scheme 2) and the other doublet belongs to the benzylic proton of the benzhydrylium moiety and it results shifted up field respect to its signal as free electrophile, due to the presence of the positive charge in the sigma intermediate.

The presence of the Wheland intermediates were confirmed by ^{13}C-NMR, DEPT, *g*-HSQC and *g*-COSY experiments that showed the direct connection of the proton H-1 to a carbon resonating in typical region for the hybridized sp^{3} carbon atoms (40-60 ppm), and its coupling with the benzylic proton indicated as H-1$'$ in Scheme 2.

In Figure 2 is reported, as an example, the ^{1}H-NMR spectrum recorded at room temperature for the reaction of **1a** with **2a.**

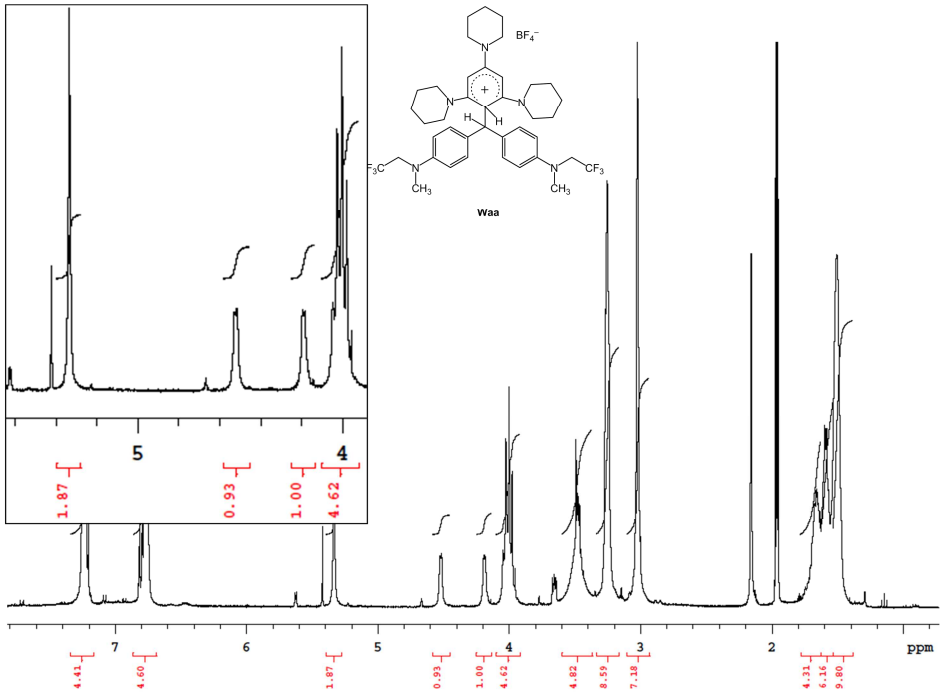


Figure 2. ^{1}H-NMR spectrum, in CD_3CN, at 25 °C of the reaction mixture from **1a** and **2a**, with expanded view of diagnostic signals belonging to **Waa**.

In Table 1 and Table 2 the ^{1}H-NMR and ^{13}C-NMR data, respectively, for selected and diagnostic signals of **Waa**, **Wab**, **Wca** and **Wcb**, are reported.

Table 1. ^{1}H-NMR selected data for **Waa**, **Wab**, **Wca** and **Wcb**, in CD_3CN at 25°C (assignement by aid of *g*-COSY experiment).

Wheland intermediate	δH-1	δH-3,5	δH-1'	δH-3',4'
Waa	4.52 (d, *J*=4.5 Hz, 1H)	5.34 (s, 2 H)	4.19 (d, *J*=4.5 Hz, 1H)	7.24 (d, *J*=8.7 Hz, 4H) 6.76 (d, *J*=8.7 Hz, 4H)
Wab	4.53 (d, *J*=3.9 Hz, 1H)	5.33 (s, 2H)	4.21 (d, *J*=3.9 Hz, 1H)	7.25 (d, *J*=8.9 Hz, 4H) 6.84 (d, *J*=8.9 Hz, 4H)
Wca	4.12 (d, *J*=5.5 Hz, 1H)	4.68 (s, 2 H)	4.34 (d, *J*=5.5 Hz, 1H)	7.32 (d, *J*=8.8 Hz, 4H) 6.74 (d, *J*=8.8 Hz, 4H)
Wcb	4.12 (d, *J*=5.3 Hz, 1H)	4.67 (s, 2H),	4.35 (d, *J*=5.3 Hz, 1H),	7.34 (d, *J*=8.3 Hz, 4H) 6.82 (d, *J*=8.3 Hz, 4H)

Table 2. ^{13}C-NMR selected data for **Waa**, **Wab**, **Wca** and **Wcb**, in CD_3CN at 25°C (assignement by aid of *g*-COSY and *g*-HSQC experiments).

Wheland intermediate	δC-1	δC-3,5	δC-1'	δC-3', 4'
Waa	61.5	90.6	46.5	113, 131.4
Wab	61.5	90.6	46.4	115.6, 131.3
Wca	51.5	88.5	58.7	112.6, 131.3
Wcb	51.3	88.5	58.8	115.3, 131.3

When the reactions were carried out at room temperature, both in acetonitrile or dichloromethane, between the less nucleophilic species **1b** (morpholinyl derivative) and **2a** or **2b** (the stronger and the medium electrophilic species, respectively), the recorded spectra showed a lot of broad signals and, apparently, no evidence of the typical doublets of the Wheland intermediates was obtained (Figure 3). Instead combining **1b** and **2c** (the less electrophilic species) no reaction was observed.

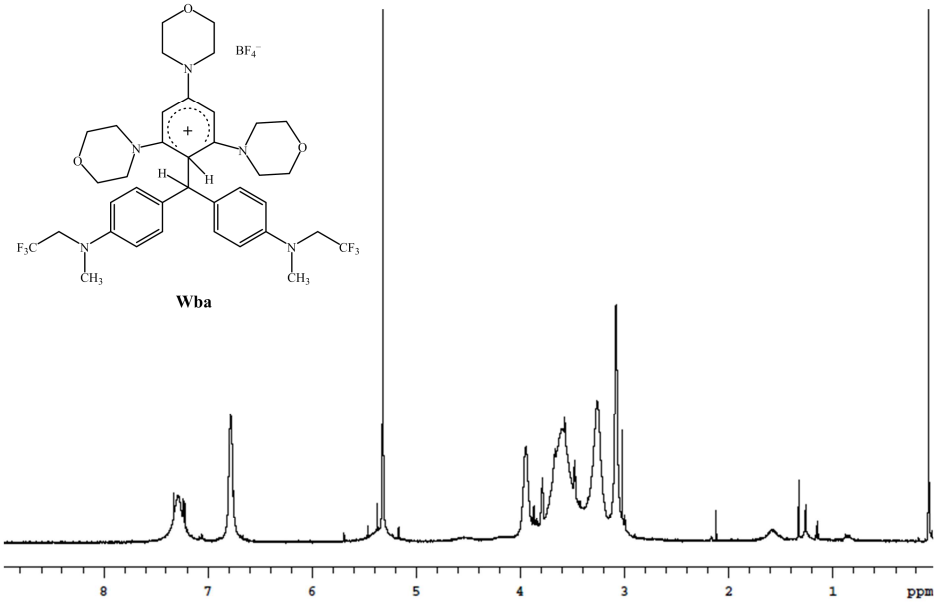
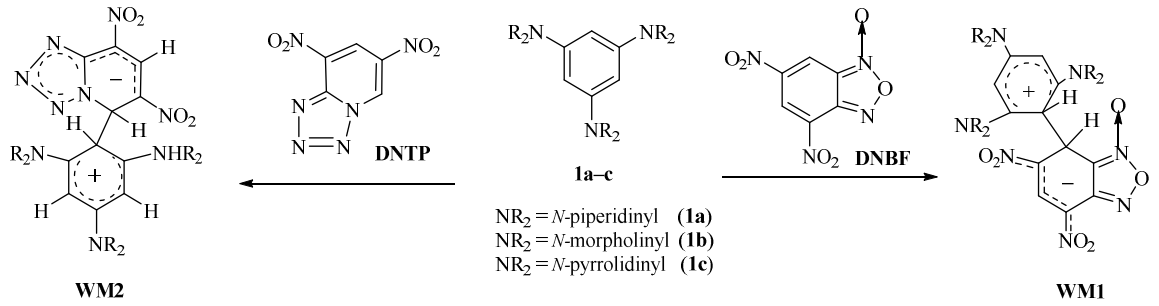


Figure 3. ^{1}H NMR spectrum, in CD_2Cl_2, at 25 °C of the reaction mixture from **1b** and **2a** in which the typical H-1 and H-1□ signals of the **Wba** are not visible.

