Synthesis of 2,3-quinoxalinedithiol

Supplementary Material

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Additional notes on the preparation of 1,2,3,4-tetrahydroquinoxaline-2,3-dione:

The average yield of this compound is 65-70%. The melting point reported in the literature is over 340ºC[1], too high to be determined in the classroom.

SM 6.1.1 - Reaction set apparatus for 1,2,3,4-tetrahydroquinoxaline-2,3-dione

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SM 6.1.2 – Reaction set apparatus for 2,3-dichloroquinoxaline

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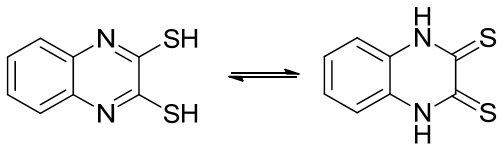
This compound is less soluble in most solvents and less acidic than other known thiols, because the dithione form is the most stable of the tautomers (Scheme **SM 6.1.4**).



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IR spectra:

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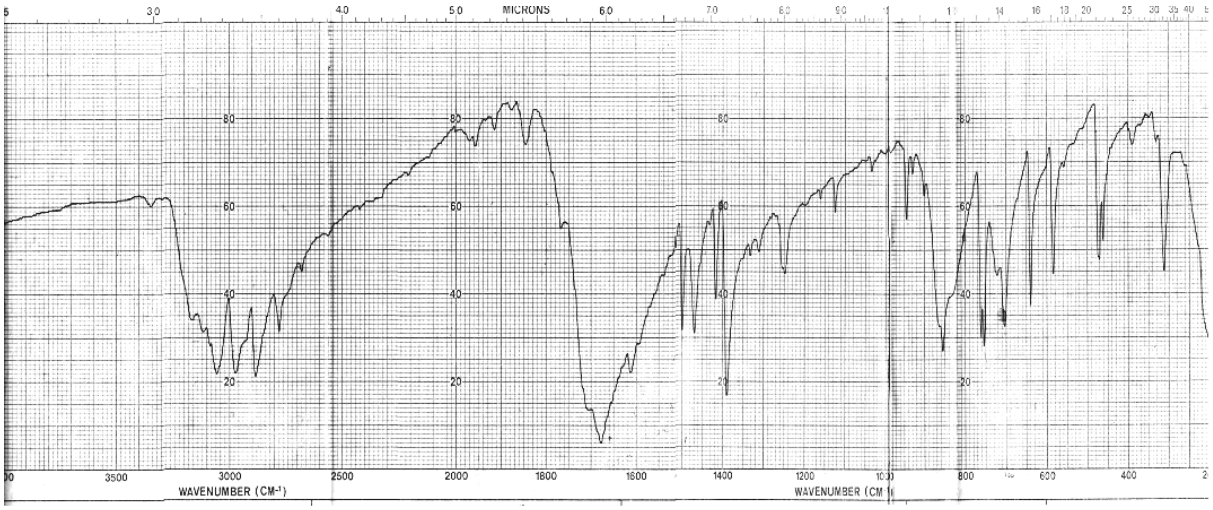

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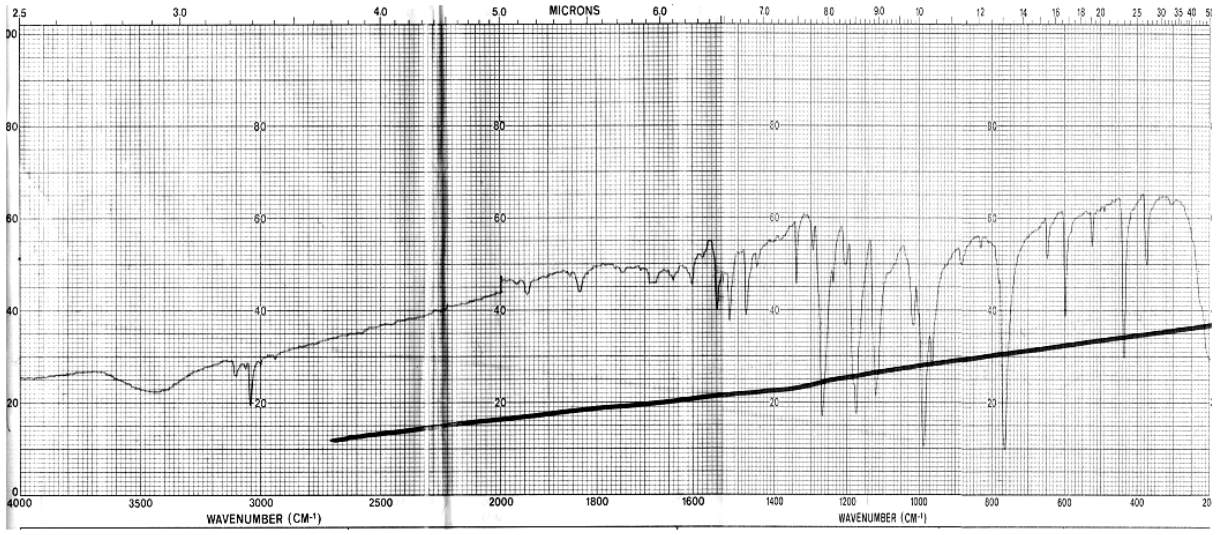

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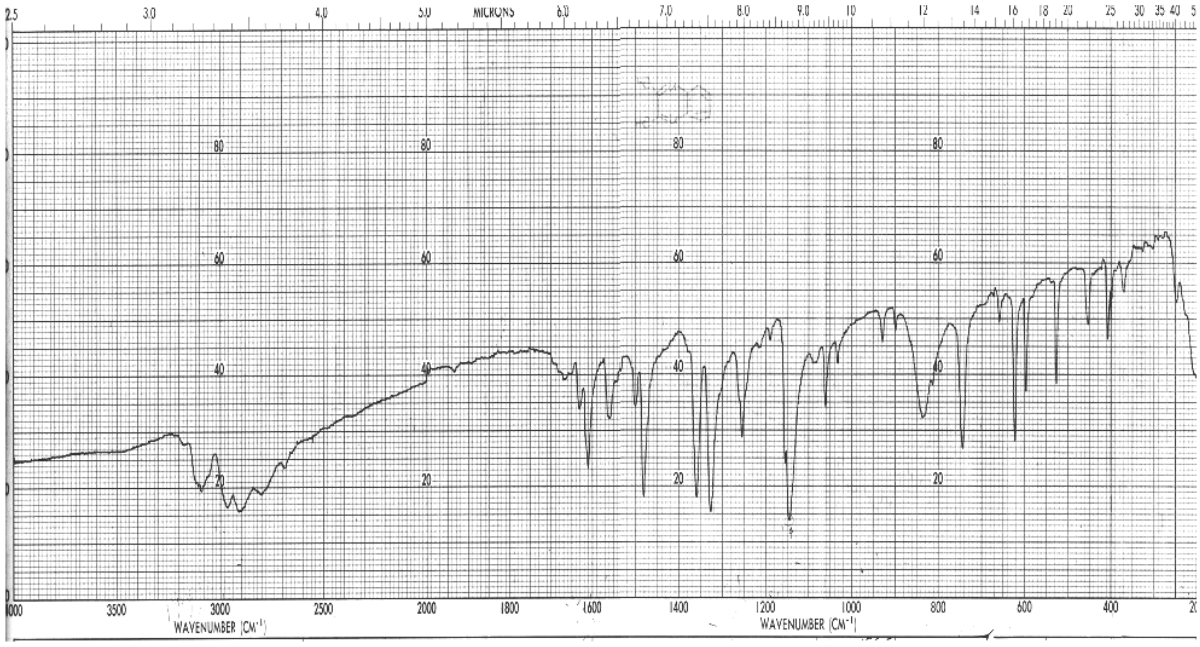

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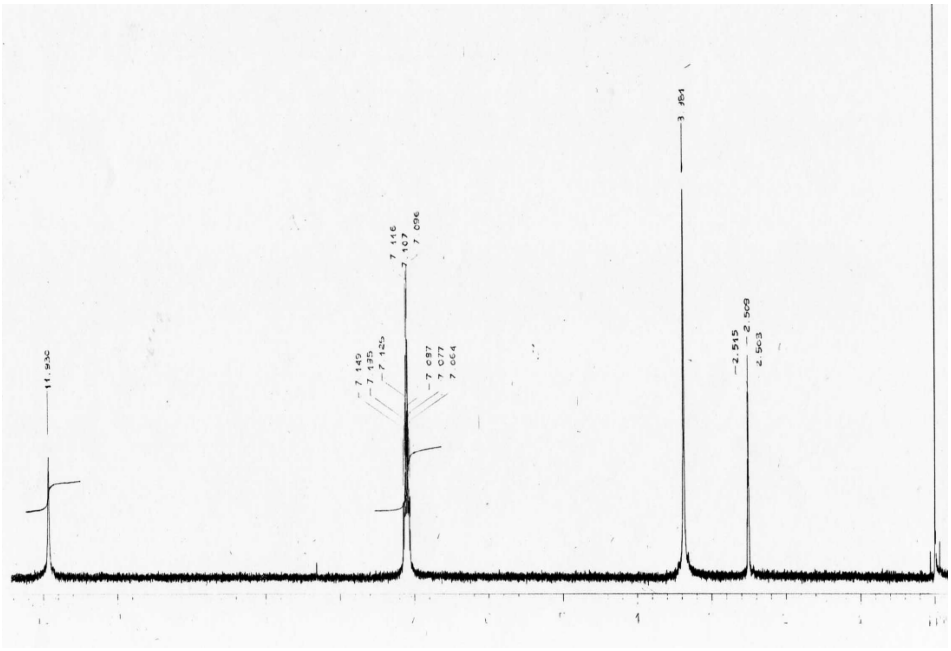
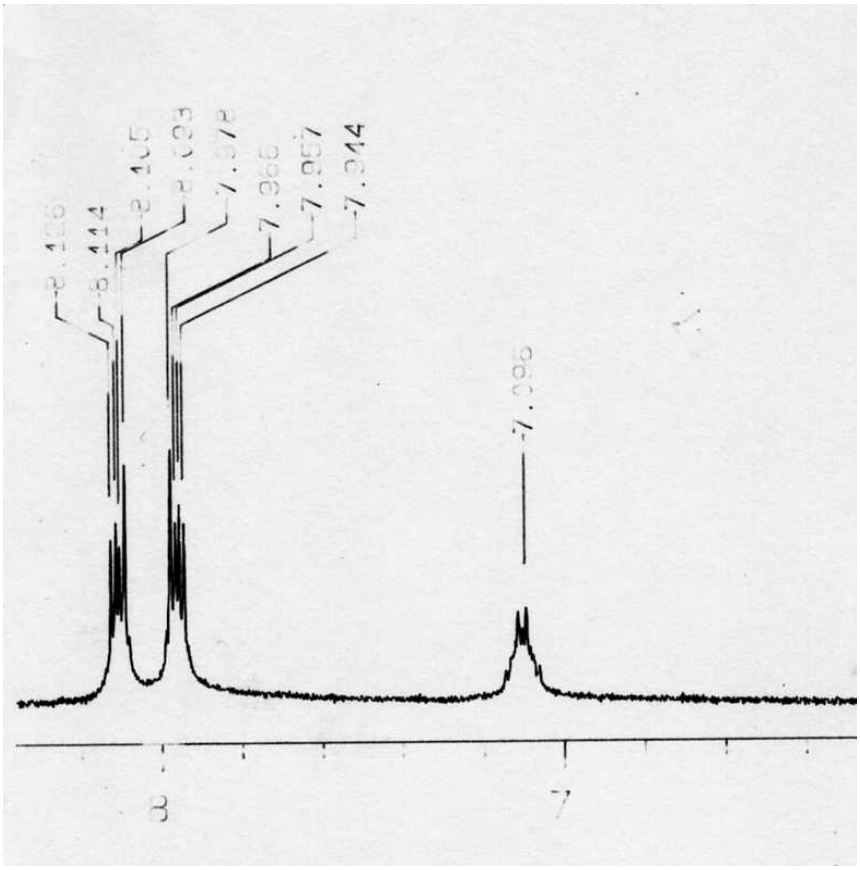