These findings reminded us a behaviour previously observed in the reactions between triaminobenzene derivatives **1a-c** and 4,6-dinitrobenzofuroxan (**DNBF**) or 4,6-dinitrotetrazolepyridine (**DNTP**). In those experiments, we detected and characterized the first Wheland-Meisenheimer species (**WM1** and **WM2**) as showed in Scheme 3.[6,7]

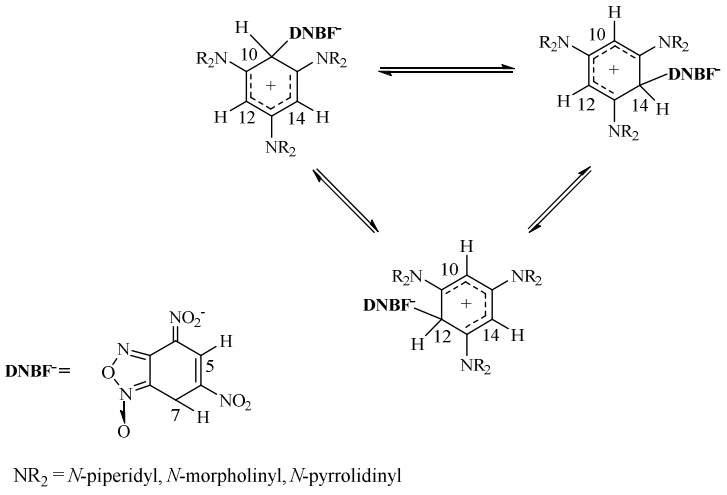


Scheme 3. Nucleophile/electrophile combination between neutral aromatic species giving moderately stable W-M intermediates.

Intermediates **WM1** and **WM2** showed sharp and well separated ^{1}H and ^{13}C-NMR signals, corresponding to the three hydrogen atoms belonging to the triaminobenzene moiety, at low temperature, whereas raising the temperature these signals became broad. A further lowering of the temperature gave again sharp signals of both **WM** intermediates.

In all cases the coalescence of the involved signals was observed and the thermodynamic activation parameters of the process were derived.

The dynamic NMR data suggested the existence, above the coalescence temperature, of **WM1** and **WM2** in three homomeric structures as depicted in Scheme 4 (for the case of **WM1**), with bonds C7/C10, C7/C12 and C7/C14 rapidly exchanging.[6]



Scheme 4. Proposed interconversion pathway for the observed reversible and temperature-dependent transformation of **WM1** structures.

In conclusion, the reported exchange process resulted in a reversible and temperature-dependent transformation of **WM1** structures.

Later, further confirmation of the reversibility of the exchange process from the reaction between triaminobenzene derivatives **1a-c** and **DNTP** (see Scheme 3) was obtained through exchange of the electrophilic moiety by addition of **DNBF** to **WM2** and also by addition of **1a** to the **WM2** derived from **DNTP** and **1b**, that produced exchange of the nucleophilic part.[7]

Based on the above results, the reactions between triaminobenzene derivatives **1b** and **2a,b** were carried out directly in the NMR spectroscopy tube, in equimolar amount of reagents, in CD_2Cl_2 at -80°C or in CD_3CN at -35°C. At these temperatures, the typical signals for **Wba** (Figure 4) and **Wbb** were observed.

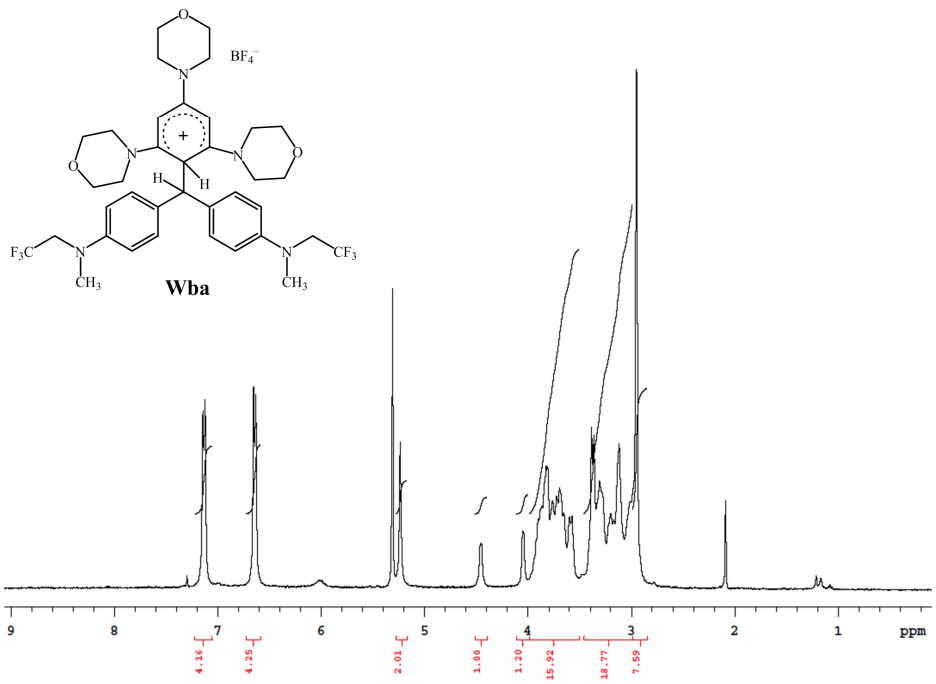


Figure 4. ^{1}H NMR spectrum, in CD_2Cl_2, at -80 °C of the reaction mixture from **1b** and **2a**, with typical signals of **Wba**.

Finally, we also combined the less electrophilic species **2c** (**dma**), with **1a** and **1c**, the stronger and the medium nucleophilic species, respectively, at room temperature and also in these two cases, the ^{1}H-NMR spectra showed broad signals, while performing the same reactions at low temperature typical signals for **Wac** and **Wcc**, appeared in the ^{1}H-NMR spectra.

In the whole, all the combinations gave the formation of the Wheland intermediates **Waa-cc** (Scheme 2), except for the case of the combination between **1b** and **2c**, in which no reaction was observed; it is interesting to note that in this case the reaction was carried out between the less electrophilic species (Figure 1) and the less nucleophilic species (inferred by considering the nitrogen nucleophilicity values for the secondary amines morpholine respect to piperidine and pyrrolidine, reported by Prof. Herbert Mayr and coworkers in Ref 8).

Finally, in all these experiments the observed dynamic processes resulted reversible: warming the solution from -35°C or -85°C (in CD_3CN or CD_2Cl_2, respectively) to room temperature and cooling again, ^{1}H-NMR spectra identical to the starting one, were obtained.

In Table 3 and Table 4 are reported the ^{1}H-NMR and ^{13}C-NMR data, respectively, for selected and diagnostic signals of **Wba**, **Wbb**, **Wac** and **Wcc**, in acetonitrile, at low temperature (-35°C).

The full spectroscopic characterization for all the obtained Wheland intermediates, in both solvents, are reported in the Experimental section of this chapter.

Table 3. ^{1}H-NMR data for selected signals for **Wba**, **Wbb**, **Wac**, **Wcc**, in CD_3CN at low temperature.

Wheland intermediate	δH-1	δH-3,5	δH-1'	δH-3',4'
Wba	4.48-4.40 [a]	5.31 (s, 2H)	4.18 (d, *J*=5.2 Hz, 1H)	7.23 (d, *J*=8.1 Hz, 4H) 6.75 (d, *J*=8.1 Hz, 4H)
Wbb	4.46 (d, *J*=5.8 Hz, 1H)	5.32 (s, 2H)	4.22 (d, *J*=5.8 Hz, 1H)	7.29 (d, *J*=8.9 Hz, 4H) 6.89 (d, *J*=8.9 Hz, 4H)
Wac	4.48 (d, *J*=5.4 Hz, 1H)	5.30 (s, 2H)	4.17 (d, *J*=5.4 Hz, 1H)	7.17 (d, *J*=8.5 Hz, 4H) 6.62 (d, *J*=8.5 Hz, 4H)
Wcc	4.07 (d, *J*=5.4 Hz, 1H)	4.62 (s, 2H)	4.30 (d, *J*=5.4 Hz, 1H)	7.26 (d, *J*=8.7 Hz, 4H) 6.61 d, *J*=8.7 Hz, 4H)

[a] (m, 1H) two signals overlapped: signal ascribed to H-1 overlapped to the CH_2-CF_3 signal of the unreacted electrophile **2a**.

Table 4. ^{13}C-NMR for selected signals for **Wba**, **Wbb**, **Wac**, **Wcc**, in CD_3CN at low temperature.

Wheland intermediate	δC-1	δC-3,5	δC-1'	δC-3', 4'
Wba	60.4	90.2	45.6	112.0, 130.8
Wbb	60.4	90.3	45.3	115.2, 131.0
Wac	60.4	89.5	45.5	112.5, 130.6
Wcc	51.3	87.9	58.1	112.0, 130.8

The behaviour of the new **W** complexes involving benzhydrylium ions at different temperature is similar to that previously found for **WM1** and **WM2.** This prompted us to

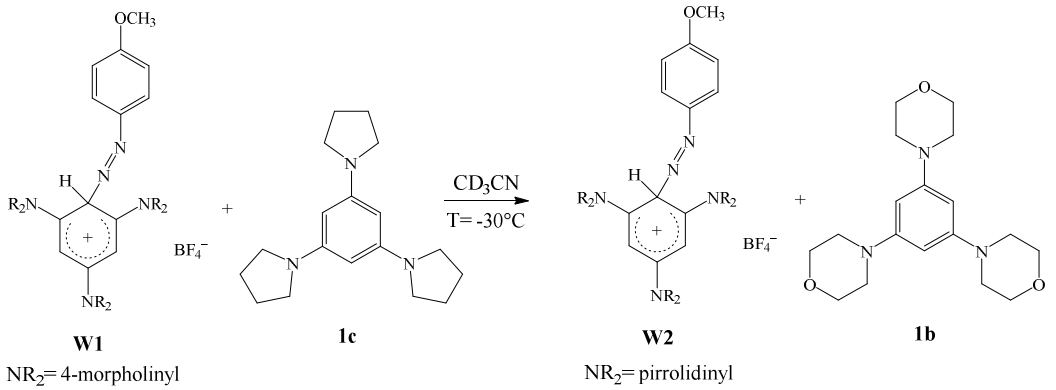
derive the coalescence temperature and the related thermodynamic activation parameters for the new stable intermediates. Work is in progress on this part of the study.

It is interested to note that, various attempts to obtain the substitution products from the reactions between **1a-c** and **2a-c**, were performed, working with an excess of the nucleophile or in the presence of different bases (DBU, triethylamine, pyrrolidine, basic Al_2O_3), but in all cases the Wheland intermediates resulted stable and no substitution products were obtained.

The behaviour of the new intermediates suggests the reversibility of their formation.

In the past the research group collected important informations about the mechanism of the S_EAr and the reversibility of the formation of the Wheland complex, during a study involving triaminobenzene derivatives **1a-c** and different aryldiazonium salts.[9] In that case the reversibility of the electrophilic aromatic substitution reaction was confirmed performing an exchange reaction in which the replacement of the nucleophilic moiety on the Wheland complex, was observed (Scheme 5).

A similar behaviour was also observed in the case of the reactions between triaminobenzene derivatives (**1a-c)** and **DNTP**, that gave **WM2** in Scheme 3; in that case was performed the exchange of both the electrophilic (with **DNBF**) and nucleophilic (**1b** was exchanged with **1a**) partners.[6] This prompted us to try to exchange the electron-donor moiety of some intermediates.

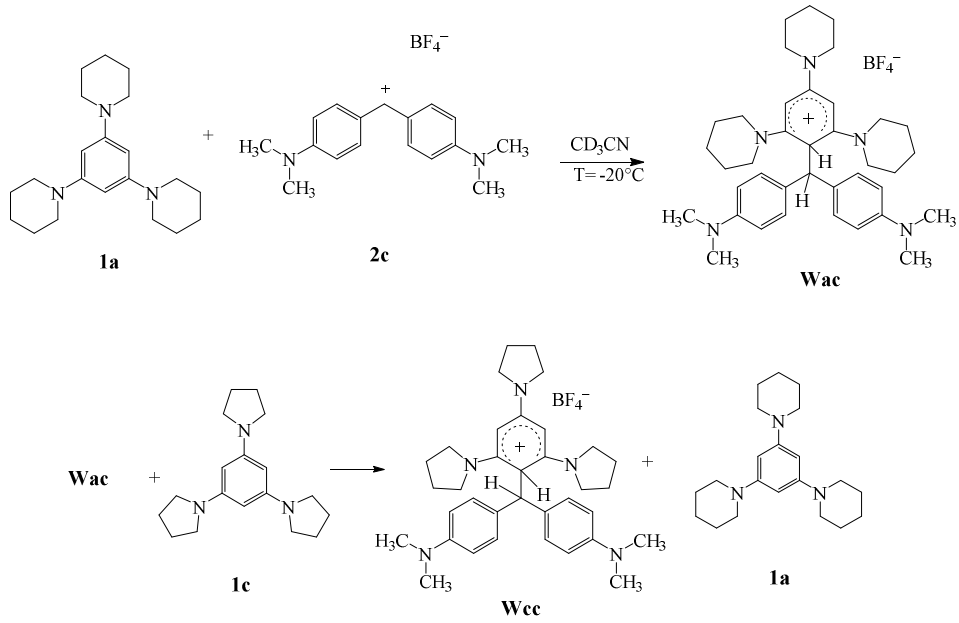


Scheme 5. Exchange of the nucleophilic partner in the reaction between triaminobenzene derivatives and 4-methoxybenzenediazonium tetrafluoroborate.

In particular, two exchange reactions were performed, the first between **Wba** and **1c**, and the second between **Wac** and **1c**.

The triaminobenzene moiety exchange was carried out, for both combinations, directly in the NMR spectroscopy tube, in CD_3CN at -20°C.

After the formation of the Wheland intermediates **Wba** or **Wac**, respectively, an equimolar amount of the pyrrolidinyl derivative **1c**, was added to the reaction mixture (Scheme 6, for the case of **Wac**).