SM 6.1.8 - ^{1}H NMR (DMSO-d_6) of 1,2,3,4-tetrahydroquinoxaline-2,3-dione



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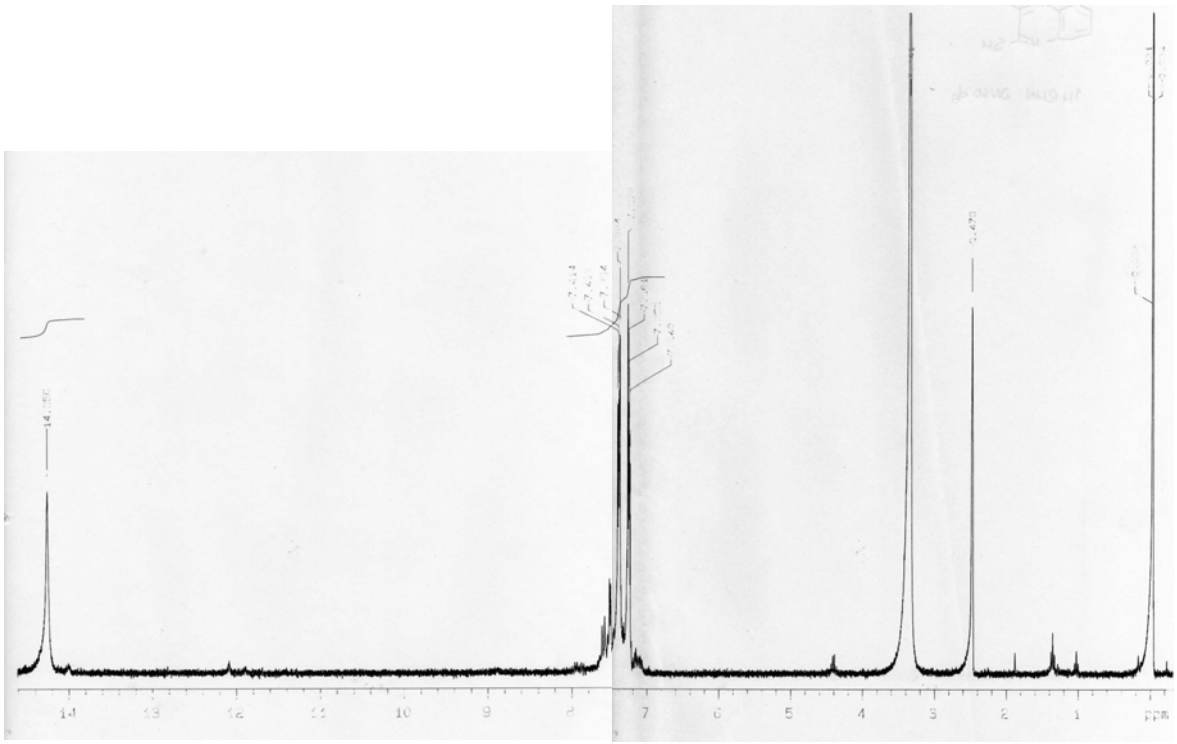

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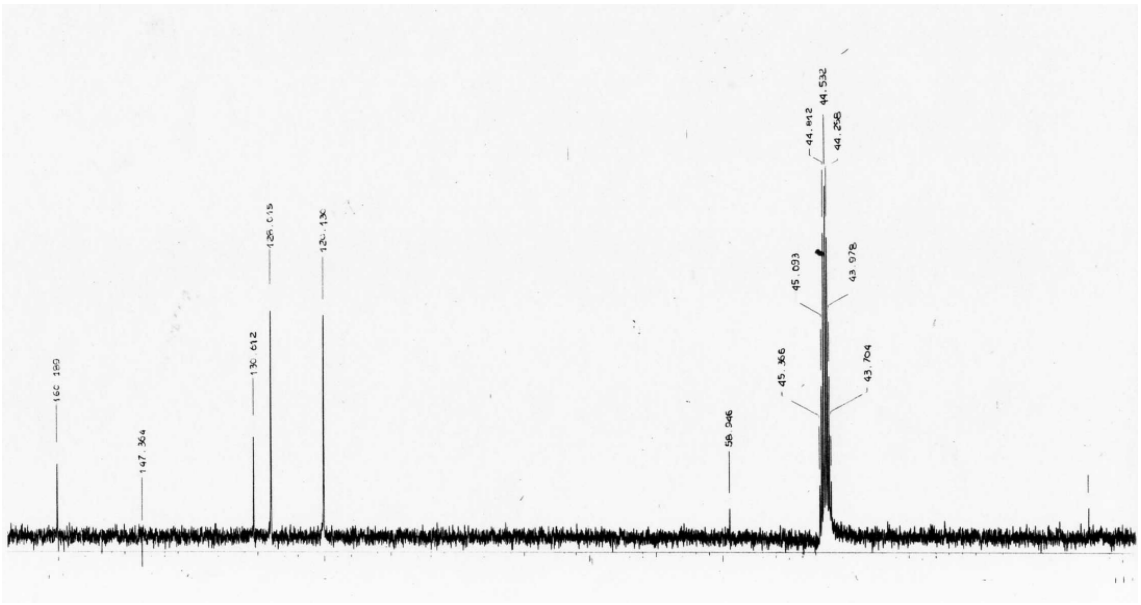

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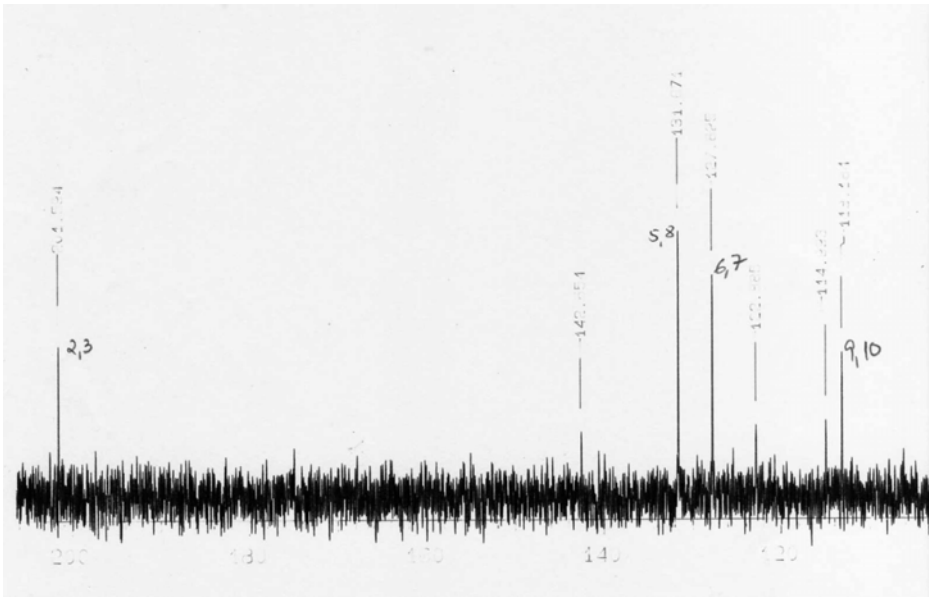

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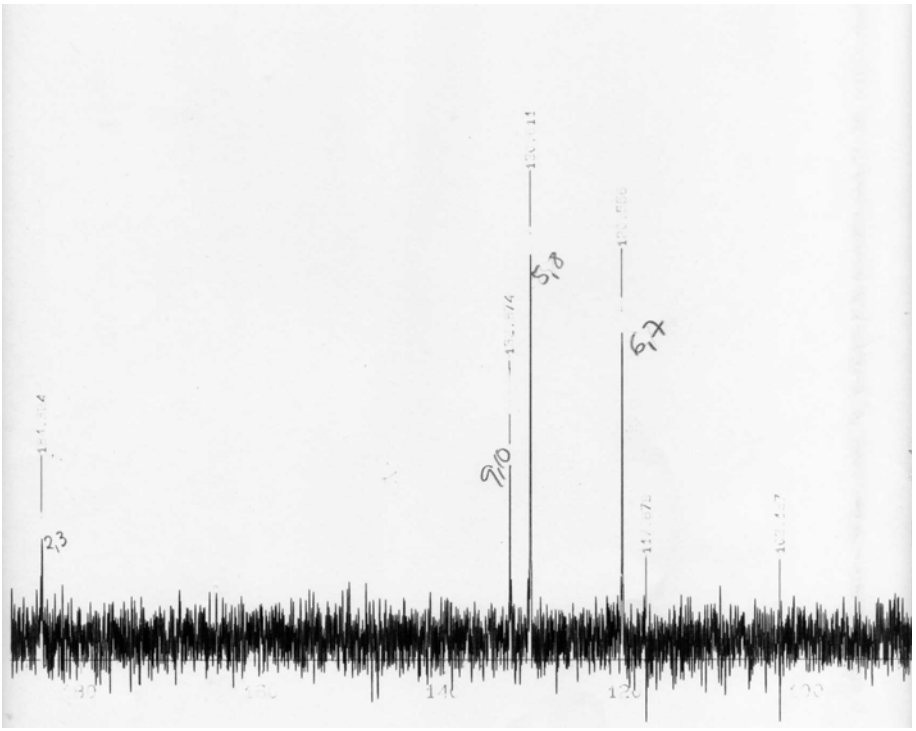

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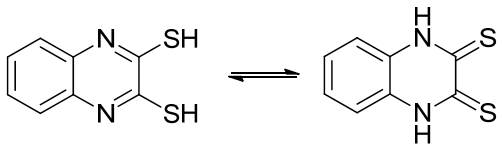

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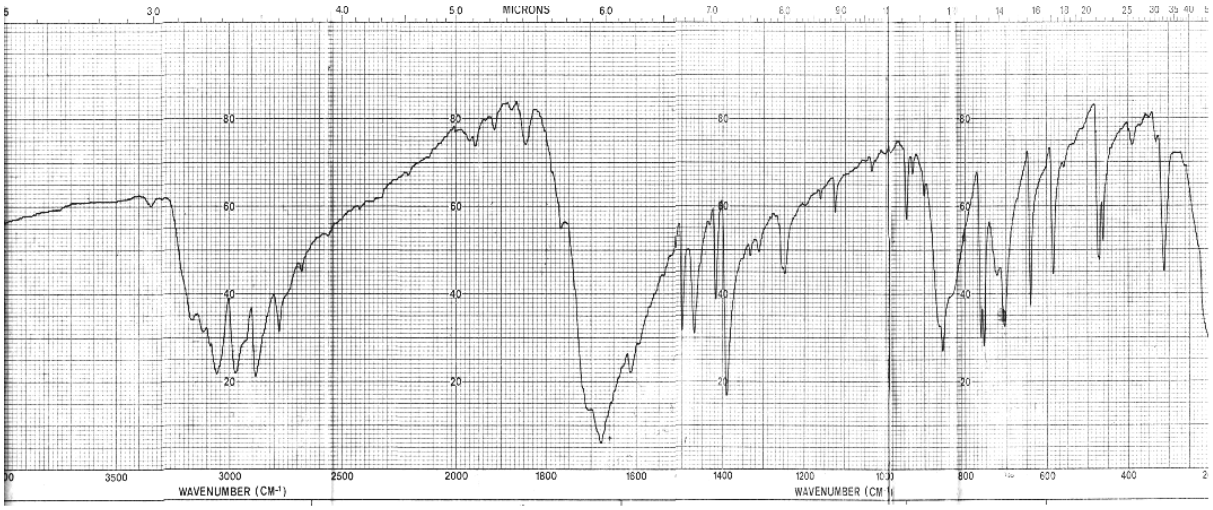

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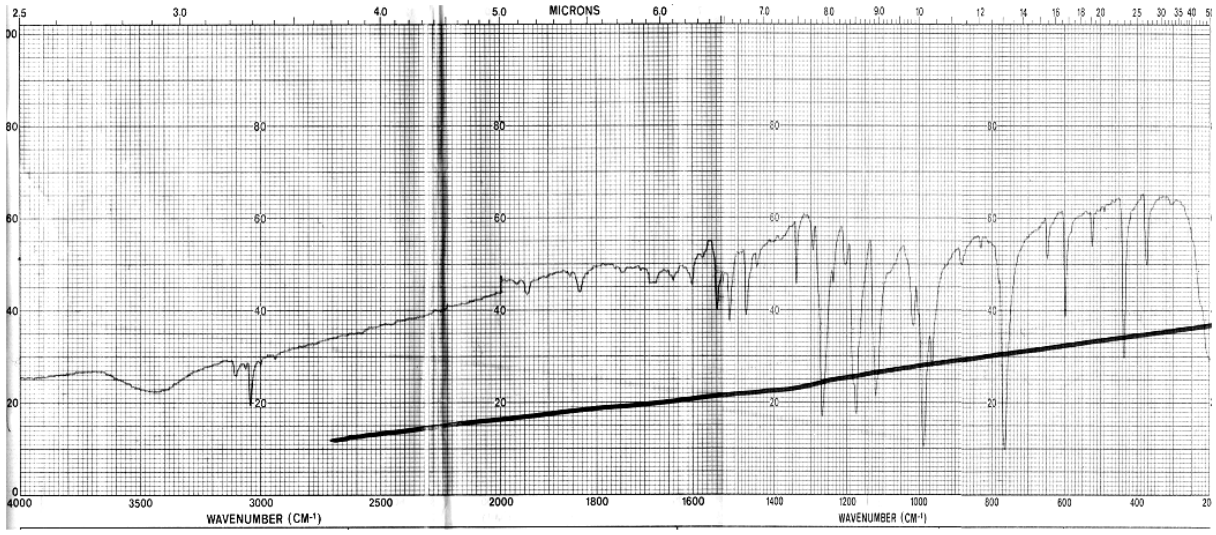