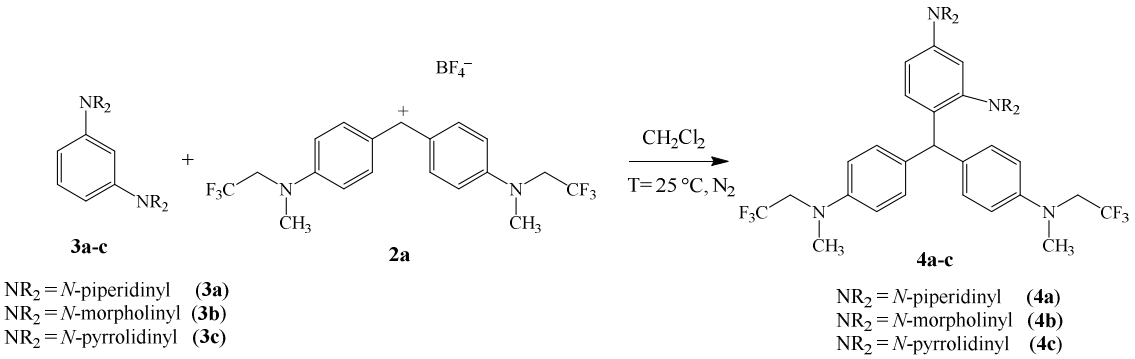
Scheme 6. Nucleophile exchange in the reaction between **1a** with **2c**.

The ^{1}H-NMR spectrum, recorded after the addition of the stronger nucleophile, showed the disappearance of signals related to **Wac** (or **Wba**), and the concomitant appearance of those related to Wheland complex **Wcc** (or **Wca**), together with those the less nucleophilic species **1a** (or **1b**), as reported in Scheme 6. So the more powerful nucleophilic reagent **1c**, replaced the less one, resulting again as an indirect evidence of the reversibility of the Wheland formation.

-Reactions between 1,3-bis(N,N-dialkylamino)benzene and benzhydrylium ions

The reactions between the diaminobenzene derivatives **3a-c** and bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methylium tetrafluoroborate (**2a**) were performed, in dichloromethane, at room temperature and under nitrogen atmosphere, with a two fold

excess of the nucleophile to neutralize the tetrafluoroboric acid produced. In all cases products **4a-c**, were obtained in high yiels, after purification on silica gel (Scheme 7).

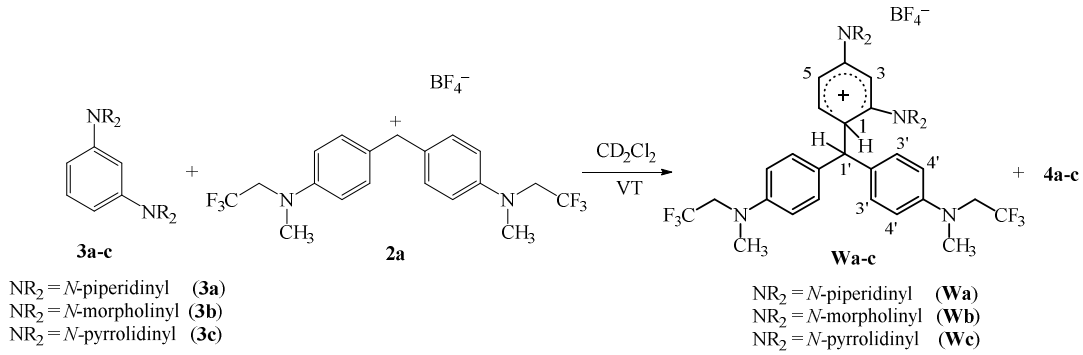


Scheme 7. Reactions between diaminobenzene derivatives **3a-c** and the benzhydrylium ion **2** to obtain the substitution products **4a-c**.

In all cases, as in the case of the reactions between diaminobenzene derivatives and benzofuroxan derivatives (see Chapter 3), the final products derived from the attack of the electrophilic species in 4 position of the nucleophile, giving the unsymmetric products **4a-c**, fully characterized by usual spectroscopyc methods.

With the purpose to investigate on the possibility to detect σ–intermediates from the reactions between **3a-c** and **2a**, we performed the reactions directly in the NMR spectroscopy tube, combining the reagents in equimolar amount, at different temperatures (from -80°C to 25°C), in CD_2Cl_2.

In all cases the formation of the Wheland intermediates **Wa-c** was observed (Scheme 8) at low temperature, where them resulted stable.



Scheme 8. Formation of **Wa-c** from the reactions between **3a-c** and **2a** in the NMR tube, at low temperature.

The **Wa-c** formation was deduced owing to the presence, in the ^{1}H-NMR spectra, of two new signals in the range of 4.3-4.5 ppm, a triplet and a doublet, integrating each for one proton (Figure 5A); the triplet in particular was ascribed to H-1. This attribution was confirmed by ^{13}C-NMR, DEPT and *g*-HSQC experiments, at low temperature, that showed that the triplet is directly connected to C-1, resonating in the typical region for the hybridized sp^{3} carbon atoms (40-55 ppm).

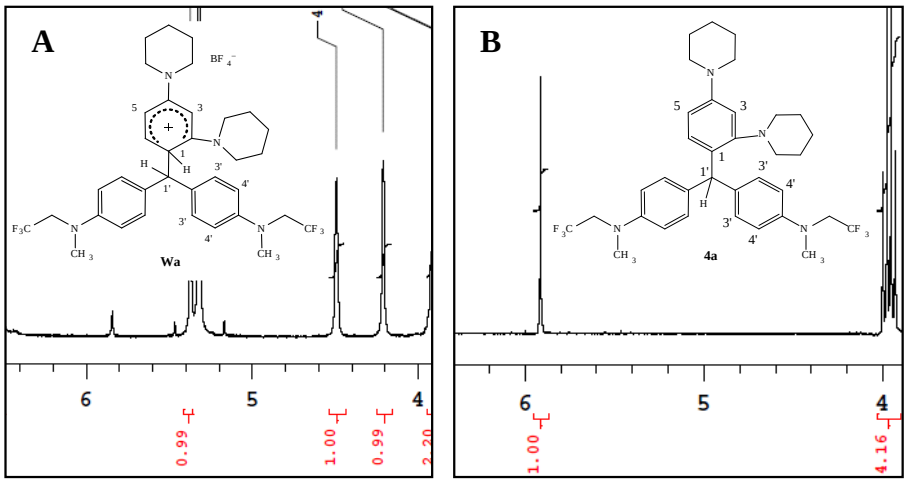


Figure 5. Comparison of the ^{1}H-NMR spectra in the 4-6 ppm region, between **Wa** (A) and its substitution product **4a** (B).

The presence of the doublet in the same region, it's another confirmation for the **Wa-c** formation, in fact, this signal belongs to the benzylic proton of the benzhydrylium moiety, and it results shifted up field respect to its signal in the substitution product **4a-c** (the singlet at about 6 ppm, visible in Figure 5B in the case of **4a**); this behaviour depends on the presence of the positive charge in the sigma intermediate respect to the substitution product.

Increasing the temperature, signals ascribed to the Wheland intermediates became broad until they disappeared at room temperature; contemporaneously the formation of the substitution products **4a-c** was observed and these became the only species in solution at room temperature, in the case of **a** and **b**.

It is interesting to note that in the case of **Wb**, the morpholinyl derivative, the sigma intermediate was present in very low concentration also at low temperature and the substitution product **4b** was already present in the solution immediately after the mixing of the reagents at -80°C. Instead, in the case of **Wc**, the pyrrolidinyl derivative, typical signals

of this sigma complex were present also at room temperature, together with signals ascribed to the substitution product **4c**.

Therefore, this can be considered an indication that **Wc** is probably the more stable intermediate with respect to the others (**Wa,b**), thanks to the stronger ability of the pyrrolidinyl groups respect to the piperidinyl (case **a**) and morpholinyl (case **b**) to stabilize the positive charge of the sigma intermediate on the ring. These results are again in agreement with the reported nitrogen nucleophilicity, for the secondary amines, morpholine, piperidine and pyrrolidine.[8]

4.3 CONCLUSIONS

The reported study concerns the investigation on the reactivity of triaminobenzene derivatives and diaminobenzene derivatives with a set of charged carbon electrophiles, selected from the Mayr's electrophilicity scale and allowed to evidence and characterize new σ-intermediates of the aromatic substitution reaction, when the nucleophilic species were both di- and triaminobenzene derivatives, and to synthesize new products when the nucleophilic species were diaminobenzene derivatives.