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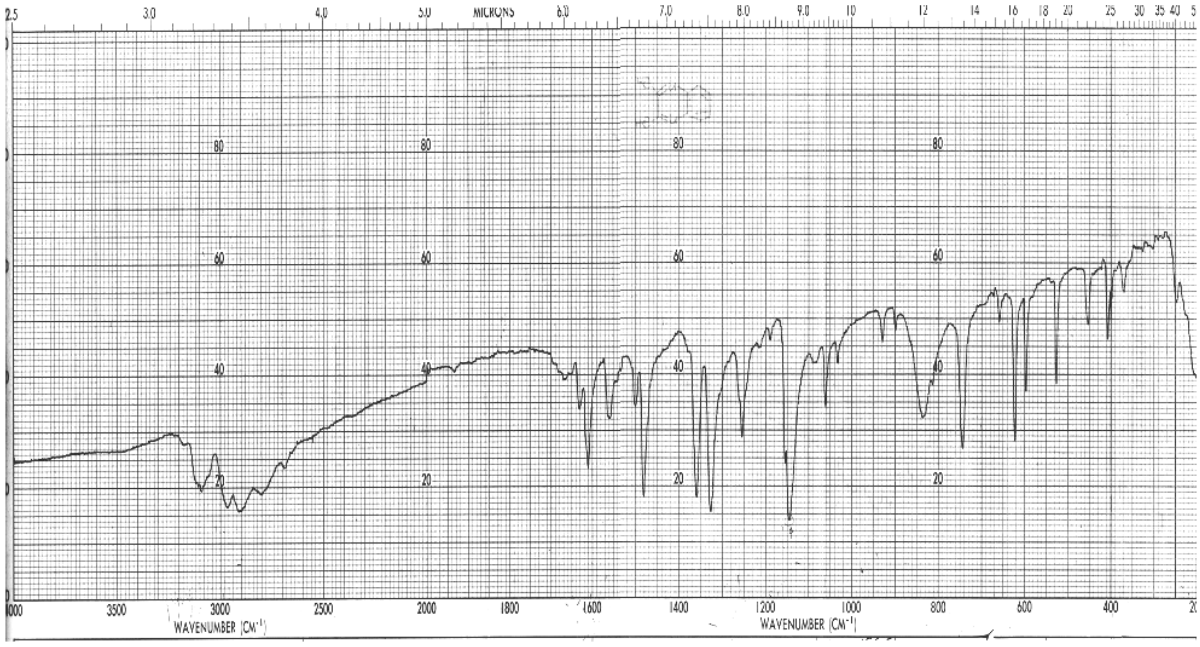

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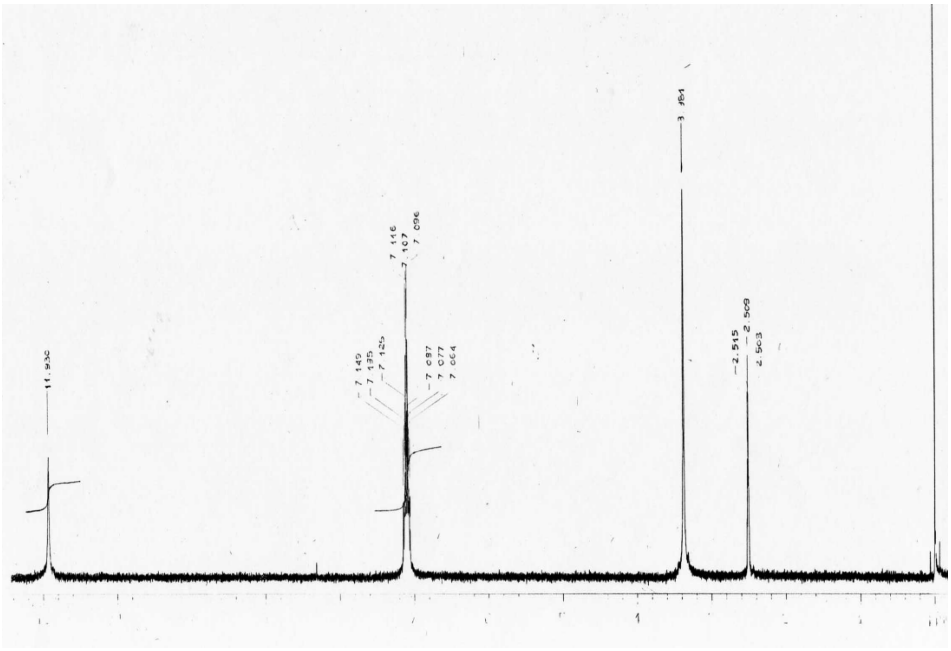

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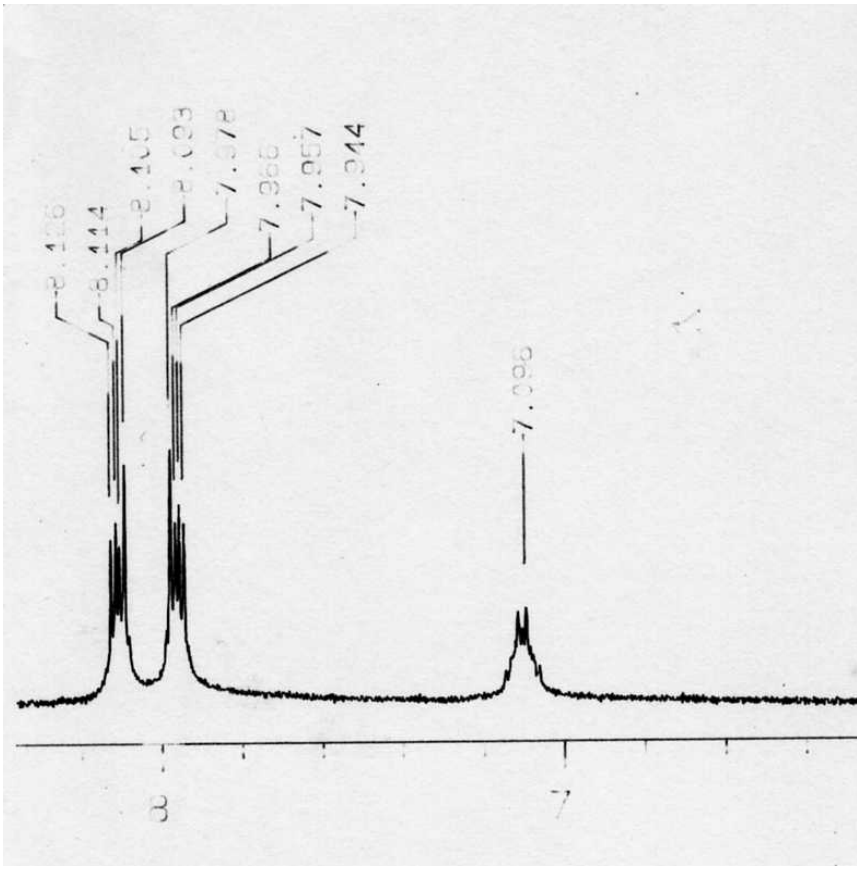

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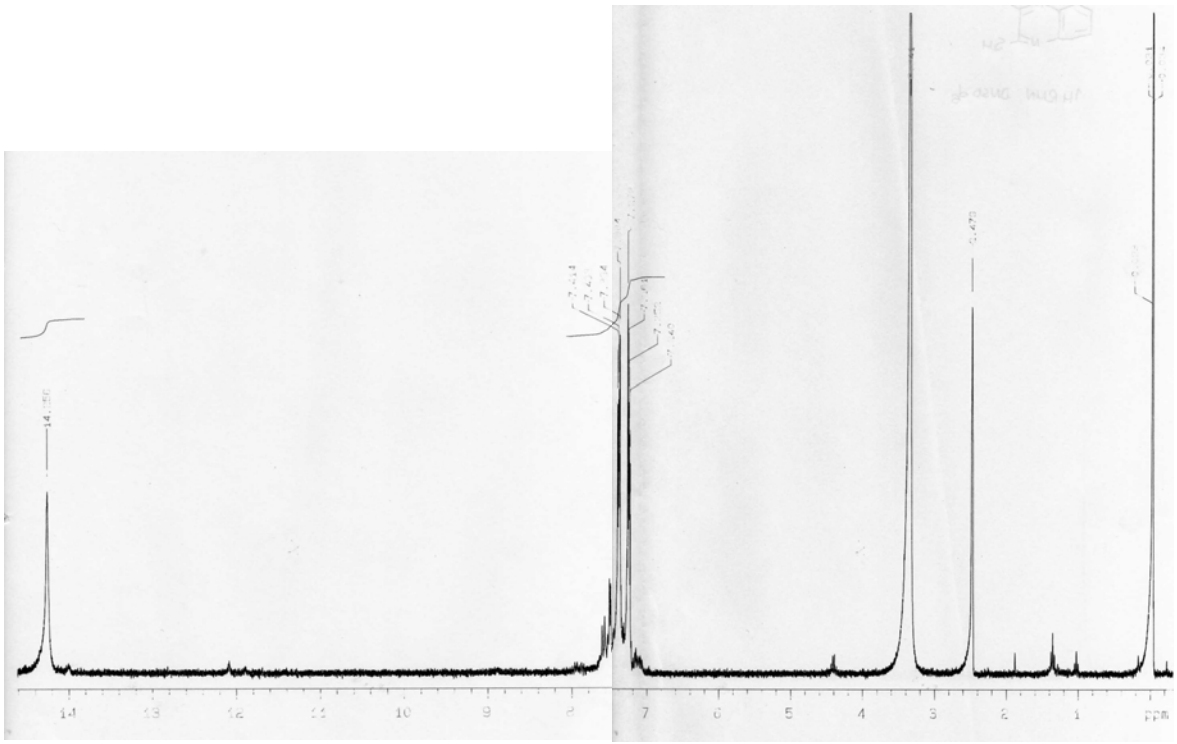

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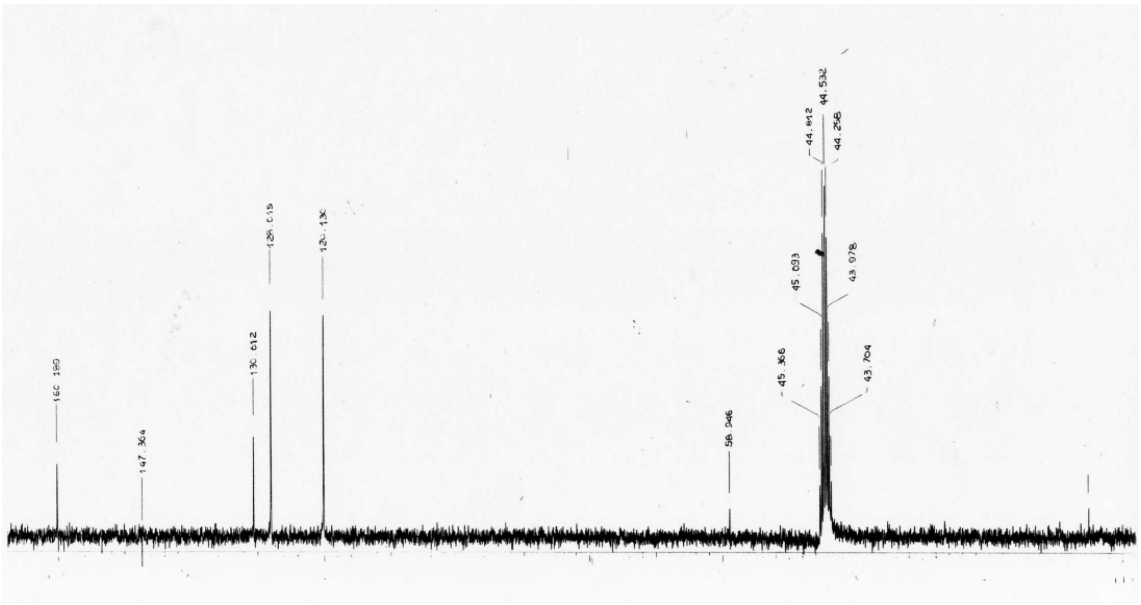