In the case of triaminobenzene derivatives **1a-c**, their reactions with the electrophilic species **2a-c**, gave only the Wheland intermediates **Waa-Wcc** whose stability depends on the electrophile/nucleophile combinations and on the experimental conditions.

In particular, stable Wheland complexes, at room temperature, where observed only when the stronger electrophiles were coupled with the stronger nucleophiles.

When one of the two reagents possess the lower electrophilic or nucleophilic power, a peculiar behaviour was observed: typical signals of Wheland intermediates with triaminobenzene derivatives were present in the spectrum only at low temperature and their gradually broadening was observed increasing the temperature; as a result, at room temperature the Wheland intermediate appears not evident in the ^{1}H-NMR spectrum.

At last, once again, the reversibility of the Wheland complex formation was observed and confirmed by exchange reactions of the nucleophilic partner in the reactions between triaminobenzene derivatives **1a,b** and **2a,c**.

With respect to the reactions between diaminobenzene derivatives **3a-c** and the benzhydrylium ion **2a**, both substitution products and Wheland intermediates were obtained.

In particular, performing the reactions between **3a-c** and **2a**, the unsymmetric products **4a-c** were synthesized; instead, coupling **3a-c** with **2a**, at low temperature, directly in the NMR spectroscopy tube, using a variable temperature experiment, the Wheland complexes **Wa-c** were obtained. **Wa-c** resulted stable only at low temperature and their signals disappeared increasing the temperature while other signals ascribed to the substitution product **4a-c** appeared until became the only species in the reaction mixture at room temperature. The presence of only two amino substituents on the diamino derivatives respect to triaminobenzene derivatives makes these nucleophilic species less able to stabilize the positive charge of the Wheland intermediates from the reactions with benzhydrylium ions.

The obtained results, in the case of both di- and triaminobenzene derivatives, showed that the Wheland intermediate stability and its evolution to the final substitution product, depends from the ability of the amino-substituent on the aromatic ring of the di- and triaminobenzene derivatives, to stabilize the σ-intermediate.

4.4 EXPERIMENTAL SECTION

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Mercury 400 and Inova 600 (Varian, Palo Alto USA) spectrometers operating at 400, or 600 MHz (for ^{1}H-NMR) and 100.56, or 150.80 MHz (for ^{13}C-NMR), respectively. Chemical shifts were measured in δ (ppm) with reference to the solvent (for ^{1}H- and ^{13}C-NMR, respectively: δ= 5.32 ppm and 53.8 ppm for CD_2Cl_2; δ= 1.96 ppm and 118.20 ppm for CD_3CN). *J* values are given in Hz. Signal multiplicities were established by DEPT experiments. The variable–temperature NMR spectra and 2D low-temperature spectra (*g*-COSY and *g*-HSQC) were recorded on a Mercury 400 or Inova 600 spectrometers. ESI-MS spectra were recorded with a WATERS 2Q 4000 instrument. Chromatographic purifications were carried out on columns of silica gel (0.037-0.063 mm) or aluminium oxide, activated, basic, Brockmann I, standard grade ca. 150 mesh at medium pressure. Solvents and reagents were commercial materials (Aldrich or Fluka) if not specified. 1,3,5- tris(*N*,*N*-dialkylamino)benzene derivatives **1a-c** were synthesized as described previously by the research group in Ref 6. 1,3-bis(*N*,*N*-dialkylamino)benzene derivatives **3a-c** were prepared from 1,3-dichlorobenzen (Sigma-Aldrich) with a modification of the reported literature[10,11] methods, as reported in the previous Chapters. Benzhydrylium ions **2a-c** were synthesized from the Professor Mayr's research group in Munich.

Typical procedure for the detection of the σ–complexes Waa, Wab, Wca and Wcb: The reactions between the triaminobenzene derivatives **1a,c** (0.02 mmol) with the benzhydrylium ions **2a,b** (0.02 mmol), were carried out directly in the NMR spectroscopy tube, in CD_3CN (1 mL) and at room temperature.

In these cases the triaminobenzene derivative was weighted directly into the tube and dissolved in the minimum amount of solvent. Then to this solution, an equimolar amount of the benzhydrylium derivative, dissolved in the minimum amount of solvent, was added and the solution was analyzed by NMR spectroscopy.

Immediately after mixing reagents, the Wheland complex formation was confirmed by the appearance of its typical signals in the ^{1}H-NMR spectrum and by the aid of ^{13}C-NMR, and in some cases also of *g*-COSY and *g*-HSQC experiments. Chemico physical data for the detected Wheland complexes are reported as follows.

1-(4-(bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methyl)-3,5-di(piperidin-1-yl) cyclohexa-2,5-dien-1-ylidene)piperidin-1-ium tetrafluoroborate (Waa): ^{1}H NMR (400 MHz, CD_3CN, 25 °C) δ (ppm): 7.24 (d, *J* = 8.7 Hz, 4H), 6.76 (d, *J* = 8.7 Hz, 4H), 5.34 (s, 2H), 4.52 (d, *J* = 4.5 Hz, 1H), 4.19 (d, *J* = 4.5 Hz, 1H), 4.00 (q, *J* = 9.4 Hz, 4H), 3.60-3.40 (m, 4H), 3.25 (t, *J* = 5.4 Hz, 8H), 3.02 (s, 6H), 1.73-1.62 (m, 4H), 1.62-1.55 (m, 6H), 1.55-1.45 (m, 8H). **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 164.9, 163.4, 148.7, 131.4, 125.7, 113.0, 90.6, 61.5, 53.9 (q, J_{C-F} = 31.9 Hz), 50.2, 49.9, 46.5, 39.6, 27.1, 27.0, 26.3, 24.7. **^{1}H NMR** (400 MHz, CD_2Cl_2, -85 °C) δ (ppm): 7.08 (d, *J* = 8.6 Hz, 4H), 6.62 (d, *J* = 8.6 Hz, 4H), 5.18 (s, 2H), 4.40 (d, *J* = 3.5 Hz, 1H), 4.19 (d, *J* = 3.5 Hz, 1H), 3.85 (q, *J* = 8.2 Hz, 4H), 3.70 (d, *J* = 11.5, 2H), 3.23 (br.s, 4H), 3.12 (br.s, 4H), 3.08-3.00 (m, 2H), 2.94 (s, 6H), 1.68-1.37 (m, 18H). ε = 7696 $M^{-1}cm^{-1}$ (λ_{max}=412.5 nm) in CH_3CN at 20°C.

1-(4-(bis(4-morpholinophenyl)methyl)-3,5-di(piperidin-1-yl)cyclohexa-2,5-dien-1-ylidene)piperidin-1-ium tetrafluoroborate (Wab): ^{1}H NMR (400 MHz, CD_3CN, 25 °C) δ (ppm): 7.25 (d, *J* = 8.9 Hz, 4H), 6.84 (d, *J* = 8.9 Hz, 4H), 5.33 (s, 2H), 4.53 (d, *J* = 3.9 Hz, 1H), 4.21 (d, *J* = 3.9 Hz, 1H), 3.80 (t, *J* = 4.2, 8H), 3.70 (t, *J* = 3.7, 8H), 3.48 (t, *J* = 5.6, 4H), 3.26 (*t*, *J* = 4.6, 8H), 1.76-1.42 (m, 18H). **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 164.8,163.3, 151.5, 131.3, 130.3, 115.6, 90.6, 67.3, 61.5, 50.2, 49.8, 48.6, 46.4, 27.1, 26.3, 24.7, 24.6.

1-(4-(bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methyl)-3,5-di(pyrrolidin-1-yl) cyclohexa-2,5-dien-1-ylidene)pyrrolidin-1-ium tetrafluoroborate (Wca): ^{1}H NMR (400 MHz, CD_3CN, 25 °C) δ (ppm): 7.32 (d, *J* = 8.8 Hz, 4H), 6.74 (d, *J* = 8.8 Hz, 4H), 4.68 (s, 2H), 4.34 (d, *J* = 5.5 Hz, 1H), 4.12 (d, *J* = 5.5 Hz, 1H), 4.01 (q, *J* = 9.6 Hz, 4H), 3.57-3.44 (m, 2H), 3.44-3.35 (m, 2H), 3.25-3.11 (m, 6H), 3.02 (s, 8H, two signals overlapped), 1.93-1.67 (m, 12H). **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 161.4, 161.3, 148.7, 131.3, 129.0, 126.5 (q, J_{C-F} = 283.1 Hz),, 112.6, 88.5, 58.7, 53.7 (q, J_{C-F} = 31.9 Hz),, 51.5, 49.5, 49.5, 39.6, 26.0, 25.1. ε = 16108 $M^{-1}cm^{-1}$ (λ_{max}=421.5 nm) in CH_3CN at 20°C.

1-(4-(bis(4-morpholinophenyl)methyl)-3,5-di(pyrrolidin-1-yl)cyclohexa-2,5-dien-1-ylidene)pyrrolidin-1-ium tetrafluoroborate (Wcb):^{1}H NMR (600 MHz, CD_3CN, 25 °C) δ (ppm): 7.34 (d, *J* = 8.3 Hz, 4H), 6.82 (d, *J* = 8.3 Hz, 4H), 4.67 (s, 2H), 4.35 (d, *J* = 5.3 Hz, 1H), 4.12 (d, *J* = 5.3 Hz, 1H), 3.78 (t, *J* = 4.8, 8H), 3.56-3.30 (m, 8H), 3.22-3.13 (m, 4H), 3.09 (t, *J*=4.8, 8H), 2.01 (br.s, 4H), 1.95-1.68 (m, 8H). **^{13}C NMR** (150.80 MHz, CD_3CN, 25 °C) δ (ppm):161.5, 161.2, 151.8, 131.3, 130.6, 115.3, 88.5, 67.3, 58.8, 51.3, 49.8, 49.7, 49.6, 25.6, 25.5.

Typical procedure for the detection of the σ–complexes Wba, Wbb, Wac and Wcc, at low temperature:

A solution of 1,3,5-triaminobenzene derivative **1a-c** (0.02 mmol), was dissolved in 1 mL of CD_2Cl_2 or in CD_3CN, and introduced in the NMR spectroscopy tube that was inserted in the NMR probe. When the probe temperature reached −80°C for the reactions carried out in CD_2Cl_2, or -30°C if acetonitrile was used as solvent, an equimolar amount of the benzhydrylium ions **2a-c** (0.02 mmol) was added to the solution, that became orange/yellow, and the ^{1}H-NMR spectrum of the resulting solution was quickly recorded. The system was monitored over time and at different temperatures until 25 °C.

Immediately after mixing reagents at low temperature, the Wheland complex formation was confirmed by the appearance of its typical signals in the ^{1}H-NMR spectrum and by the aid of ^{13}C-NMR, and in some cases also of *g*-COSY and *g*-HSQC experiments. On raising the temperature, signals belonging to the Wheland complex gradually broadened until disappeared at room temperature. A further lowering of the temperature gave again sharp signal of **W** complexes. Chemico physical data for the detected Wheland complexes, are reported as follows, in both the reaction solvents (CD_2Cl_2 and CD_3CN).

4-(4-(bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methyl)-3,5-dimorpholinocyclohexa-2,5-dien-1-ylidene)morpholin-4-ium tetrafluoroborate (Wba): ^{1}H NMR (600 MHz, CD_2Cl_2, -85 °C) δ (ppm): 7.13 (d, *J* = 8.3 Hz, 4H), 6.64 (d, *J* = 8.3 Hz, 4H), 5.24 (s, 2H), 4.46 (d, *J* = 3.3 Hz, 1H), 4.05 (d, *J* = 3.3 Hz, 1H), 3.98-3.51 (m, 16H), 3.44-3.05 8m, 12H), 2.95 (s, 6H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, -85 °C) δ (ppm): 164.8, 161.1, 146.7, 129.7, 125.2, 125.1 (q, $J_{C\text{-}F}$ = 283.5 Hz), 111.0, 89.0, 66.0, 65.6, 64.8, 60.8, 52.6 (q, $J_{C\text{-}F}$ = 33.8 Hz),, 48.1, 47.1, 46.7, 44.8, 39.8.

^{1}H NMR (600 MHz, CD_3CN, -35 °C) δ (ppm): Signals tentatively assigned due to the presence in the reaction mixture of the unreacted mfa.7.23 (d, *J* = 8.1 Hz, 4H), 6.75 (d, *J* = 8.1 Hz, 4H), 5.31 (s, 2H), 4.48-4.40 (m, 1H, two signals overlapped), 4.18 (d, *J* = 5.2 Hz, 1H), 4.12-3.96 (m, 4H), 3.76-3.67 (m, 4H),), 3.65-3.58 (m, 4H),), 3.56-3.39 (m, 8H),), 3.34-3.13 (m, 4H), 3.01 (s, 6H). **^{13}C NMR** (150.80 MHz, CD_3CN, -35 °C) δ (ppm): 165.1, 163.0, 147.8, 130.8, 127.2, 126.5 (q, $J_{C\text{-}F}$ = 285.0 Hz),, 112.0, 90.2, 66.6, 65.8, 60.4, 52.7 (q, $J_{C\text{-}F}$ = 32.3 Hz),, 48.1, 45.6, 40.9, 39.0.

4-(4-(bis(4-morpholinophenyl)methyl)-3,5-dimorpholinocyclohexa-2,5-dien-1-ylidene)morpholin-4-ium tetrafluoroborate (Wbb): ^{1}H NMR (600 MHz, CD_3CN, -35 °C) δ (ppm): 7.29 (d, *J* = 8.9 Hz, 4H), 6.89 (d, *J* = 8.9 Hz, 4H), 5.32 (s, 2H), 4.46 (d, *J* = 5.8 Hz, 1H), 4.22 (d, *J* = 5.8 Hz, 1H), 3.78 (t, *J* = 4.5, 8H), 3.86-3.62 (m, 8H), 3.60-3.37 (m, 8H), 3.34-3.16 (m, 8H), 3.15-3.06 (m, 8H). **^{13}C NMR** (150.80 MHz, CD_3CN, -35 °C) δ (ppm): 164.9, 162.7, 150.3, 131.0, 130.0, 115.2, 90.3, 66.6, 66.4, 65.8, 60.4, 49.3, 48.1, 48.0, 45.3.

1-(4-(bis(4-(dimethylamino)phenyl)methyl)-3,5-di(piperidin-1-yl)cyclohexa-2,5-dien-1-ylidene)piperidin-1-ium tetrafluoroborate (Wac): ^{1}H NMR: (600 MHz, CD_2Cl_2, -85 °C) δ (ppm): 7.06 (d, *J* = 8.8 Hz, 4H), 6.53 (d, *J* = 8.8 Hz, 4H), 5.18 (s, 2H), 4.38 (d, *J* = 4.0 Hz, 1H), 4.17 (d, *J* = 4.0 Hz, 1H), 3.32-3.00 (m, 12H), 2.84 (s, 12H), 1.74-1.40 (m, 18H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, -80 °C) δ (ppm): 161.2, 159.8, 148.3, 129.4, 123.5, 110.6, 88.3, 58.9, 48.6, 47.9, 44.7, 39.8, 25.8, 25.5, 23.5, 23.4. **^{1}H NMR** (600 MHz, CD_3CN, -30 °C) δ (ppm): 7.17 (d, *J* = 8.5 Hz, 4H), 6.62 (d, *J* = 8.5 Hz, 4H), 5.30 (s, 2H), 4.48 (d, *J* = 5.4 Hz, 1H), 4.17 (d, *J* = 5.4 Hz, 1H), 3.45 (t, *J* = 4.9 Hz, 4H), 3.27-3.12 (m, 8H), 2.87 (s, 12H), 1.76-1.42 (m, 18H). **^{13}C NMR** (150.80 MHz, CD_3CN, -35 °C) δ (ppm): signals tentatively assigned: 162.1, 158.5, 149.8, 130.6, 126.3, 112.5, 89.5, 60.4, 52.10, 49.2, 47.9, 45.5, 40.2, 26.7, 26.6, 25.1, 24.4, 24.3, 24.32, 24.26.