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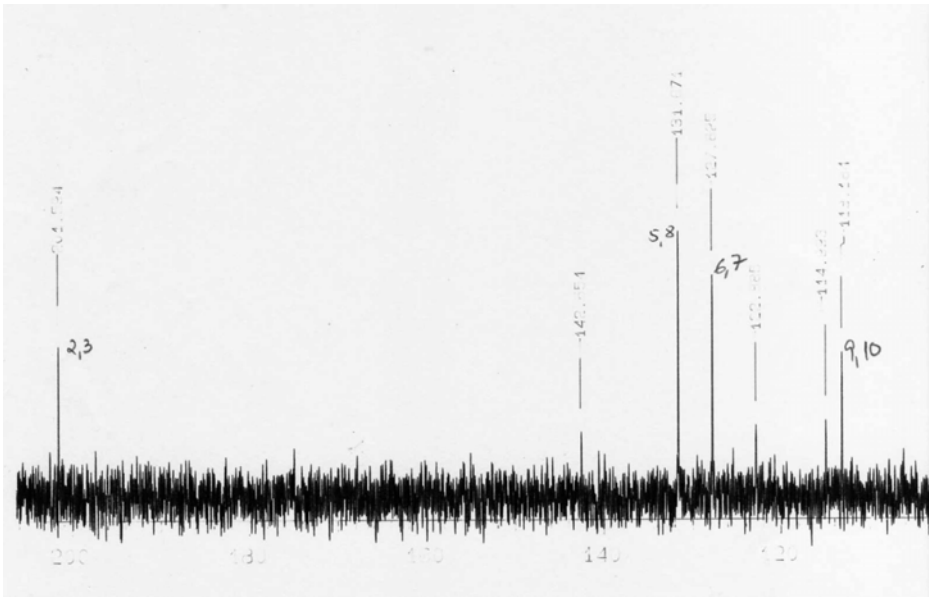

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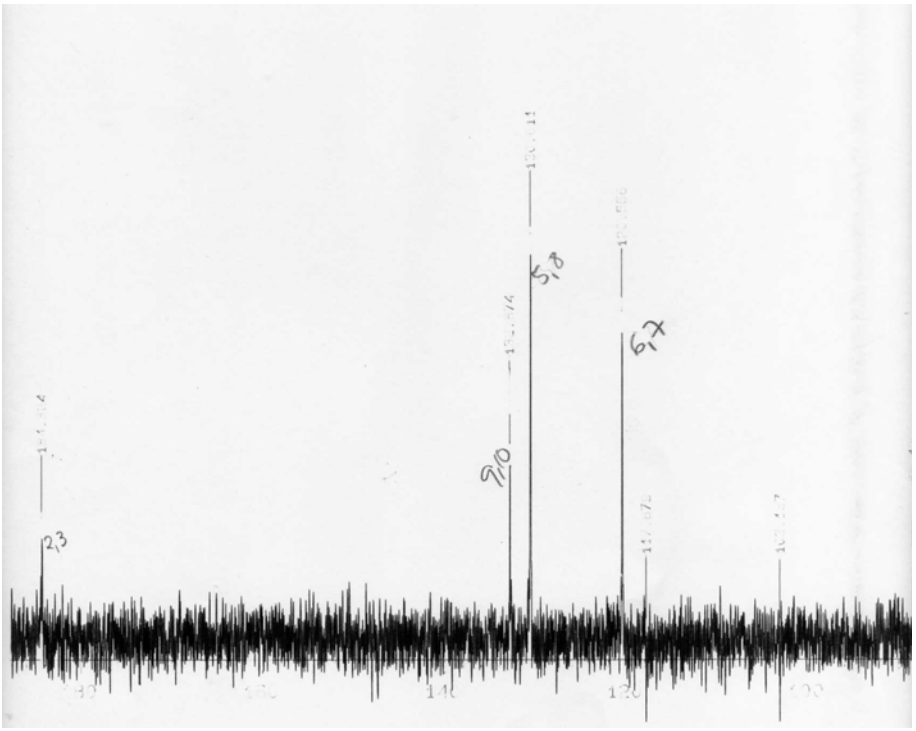

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Synthesis of the antitumoral drug 2,4,6-tris(dimethylamino)-1,3,5-triazine *via* sequential nucleophilic substitution

Supplementary Material

1. Experiments reproduction and students results.

HMM was first synthesized by the authors according to the conditions develop by Kolesinska *et. al.*,[1] (**Table SM X.1, Entry 1**), followed by optimization of the reactions conditions aiming the search for more environmental conditions and execution by the students. (**Table SM X.1 and Table SM X.2**). The reported method[1] implies the isolation of the product HMM by vacuum distillation (2.7 mbar) or under high temperature, which is not feasible in the teaching laboratory. In order to circumvent this limitation, after different approaches we found that the HMM can be easily isolated although with considerable yield erosion, just by immobilization of the reaction mixture in silica followed by selective dissolution of HMM by hexane and by crystallization in hexane.

Table SM X.1. Selected results obtained by the authors during the optimization of HMM conditions.

Entry	Solvent	Reaction time	Isolated product Yield (%)
1[a]	Dichloromethane	1h30 min	62
2	Dichloromethane	30 min	28[b]
3	Ethyl acetate	30 min	24
4	1,2-dicloroethane	30 min	22

Entry 1- [a] Followed reported method which required further vacuum distillation of benzyl chloride [(2.7 mbar), boiling point (30 ºC)].[1] General method (Entries 2 - 4): Cyanuric chloride (500 mg, 2.7 mmol) was dissolved in the appropriated solvent and *N*,*N*-dimethylbenzylamine (3 moles eq.) was added dropwise. The reaction mixture was reflux for 30 min. The product was obtained as a crystalline white solid after crystallization from hexane. [b] Complete conversion from ^{1}H NMR of the crude reaction was observed.

The authors also investigated the reaction conditions to prepare HMM under microwave irradiation and compared it with the thermal heating. The microwave reaction was studied in a CEM-equipment (**Table SM X.2, Entry 3**) and in a domestic microwave oven (**Table SM X.2, Entries 2 and 5**) in order to become more easily implemented in a teaching laboratory. The conversion observed during microwave irradiation using 4 and 7 amine moles equivalents are very similar providing yields above 95% (**Table SM X.2, Entry 2 vs 5**). Furthermore the reaction is more efficient with microwave irradiation then under thermal heating since complete conversion is achieved while under thermal heating the product was formed in only 74% (**Table SM X.2, Entry 4 vs 5**). Nevertheless these results show that the domestic microwave oven is an excellent alternative apparatus to perform this transformation.

Table SM X.2. Selected results under solvent free conditions obtained by the authors during the optimization of HMM conditions using thermal heating and microwave irradiation.

Entry	Heating mode	Amine moles (eq.)	Isolated product Yield (%)[a];[Conversion (%)][b]
1	Thermal	4	16[a]
2	Domestic microwave oven	4	69[a]; [96][b]
3	CEM-equipment	4	75[a]
4	Thermal	7	[74][b]
5	Domestic microwave oven	7	[100][b]

General reaction method: *N*,*N*-dimethylbenzylamine (4 or 7 moles eq.) were added to cyanuric chloride (500 mg, 2.7 mmol) the reaction mixture was placed under thermal (150 ºC) for 30 min or microwave irradiation (CEM-equipment adjusted to170 ºC, Power 300 W or Domestic microwave oven adjusted to med high) for 3 min. The product was purified by adding to the reaction mixture dichloromethane and ethanol, evaporation of the solvent and adsorption of the reaction mixture in silica followed by addition of hexane and solvent evaporation. The product was obtained as a white solid after crystallization using hexane; [b]Conversion observed by ^{1}H NMR of the crude reaction.

In the next stage the experiment was implemented in the teaching laboratory environment (3 hours) by undergraduate students from the 2nd year of pharmaceutical sciences course (5 years course), they worked as teams of 2 students (12 groups in four classes), and the results are presented in **Table SM X.3**. During the laboratory sessions the students performed the synthesis of HMM using different reaction conditions, such as the quantity of amine and the reaction time in the domestic microwave oven. The purity of the products obtained by the students were determined during the laboratory class by melting point and later by ^{1}H and ^{13}C NMR. The melting points presented in some cases are lower (up to 10ºC) than the ones reported in the literature [mp=172-174 ºC][2].

Table SM X.3. Results from the students experiments in domestic kitchen-Crown Japan microwave instrument.

Entry	Amine (moles eq.)	Reaction time (min)	Isolated product Yield (%)
1[a]	4	3	15
2[a]	4	5	27
3[a]	4	3	26
4[a]	7	6	22
5[b]	variation conditions		<22

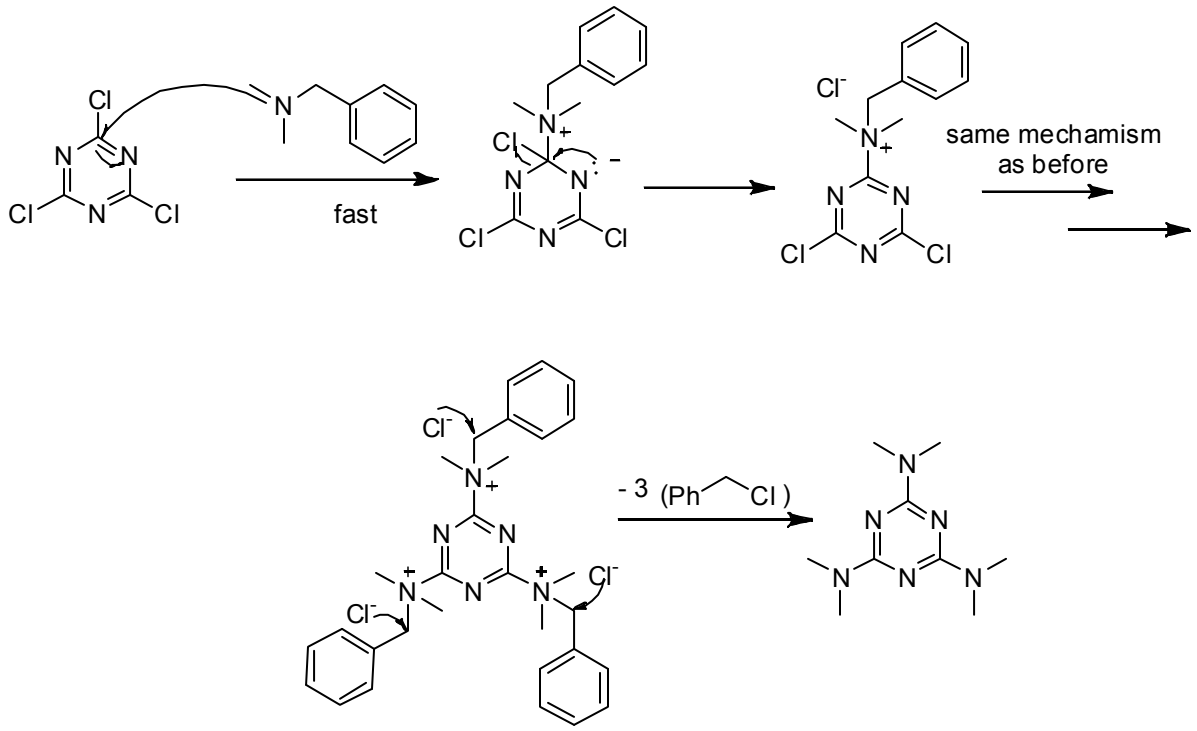
General reaction method: *N*,*N*-dimethylbenzylamine (4 or 7 moles eq.) were added to cyanuric chloride (500 mg, 2.7 mmol) the reaction mixture was placed in a domestic microwave instrument a kitchen-Crown Japan. The product was purified by adding to the reaction mixture dichloromethane and ethanol, evaporation of the solvent and adsorption of the reaction mixture in silica followed by addition of hexane and solvent evaporation. The product was obtained as a white solid after crystallization from hexane; [a] Experiment performed by each student group; [b] Experiments done by the remaining groups using different experiment conditions such as amine moles equivalents (3), and reaction time (2 min).

2. Answers of the hints for students' discussion

1. Explain the reaction mechanism for the synthesis of HMM using N,N-dimethylbenzylamine, a tertiary amine.[1] Compare the reaction mechanism with a tertiary amine to the reaction with secondary or primary amines.

The mechanism for HMM is presented on **Scheme SM X.2**. The mechanism was based on the studies of Kolesinska and co-workers. [1]

Scheme SM X.2. Proposed reaction mechanism for HMM synthesis from cyanuric chloride and *N,N*-dimethylbenzylamine.



The reactivity of the triazine aromatic ring after the first nucleophilic substitution is completely different in case of using primary/secondary amines or tertiary amines. In case of primary/secondary amines (electron donating groups) after the nucleophilic addition to triazine carbons the reactivity of the triazine ring decrease and the subsequent nucleophilic reaction in more difficult. Using tertiary amines a quaternary ammonium is formed (electron withdrawing substituent) after the first addition to the triazine ring and the next aromatic nucleophilic reaction is favored due to the accumulation of the positive charge in the triazine ring.

2. Discuss the competition between the benzyl and the methyl group on the nucleophilic substitution of the ammonium salt.

A Density Functional Theory study[3] on the nucleophilic attack of the chloride to the ammonium salt **A** was performed (**Figure SM X.1**). It was determined that the energy barrier for the nucleophilic attack of the chloride ion to the benzylic position (path a) is 4.4 kcal/mol lower than the energy barrier that corresponds to the nucleophilic attack at the methyl group (path b). In both paths, a secondary amine is the leaving group of the reaction. However, a nucleophilic attack at the benzylic position is favored due to the stabilization of the transition state through overlap of the transition state π-type orbital and the π-system of the phenyl ring. A planarity of the chloride ion and the π system of the aromatic ring is visible in the DFT optimized transition state of the benzylic nucleophilic attack (**Figure SM X.2**).

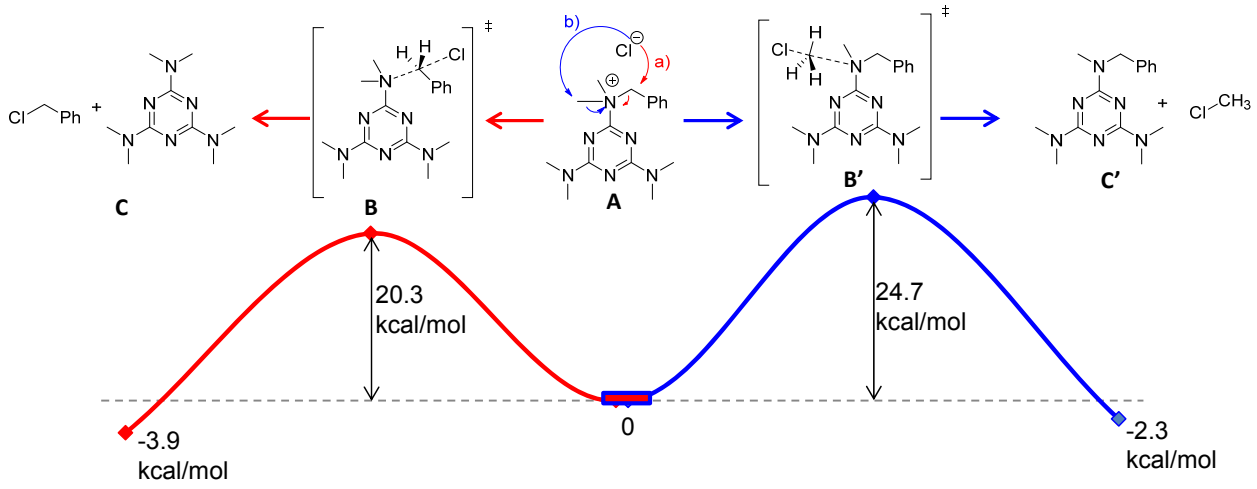


Figure SM X.1. Energy profiles calculated for the chloride nucleophilic substitution of the benzyl and methyl groups of ammonium chloride **A**. The minima and the transition states were optimized and the energy values (kcal/mol) are referred to **A** in dichloromethane.

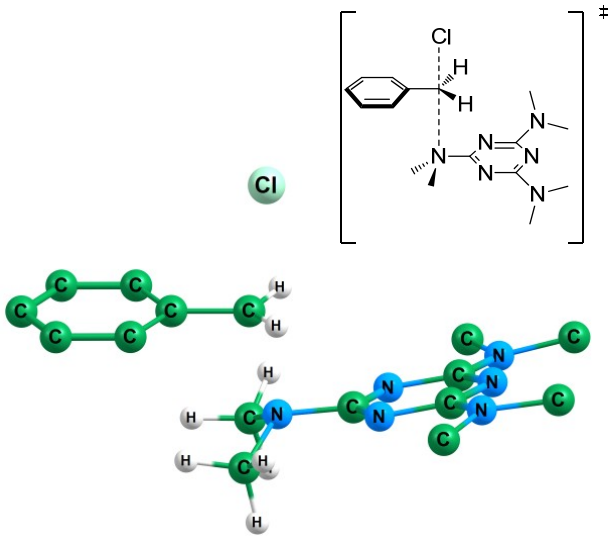


Figure SM X.2. Tridimensional and planar representation of the optimized transition state for the chloride benzylic nucleophilic substitution (**B**). Some of the hydrogen atoms were omitted for sake of clarity.

3. *Considering the use of N,N-dimethylbenzylamine, 4-nitro-N,N-dimethylbenzylamine and 4-methoxy-N,N-dimethylbenzylamine in the synthesis HMM. Analyzed the* ^{1}*H-NMR data for each reaction obtained after 30 minutes reaction at 150 °C of cyanuric chloride with 7 moles equivalents of the correspondent amine* **(Figure SM X.7, Figure SM X.8 and Figure SM X.9).** Discuss the influence of the substituent in *para* position of the amine aromatic ring for the preparation of HMM.

The HMM formation was evaluated taking into account the influence of an electron donating and an electron withdrawing group in the *para* position in the amine aromatic ring. The amines 4-nitro-*N,N*-dimethylbenzylamine (as a strongly electron withdrawing group), 4-methoxy-*N,N*-dimethylbenzylamine (as a moderately electron donating group) and *N,N*-dimethylbenzylamine were used in the same reactions conditions, i.e. 7 moles equivalents of amine with cyanuric chloride during 30 min at 150ºC, after this period a ^{1}H NMR was performed. The ^{1}H NMR data present in **Figure SM X.7, Figure SM X.8 and Figure SM X.9** show some differences regarding the product integration that can be assigned to the amine reactivity. In this sense using *N,N*-

dimethylbenzylamine the product is integrated to 2.57 corresponding to 70%[1] of conversion, using the amine 4-nitro-*N,N*-dimethylbenzylamine the product gave an integration of 2.26 corresponding to a conversion of 64%. This small difference should be related to the presence of the nitro group in the *para* position. In case of using 4-methoxy-*N,N*-dimethylbenzylamine in the same reaction conditions, the ^{1}H NMR data present a product integration of 4.32 that correspond to 97% yield. This can be due to the donating character of the methoxy group that favors the nucleophilic substitution by inductive effect. Once the reactivity is determined by the first nucleophilic substitution to the cyanuric chloride and since the amines used in this studied have slight differences in nucleophilicity, as expected the more nucleophic amine gives rise to more product.

4. Please explain why microwave irradiation is more advantageous in some reaction when compared to conventional heating.

The advantages of the use of microwave irradiations instead of a thermal bath are: [4-6]

- Depending on the polarity of the species involved in the reaction, the reaction time is usually much faster for polar molecules due to more efficient interactions with microwave irradiation. Taken in consideration that a tris-quaternary *N*-triazinylammonium salt is formed along the preparation of HMM, microwave irradiations dramatically increase the reaction rate for this sequence of addition-elimination events.
- The reactions can be usually performed under free-solvent conditions or in water. The employment of silica or clays is usually more profitable in the absorption of microwaves irradiation, than the use of solvents that may lead to radiation losses depending on its dielectric constant. Due to its low molecular weight and high dielectric constant, water is a good solvent for microwave irradiation, since its temperature increases very rapidly.

[1]Since was used an excess of amine the conversion was determined by the following equation.

$$Conversion = \frac{\text{Area of HMM signal (2.57 ppm)}}{[\text{Area of HMM signal (2.57 ppm)} + \text{Area of Amine CH3 signals (6.00 ppm)}]x3/7} x100$$

3. Product NMR spectra

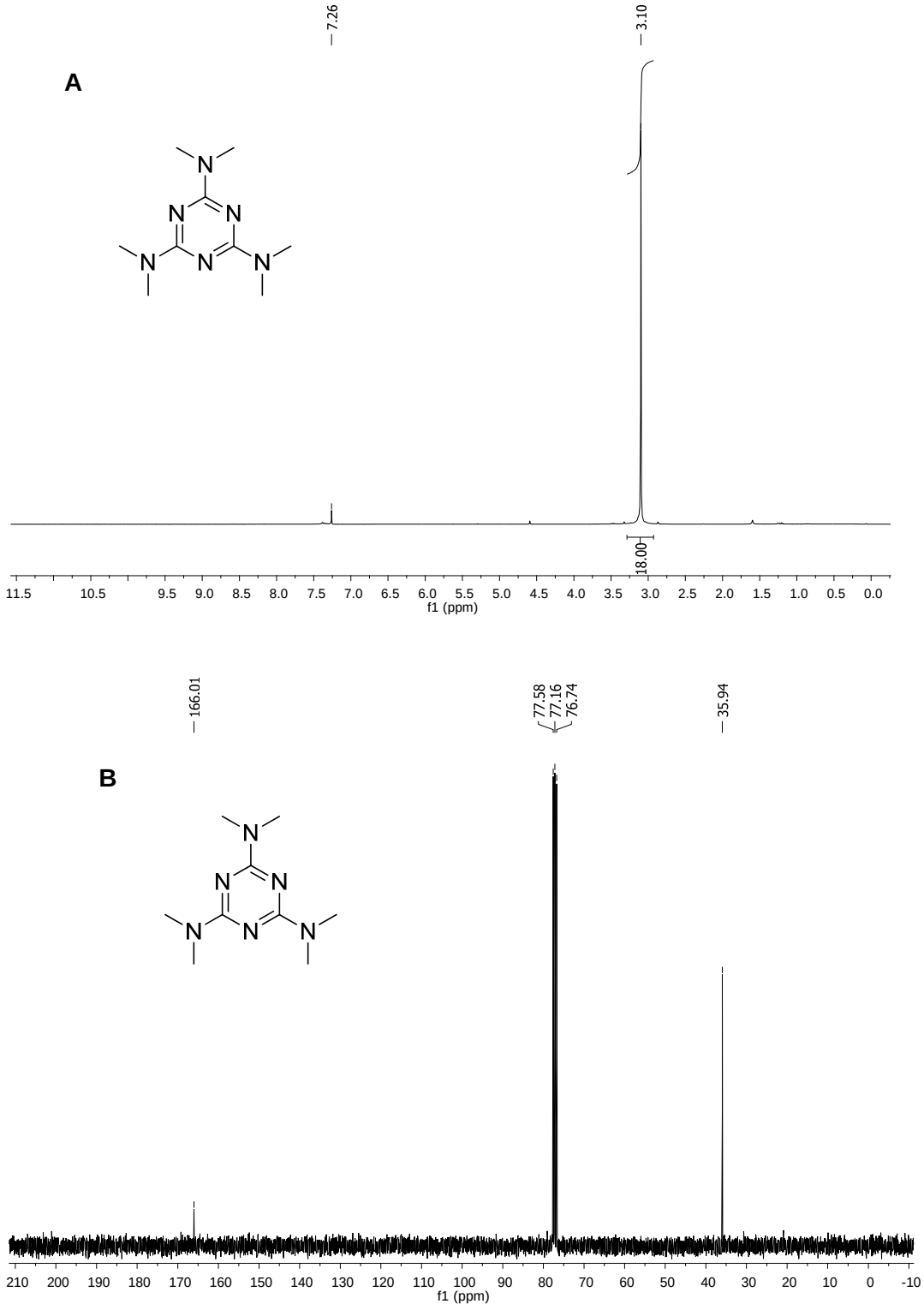


Figure SM X.3. Examples of ^{1}H NMR (400 MHz, $CDCl_3$) (A) and ^{13}C NMR (75 MHz, $CDCl_3$) (B) spectra of HMM obtained by the authors.