1-(4-(bis(4-(dimethylamino)phenyl)methyl)-3,5-di(pyrrolidin-1-yl)cyclohexa-2,5-dien-1-ylidene)pyrrolidin-1-ium tetrafluoroborate (Wcc): ^{1}H NMR (600 MHz, CD_2Cl_2, -20 °C) δ (ppm): 7.18 (d, *J* = 8.8 Hz, 4H), 6.56 (d, *J* = 8.8 Hz, 4H), 4.58 (s, 2H), 4.15 (d, *J* = 5.3 Hz, 1H), 4.02 (d, *J* = 5.3 Hz, 1H), 3.50 (t, *J* = 6.7 Hz, 2H), 3.45 (t, *J* = 6.7 Hz, 2H), 3.42-3.34 (m, 2H), 3.30 (t, *J* = 6.7 Hz, 2H), 3.20-3.07 (m, 4H), 2.88 (s, 12H), 2.04-1.7 (m, 12H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, -20 °C) δ (ppm): 160.4, 160.1, 149.8, 130.1, 125.8, 111.4, 87.1, 59.3, 51.3, 49.0, 48.9, 48.6, 48.3, 43.3, 40.4, 25.6, 25.4, 25.0, 24.7, 24.6.**^{1}H-NMR** (600 MHz, CD_3CN, -12 °C) δ (ppm): 7.26 (d, *J* = 8.7 Hz, 4H), 6.61 (d, *J* = 8.7 Hz, 4H), 4.62 (s, 2H), 4.30 (d, *J* = 5.4 Hz, 1H), 4.07 (d, *J* = 5.4 Hz, 1H), 4.01 (q, *J* = 9.6 Hz, 4H), 3.52-3.45 (m, 4H), 3.39-3.33 (m, 2H), 3.20-3.10 (m, 6H, two signals overlapped), 1.95-1.68 (m, 8H), 1.79-1.69 (m, 4H) . **^{13}C-NMR** (150.80 MHz, CD_3CN, -12 °C) δ (ppm): 161.0, 157.0, 150.4, 130.8, 127.2, 112.0, 87.9, 58.1, 51.3,49.20, 49.16, 49.08, 49.01, 48.86, 48.30, 40.3, 25.8, 25.6, 25.2, 25.1, 24.9, 24.8.

General procedure for the exchange of the nucleophilic moiety: A solution of 1,3,5-triaminobenzene derivative **1a** or **1b** ($2.0x10^{-5}$ mol), was dissolved in 0.7 mL of CD_3CN, and introduced in the NMR spectroscopy tube that was inserted in the NMR probe. When the probe temperature reached -20°C, an equimolar amount of the benzhydrylium ions **2c** or **2a**, respectively, (0.02 mmol) was added to the solution, that became orange/yellow, and the ^{1}H-NMR spectrum of the resulting **Wac** or **Wba** was recorded. Then to the obtained solution, an equivalent amount of the nucleophilic species **1c** was added. Immediately after mixing, the spectrum showed disappearance of signals ascribed to the piperidinyl or morpholinyl moiety of **Wac** and **Wba** respectively, with concomitant appearance of signals belonging to the Wheland complexes with the pyrrolidinyl derivative **1c** (**Wcc** and **Wca**) together with typical signals for the free nucleophiles **1b** or **1a**.

General procedure for the synthesis of 4a-b: To the benzhydrylium ion **2a**, dissolved in CH_2Cl_2 (4 mL), under nitrogen atmosphere and at room temperature, was added a two-fold excess of the nucleophilic species **3a** or **3b**. Immediately after mixing, the color of the reaction mixture turned to bordeaux (**4a**) or violet (**4b**). The progress of the reactions, magnetically stirred, was monitored by TLC and ^{1}H-NMR analysis. The final products were purified by flash chromatography on silica gel (dichloromethane/n-hexane 9:1 for **4a**, Et_2O/n-hexane 9.5:0.5 for **4b**).

General procedure for the synthesis of 4c: To the benzhydrylium ion **2a (**$2x10^{-5}$ mol), dissolved in CH_2Cl_2 (4 mL), under nitrogen flow and at room temperature, was added an

equimolar amount of nucleophilic species **3c** in the presence of 2 eq of basic Al_2O_3. Immediately after mixing, the color of the reaction mixture turned rom strong violet to pale red. The progress of the reaction, magnetically stirred, was monitored by TLC and ^{1}H-NMR analysis and at the end of the reaction the Al_2O_3 was filtered off and the solvent evaporated under vacuum. Finally, an equimolar amount of a $3.7x10^{-2}$M methanolic/KOH solution was added to the residue, affording the substitution product **4c**.

Compounds **4a-c** were fully characterized by usual spectroscopic methods; chemico-physical data are reported as follows.

4,4'-((2,4-di(piperidin-1-yl)phenyl)methylene)bis(N-methyl-N-(2,2,2-trifluoro ethyl)aniline) (4a): yellow liquid, 77% yield. **^{1}H NMR** (400 MHz, CD_2Cl_2, 25 °C) δ (ppm): 6.98 (d, *J* = 9.0 Hz, 4H), 6.86 (d, *J* = 8.6 Hz, 1H), 6.76 (d, *J* = 9.0 Hz, 4H), 6.74 (d, *J* = 2.7 Hz, 1H), 6.61 (dd, J_1 = 8.4, J_2 = 2.7, 1H), 5.91 (s, 1H), 3.96 (q, *J* = 9.3 Hz, 4H), 3.10 (t, *J* = 5.5 Hz, 4H), 3.00 (s, 6H), 2.69 (t, *J* = 4.5 Hz, 4H), 1.70-1.48 (m, 12H). **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 154.2, 152.3, 147.6, 136.1, 132.2, 131.6, 130.5, 127.2 (q, J_{C-F} = 283.5 Hz), 113.3, 112.6, 110.2, 55.0, 54.3 (q, J_{C-F} = 32.6 Hz), 51.3, 48.0, 39.6, 27.5, 26.6, 25.01, 24.98. Un segnale in più. **ESI MS (ES$^+$) m/z:** 633 $[M+H]^+$, 655 $[M+Na]^+$, 671 $[M+K]^+$.

4,4'-((2,4-dimorpholinophenyl)methylene)bis(N-methyl-N-(2,2,2-trifluoroethyl) aniline) (4b): pale pink liquid, 85% yield. **^{1}H NMR** (400 MHz, CD_2Cl_2, 25 °C) δ (ppm): 6.98 (d, *J* = 8.7 Hz, 4H), 6.89 (d, *J* = 6.9 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 5H, two signals overlapped), 6.65 (dd, J_1 = 8.7, J_2 = 2.6, 1H), 5.95 (s, 1H), 3.99 (q, *J* = 9.7 Hz, 4H), 3.77 (t, *J* = 4.7 Hz, 4H), 3.69 (t, *J* = 4.7 Hz, 4H), 3.10 (t, *J* = 4.8 Hz, 4H), 3.00 (s, 6H), 2.69 (t, *J* = 4.8 Hz, 4H). **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 152.7, 151.6, 147.7, 135.8, 133.1, 131.8, 130.5, 129.9 (q, J_{C-F} = 282.3 Hz), 113.3, 112.2, 109.7, 67.9, 67.4, 54.5 (q, J_{C-F} = 29.4 Hz), 53.9, 50.0, 48.3, 39.5. **ESI MS (ES$^+$) m/z:** 637 $[M+H]^+$, 659 $[M+Na]^+$.