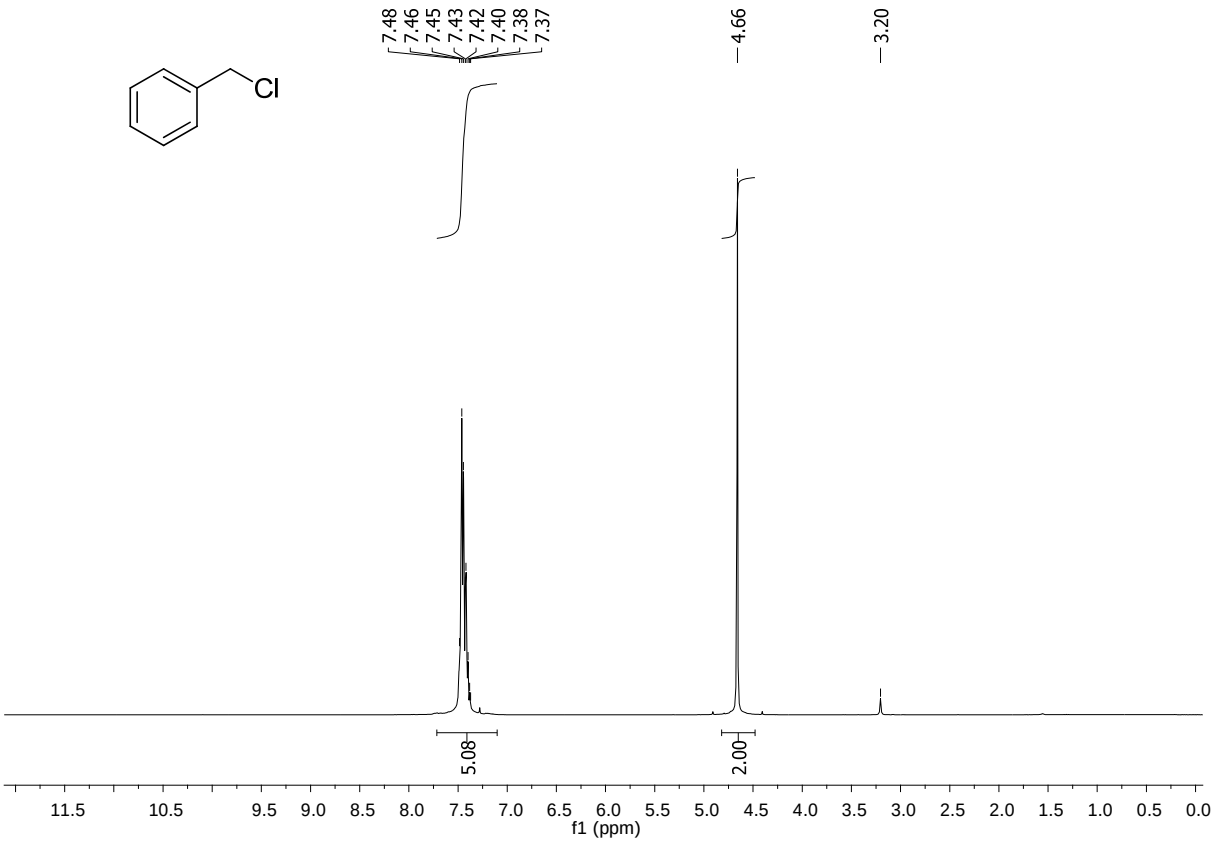


Figure SM X.5. Examples of ^{1}H NMR (400 MHz, $CDCl_3$) spectra of benzyl chloride obtained by the authors during the reproduction of Kolesinska *et. al,*[1] methodology.

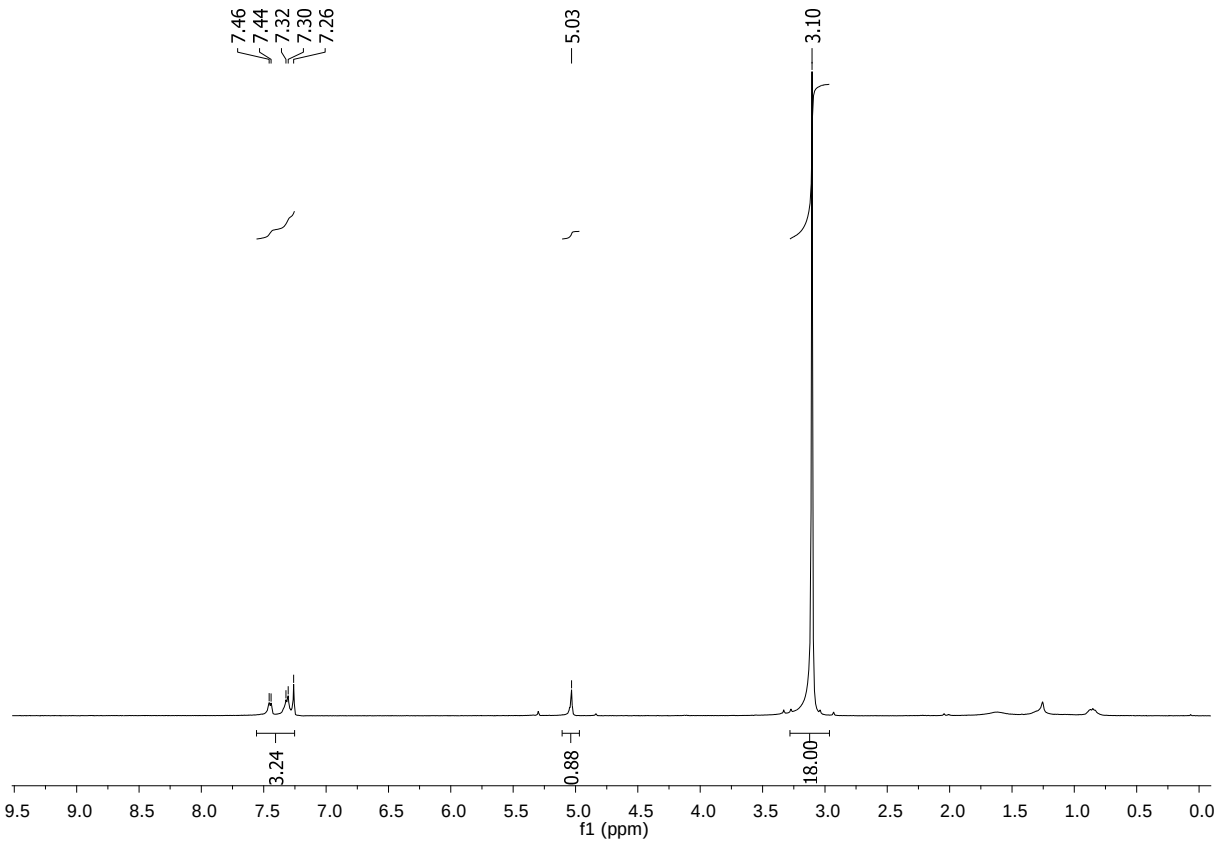
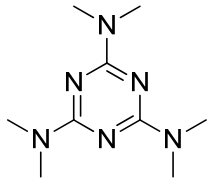


Figure SM X.6. Example of ^{1}H NMR (400 MHz, $CDCl_3$) spectra of HMM obtained by the students.



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10 (s, 1H, 18).

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.01 (C_{ar}), 35.94 (CH_3).

mp=172-175 ºC, [Lit mp=172-174 ºC][2]

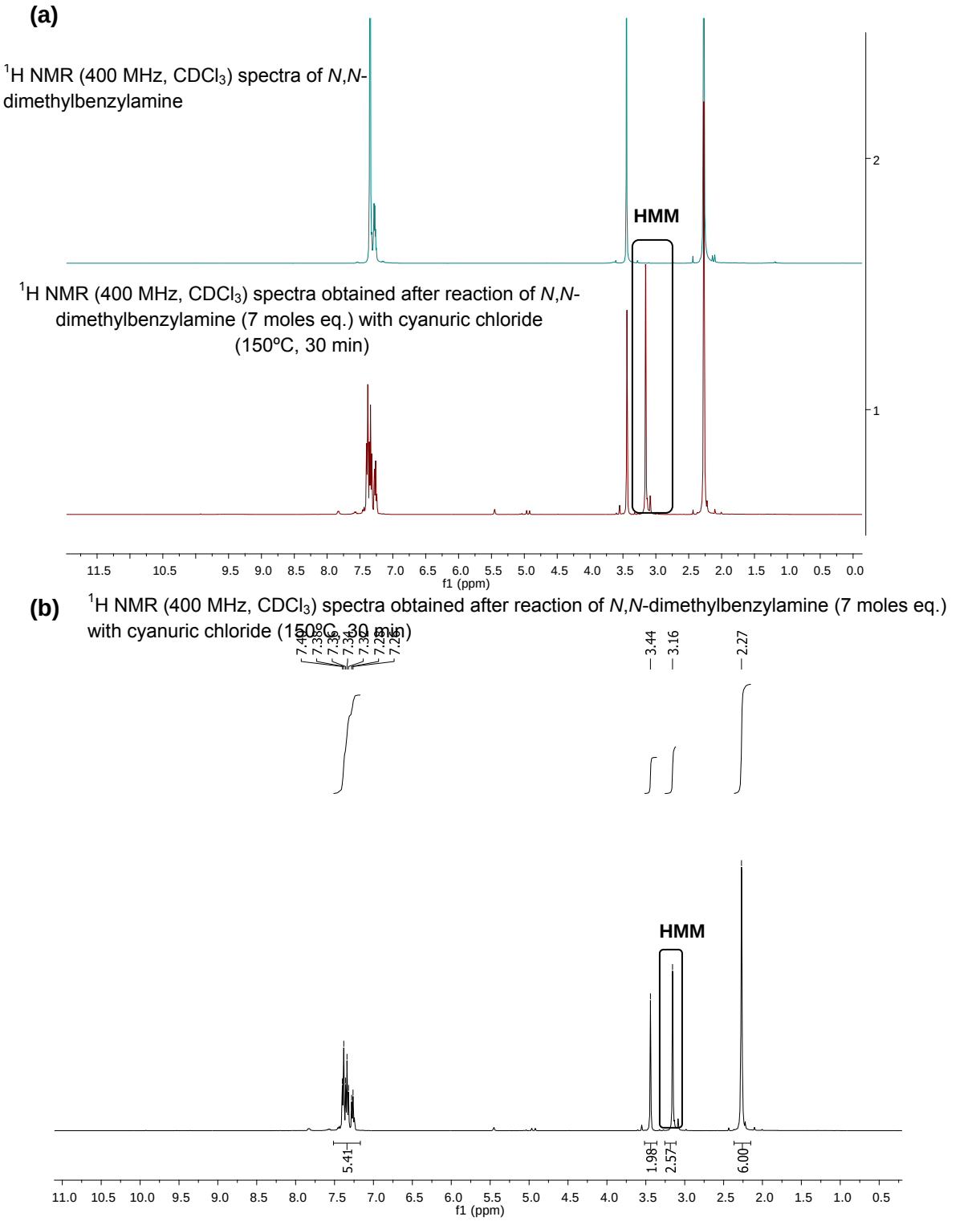


Figure SM X.7. (a) ^{1}H NMR (400 MHz, $CDCl_3$) overlap of *N*,*N*-dimethylbenzylamine spectra and reaction spectra obtained after reaction of 7 moles equivalents of *N*,*N*-dimethylbenzylamine with cyanuric chloride for 30ºmin at 150ºC. **(b)** ^{1}H NMR (400 MHz, $CDCl_3$) reaction spectra obtained after 7 moles equivalents of *N*,*N*-dimethylbenzylamine with cyanuric chloride for 30ºmin at 150ºC.

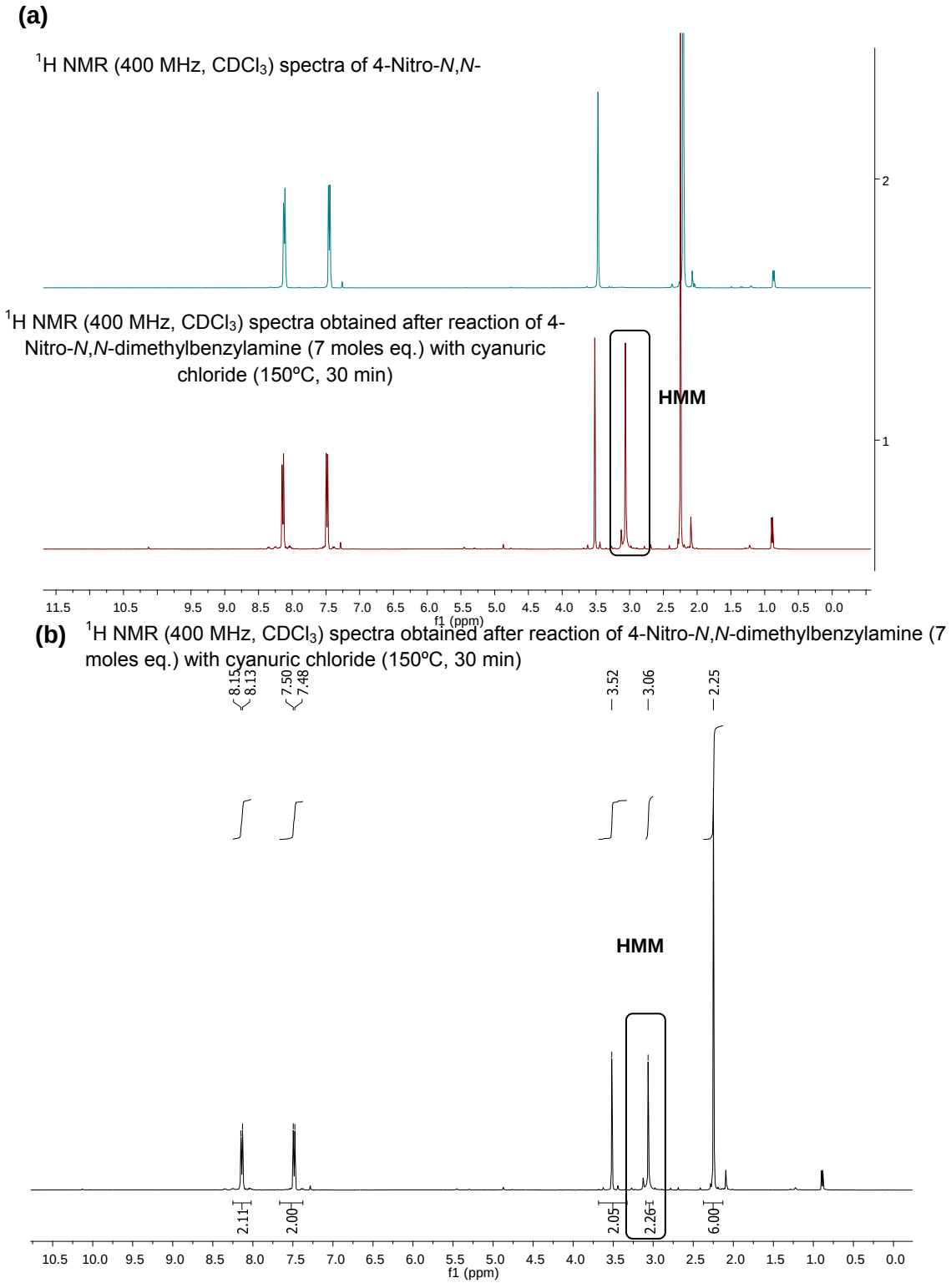
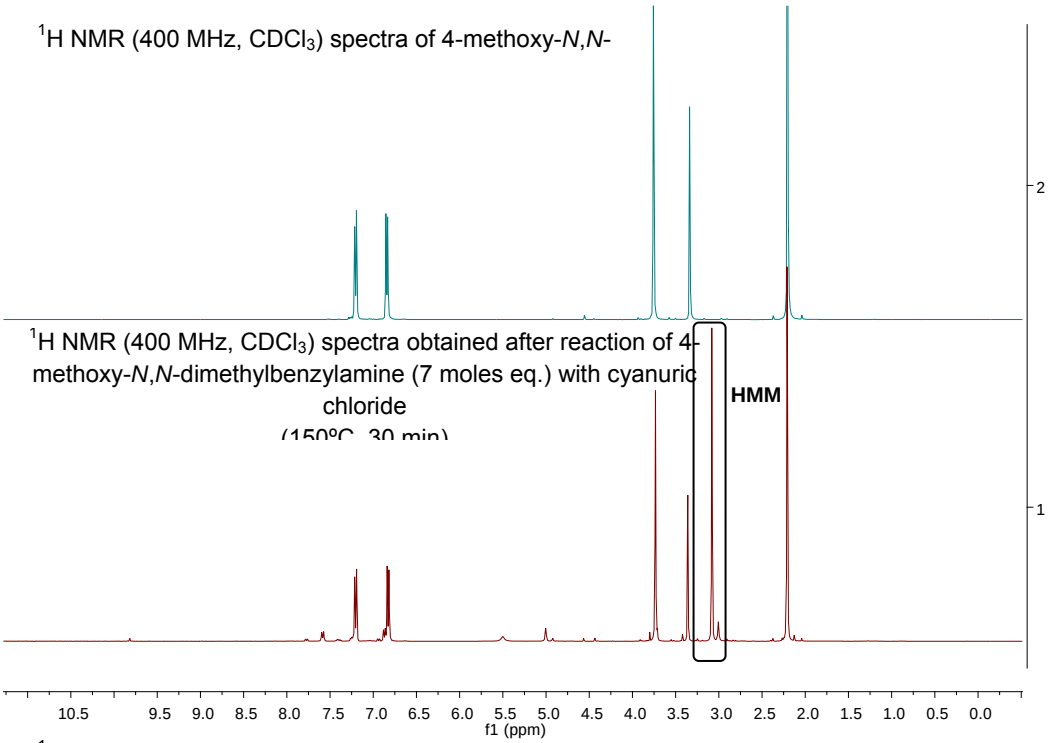


Figure SM X.8. (a) ^{1}H NMR (400 MHz, $CDCl_3$) overlap of 4-Nitro-*N*,*N*-dimethylbenzylamine spectra and reaction spectra obtained after reaction of 7 moles equivalents of 4-Nitro-*N*,*N*-dimethylbenzylamine with cyanuric chloride for 30ºmin at 150ºC. **(b)** ^{1}H NMR reaction spectra obtained after 7 moles equivalents of 4-Nitro-*N*,*N* dimethylbenzylamine with cyanuric chloride for 30ºmin at 150ºC.

(a)



^{1}H NMR (400 MHz, $CDCl_3$) spectra obtained after reaction of 4-methoxy-*N*,*N*-dimethylbenzylamine (7 moles eq.) with cyanuric chloride (150ºC, 30 min)

(b)

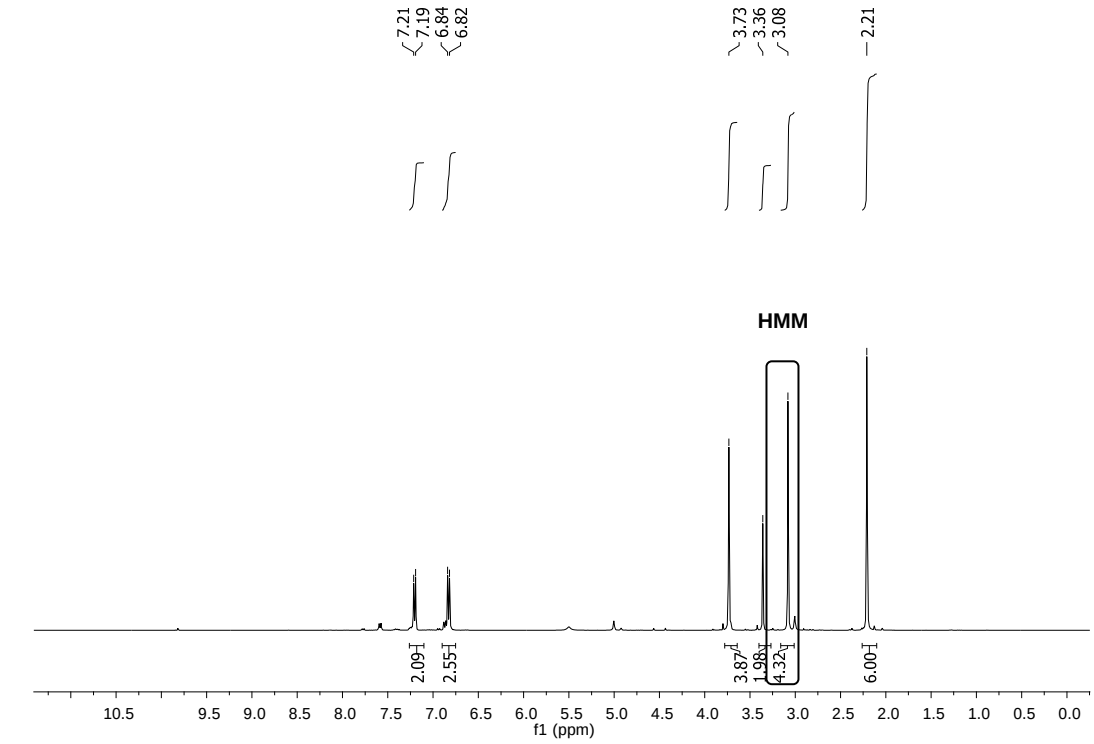


Figure SM X.9. (a) ^{1}H NMR (400 MHz, $CDCl_3$) overlap of 4-Methoxy-*N*,*N*-dimethylbenzylamine spectra and reaction spectra obtained after reaction of 7 moles equivalents of 4-methoxy-*N*,*N*-

dimethylbenzylamine with cyanuric chloride for 30ºmin at 150ºC. **(b)** ^{1}H NMR (400 MHz, $CDCl_3$) reaction spectra obtained after 7 moles equivalents of 4-methoxy-*N*,*N*-dimethylbenzylamine with cyanuric chloride for 30ºmin at 150ºC.

4. Synthesis and characterization of the compounds 4-nitro-*N,N*-dimethylbenzylamine and 4-methoxy-*N,N*-dimethylbenzylamine

4-Nitro-*N*,*N*-dimethylbenzylamine: To a solution of 4-nitrobenzyl chloride (5.0 g, 29.0 mmol) in 20 mL of methyl-*iso*-butylketone was added dropwise a solution of 40% aqueous dimethylamine (3 moles eq.) in 30 mL of methyl-*iso*-butylketone. The reaction was stirred at room temperature for 2h and at 30 ºC for 3h. Water was added to the reaction mixture and the organic phase was extracted with ethyl acetate (100 mL) and the combined organic extracts were washed with brine. The solution was dried over magnesium sulfate, filtrated and concentrated. The product was obtained as a yellow liquid in 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 3.47 (s, 2H), 2.21 (s, 6H). Spectral data ^{1}H identical to reported one.[7]

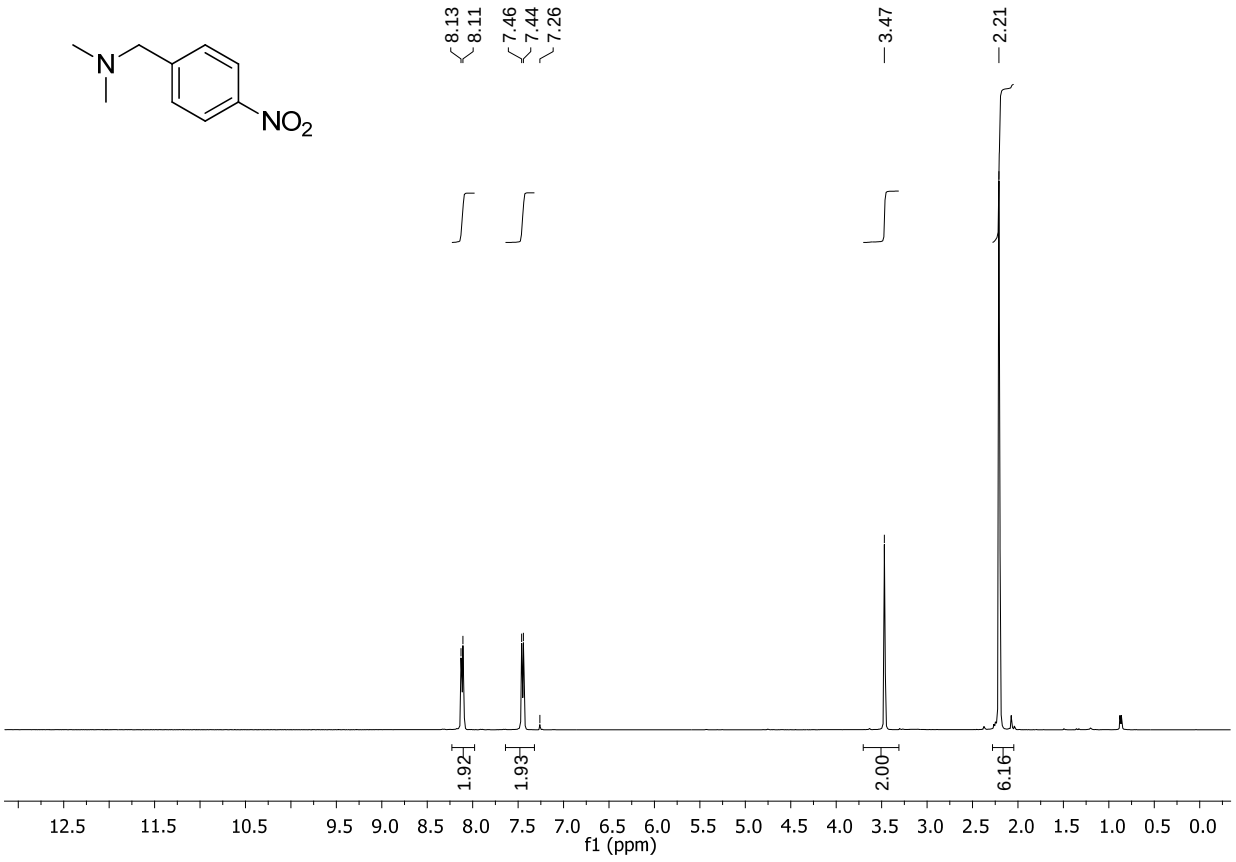


Figure SM X.10. Example of ^{1}H NMR (400 MHz, $CDCl_3$) spectra of 4-nitro-*N*,*N* dimethylbenzylamine.