4,4'-((2,4-di(pyrrolidin-1-yl)phenyl)methylene)bis(N-methyl-N-(2,2,2trifluoroethyl) aniline) (4c): yellow, 62% yield. **^{1}H NMR** (400 MHz, CD_3CN, 25 °C) δ (ppm): 6.95 (d, *J* = 8.6 Hz, 4H), 6.78 (d, *J* = 9.0 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 4H), 6.33 (d, *J* = 2.4 Hz, 1H), 6.20 (dd, J_1 = 8.5, J_2 = 2.5, 1H), 5.77 (s, 1H), 3.97 (q, *J* = 9.5 Hz, 4H), 3.23 (t, *J* = 6.9 Hz, 4H), 3.00 (s, 6H), 2.94 (t, *J* = 5.9 Hz, 4H), 2.00-1.94 (m, 4H), 1.86-1.80 (m, 4H). **^{13}C NMR** (100.56 MHz, CD_3CN, 25 °C) δ (ppm): 150.7, 148.2, 147.6, 136.7, 132.5, 130.5, 130.4,

128.5, 126.5, 125.7, 113.2, 107.0, 103.2, 54.2 (q, $J_{C\text{-}F}$ = 31.8 Hz), 53.4, 48.7, 48.3, 39.5, 25.9, 25.1. **ESI MS (ES^+) m/z:** 605 $[M+H]^+$, 627 $[M+Na]^+$, 643 $[M+K]^+$.

Formation and detection of Wheland intermediates Wa-c.

A solution of 1,3-diaminobenzene derivatives **3a-c** (0.02 mmol), was dissolved in CD_2Cl_2 (1 mL) and introduced in a NMR spectroscopy tube that was inserted in the NMR probe. When the probe temperature reached –80°C, an equimolar amount of the benzhydrylium ion **2a** (0.02 mmol) was added to the solution, that became orange/yellow, and the ^1H NMR spectrum of the resulting solution was quickly recorded. The system was monitored after various times and at different temperatures until 25 °C. Immediately after the mixing, the spectrum at –80 °C showed the appearance of signals ascribed to the substitution products **4a-c**, and signals ascribed to **Wa-c**, assigned with the aid of *g*-COSY and *g*-HSQC experiments. On raising the temperature, signals belonging to **Wa-b** gradually broadened and then disappeared at about 20°C for **Wa** and −10 °C for **Wb**, and the only signals at room temperature, were those ascribed to the substitution products **4a-b**. In case of reaction of **3c** with **2a**, signals ascribed to the **Wc** were distinguishable and remain stable at room temperature, togheter with the major product **4c**.

1-(4-(bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methyl)-3-(piperidin-1-yl)cyclo hexa-2,5-dien-1-ylidene)piperidin-1-ium tetrafluoroborate (Wa): ^1H NMR (600 MHz, CD_2Cl_2, -70 °C) δ (ppm): 7.12 (d, *J* = 8.2 Hz, 2H), 6.96-6.90 (m, 1H), 6.78 (d, *J* = 8.2 Hz, 2H), 6.71 (d, *J* = 8.2 Hz, 2H), 6.59-6.54 (m, 3H, two signals overlapped), 5.37 (br.s, 1H), 4.51-4.47 (m, 1H), 4.21 (br.s, 1H), 3.94-3.86 (m, 2H), 3.86-3.76 (m, 2H), 3.72 (d, *J*=12.5 Hz, 2H), 3.67-3.59 (m, 2H), 3.22-3.06 (m, 4H), 2.99 (s, 3H), 2.92 (s, 3H), 1.76-1.56 (m, 12H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, -70 °C) δ (ppm): 167.3, 157.4, 147.1, 146.6, 143.1, 130.4, 128.2, 127.7, 126.1, 125.3 (q, $J_{C\text{-}F}$ = 286.0 Hz),, 119.2, 111.4, 110.5, 89.3, 54.8, 53.4 (q, $J_{C\text{-}F}$ = 32.4 Hz), 52.7 (q, $J_{C\text{-}F}$ = 32.4 Hz), 50.1, 49.9, 49.5, 48.2, 41.9, 39.0, 38.8, 26.9, 26.8, 25.8, 25.2, 23.6 (two signals overlapped).

4-(4-(bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methyl)-3-morpholinocyclohexa-2,5-dien-1-ylidene)morpholin-4-ium tetrafluoroborate (Wb): ^1H NMR (600 MHz, CD_2Cl_2, -50 °C) δ (ppm): 7.05 (d, *J* = 8.1 Hz, 2H), 6.98-6.90 (m, 3H, two signals overlapped), 6.69 (d, *J* = 9.1 Hz, 2H), 6.68-6.61 (m, 3H, two signals overlapped), 5.59 (s, 1H), 4.48 (t, *J* = 6.5 Hz, 1H), 4.03 (d, *J* = 6.5 Hz, 1H), 3.98-3.76 (m, 4H), 3.76-3.67 (m, 4H), 3.68-3.60 (m, 2H), 3.60-3.51 (m, 2H), 3.51-3.42 (m, 4H), 3.17-3.09 (s, 4H), 3.02-2.97 (m, 6H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, -70 °C) δ (ppm): 169.4, 158.3, 147.1, 145.5,

129.6, 128.8, 126.8, 124.4, 117.8, 111.4, 111.06, 108.4, 104.0, 89.3, 66.0, 56.7, 52.3, 48.3, 47.9, 47.7, 46.5, 42.6, 38.9.

1-(4-(bis(4-(methyl(2,2,2-trifluoroethyl)amino)phenyl)methyl)-3-(pyrrolidin-1-yl)cyclohexa-2,5-dien-1-ylidene)pyrrolidin-1-ium (Wc): ^{1}H NMR (600 MHz, CD_2Cl_2, -70 °C) δ (ppm): 7.06 (d, *J* = 6.9 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.76-6.72 (m, 1H), 6.70 (d, *J* = 7.7 Hz, 2H), 6.64 (d, *J* = 7.7 Hz, 2H), 6.37 (d, *J* = 10.8 Hz, 1H), 6.59-4.91(s, 1H), 4.23 (t, *J* = 5.7 Hz, 1H), 3.89 (t, *J* = 8.7 Hz, 5H), 3.63 (br.s, 1H), 3.51-3.43 (m, 2H), 3.42-3.34 (m, 2H), 3.33-3.24 (m, 2H), 2.99 (s, 3H), 2.96 (s, 3H), 2.58 (br.s, 1H), 2.07-1.85 (m, 5H), 1.75-1.62 (m, 2H), 1.52 (br.s, 1H). **^{13}C NMR** (150.80 MHz, CD_2Cl_2, -70 °C) δ (ppm): 167.1, 155.0, 146.9, 146.7, 1434.2, 129.3, 128.7, 127.8, 127.6, 125.35 (q, $J_{C\text{-}F}$ = 283.1 Hz), 125.25 (q, $J_{C\text{-}F}$ = 284.4 Hz), 119.5, 111.1, 111.04, 88.5, 57.6, 53.02 (q, $J_{C\text{-}F}$ = 31.0 Hz), 52.42 (q, $J_{C\text{-}F}$ = 32.4 Hz), 49.6, 49.34, 49.30, 49.28, 45.9, 38.98, 38.97, 24.7, 24.6, 24.2, 23.8.

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