4-Methoxy-*N*,*N*-dimethylbenzylamine: To a solution of 4-methoxybenzyl chloride (4.1 g, 25.5 mmol) in 20 mL of ether was added dropwise a solution of 40% aqueous dimethylamine (5 moles eq.) in 30 mL of ether. The reaction was stirred overnight at room temperature. After that time, water was added to the reaction mixture. The organic phase was extracted with ether (100 mL) and the combined organic extracts were washed with brine. The solution was dried over magnesium sulfate, filtrated and concentrated. The product was obtained as a yellow pale liquid in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.3 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 3.76 (s, 3H), 3.34 (s, 2H), 2.21 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.69 (C_{ar}), 130.82 (C_{ar}), 130.27 (C_{ar}), 113.57 (C_{ar}), 63.68 (CH_2), 55.16 (OCH_3), 45.15 (CH_3). Spectral data ^{1}H identical to reported one.[8]

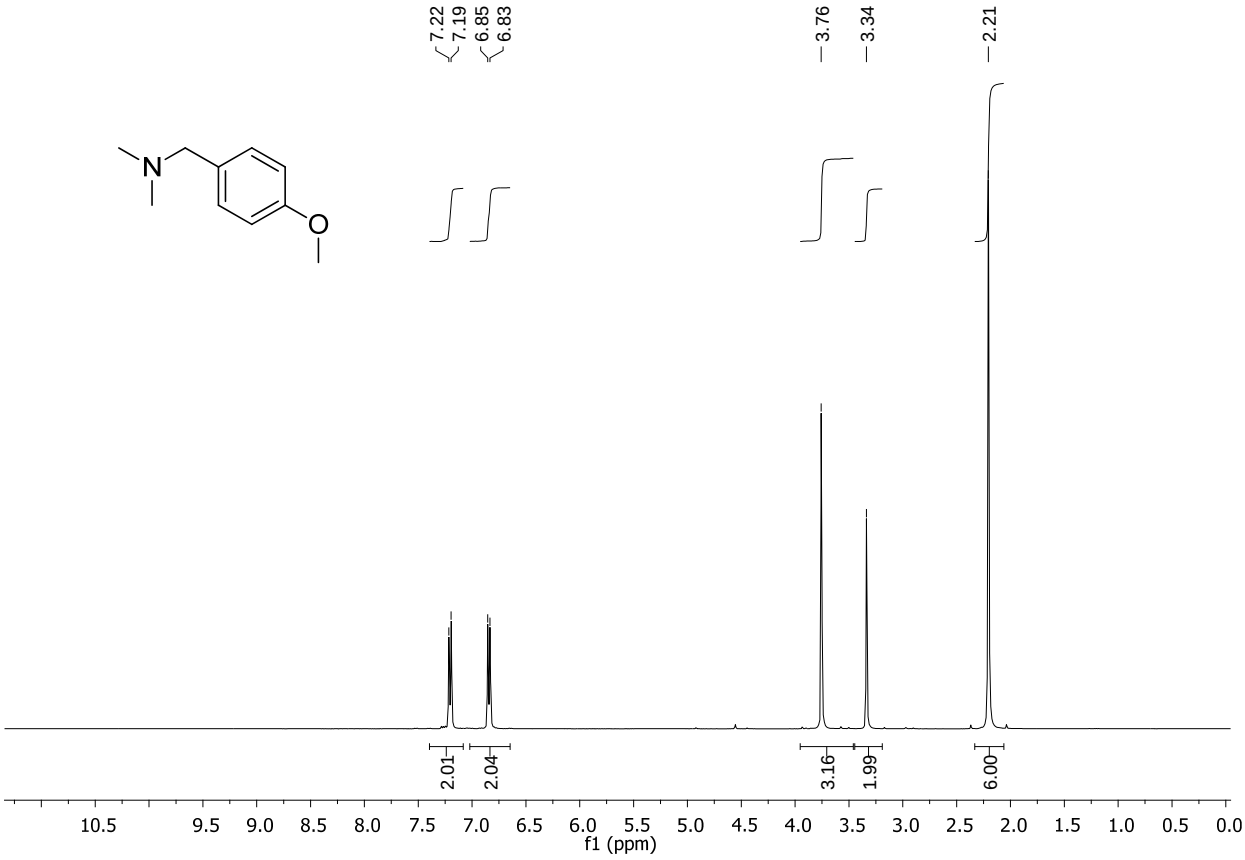


Figure SM X.11. Example of ^{1}H NMR (400 MHz, $CDCl_3$) spectra of 4-methoxy-*N*,*N*-dimethylbenzylamine.

5. Experiment's photos

Figure SM X.12. Photograph of the experiment vial in the domestic kitchen-type microwave instrument before the irradiation. The solid in the vial is cyanuric chloride and the liquid *N*,*N*-dimethylbenzylamine.

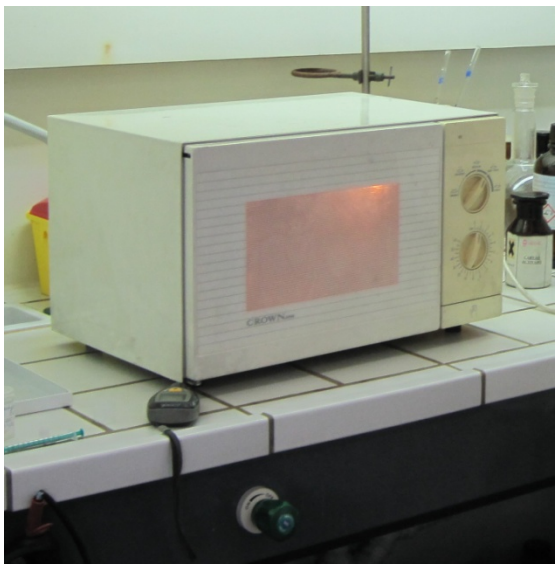


Figure SM X.13. Photograph of the domestic kitchen-type microwave instrument used for the reactions.

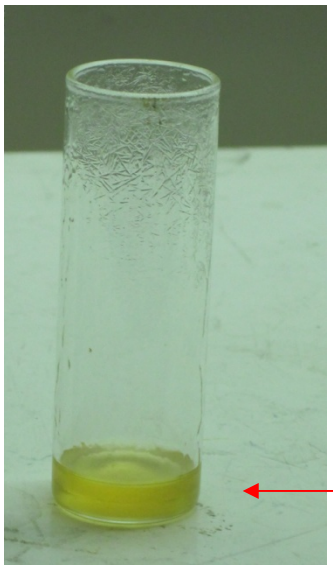


Figure SM X.14. Photograph of the experiment vial after the irradiation in the domestic kitchen-type microwave instrument.

6. Computational details

All calculations were performed using the GAUSSIAN 09 software package[9], and the PBE1PBE functional, without symmetry constraints. That functional uses a hybrid generalized gradient approximation (GGA), including 25 % mixture of Hartree-Fock[10] exchange with DFT[11] exchange-correlation, given by Perdew, Burke and Ernzerhof functional (PBE).[12, 13] The optimized geometries were obtained with a standard 6-31G(d,p)[14-18]. Transition state optimizations were performed with the Synchronous Transit-Guided Quasi-Newton Method (STQN) developed by Schlegel *et al*,[19, 20] following extensive searches of the Potential Energy Surface. Frequency calculations were performed to confirm the nature of the stationary points, yielding one imaginary frequency for the transition states and none for the minima. Each transition state was further confirmed by following its vibrational mode downhill on both sides and obtaining the minima presented on the energy profile. Single point energy calculations were performed using 6-311++G(d,p)[21-28] basis set for the geometries optimized at the PBE1PBE/31G(d,p) level. Solvent effects (dichloromethane) were considered in the PBE1PBE/6-311++G(d,p)//PBE1PBE/6-31G(d,p) energy calculations using the Polarizable Continuum Model (PCM) as implemented on Gaussian 09.

Atomic coordinates for all the optimized species (PBE1PBE/31G(d,p))

A				6	13.536572	3.059281	-12.289747
7	14.473179	7.729449	-7.688428	6	13.515501	3.885113	-11.168864
6	14.349146	8.255484	-6.435449	1	16.239496	2.655310	-9.546964
7	14.839176	7.677712	-5.331432	1	16.281329	1.197016	-11.547336
6	15.484093	6.517898	-5.507449	1	14.548481	1.452881	-13.307594
7	15.646141	5.911929	-6.715873	1	12.765367	3.160817	-13.048380
6	15.111788	6.592830	-7.699138	1	12.721944	4.619263	-11.030583
7	15.246212	5.926520	-9.038914	7	13.686173	9.415248	-6.320300
6	14.439444	4.617759	-8.952853	6	13.047103	10.062127	-7.449214
6	16.692867	5.652108	-9.2976476	6	13.538143	10.084836	-5.044546
	14.705705	6.779301	-10.138431	1	12.963560	9.370442	-8.285002
17	11.128663	6.205896	-9.510125	1	12.044256	10.383848	-7.151945
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1	16.775229	5.142773	-10.256615	7	15.974244	5.906902	-4.418474
1	17.222033	6.604741	-9.338505	6	16.747095	4.683455	-4.483324
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B

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C

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1	15.915996	1.379490	-11.497277
1	14.862655	2.434112	-13.483798
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B'

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1	16.179995	3.674093	-9.460290
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1	16.401068	5.777047	-10.594140
1	15.493630	7.268396	-9.909254
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6	13.977015	2.232965	-11.955403
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6	14.171292	5.008997	-11.896690
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1	14.190602	2.413416	-14.091710
1	14.356618	4.891341	-14.035144
1	14.228489	6.094418	-11.881676
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6	12.065687	8.969157	-7.556953
6	12.832149	9.653168	-5.326011
1	12.317817	8.309489	-8.384241
1	11.051603	8.736065	-7.208705
1	12.077972	10.006857	-7.907452
1	13.771096	9.769821	-4.786327
1	12.487176	10.633750	-5.664752
1	12.081132	9.239089	-4.640365
7	16.427444	6.544830	-4.370835
6	17.555431	5.637578	-4.384121
6	16.329297	7.439842	-3.236207
1	18.500407	6.187464	-4.483657
1	17.582010	5.074695	-3.445930
1	17.461879	4.942510	-5.215917
1	15.288946	7.708970	-3.053986
1	16.726584	6.929339	-2.355908
1	16.905879	8.360839	-3.394378
7	13.736697	6.442211	-7.510248
6	13.906001	7.524967	-6.734252
7	14.824924	7.647627	-5.763074
6	15.613686	6.574663	-5.595911
7	15.536544	5.436276	-6.307003
6	14.578448	5.429600	-7.244643
7	14.467193	4.303308	-7.997008
6	13.330900	4.076440	-8.875892
6	15.349819	3.178612	-7.769057
6	17.042372	7.848409	-9.813936
17	18.360514	8.900550	-10.419939
1	12.616897	4.883092	-8.700244
1	12.850410	3.133065	-8.587358
6	13.701841	4.020228	-10.340674
1	15.036744	2.577462	-6.904728
1	15.337276	2.541635	-8.656711
1	16.371338	3.520236	-7.598238
1	17.336586	7.455246	-8.841355
1	16.897968	7.035705	-10.524901
1	16.136500	8.447378	-9.724822
6	13.503978	2.854331	-11.082803
6	13.836942	2.804938	-12.436696
6	14.375697	3.925276	-13.062579
6	14.575413	5.096465	-12.330257
6	14.240755	5.142725	-10.980462
1	13.082685	1.977569	-10.595572
1	13.677266	1.888897	-12.999243
1	14.637172	3.889745	-14.116656
1	14.988288	5.977076	-12.815487
1	14.387962	6.060062	-10.415375
7	13.095737	8.590836	-6.963547
6	11.974260	8.494021	-7.872064
6	13.113260	9.743316	-6.089748
1	12.196947	7.785303	-8.668330
1	11.059211	8.166831	-7.357993
1	11.786455	9.477733	-8.311749
1	14.104473	9.869320	-5.657452
1	12.860302	10.634584	-6.671451
1	12.383778	9.648089	-5.272789
7	16.551840	6.634532	-4.617199
6	17.534555	5.585182	-4.457537
6	16.812992	7.866406	-3.906216
1	18.449022	5.792774	-5.031391
1	17.804930	5.508021	-3.400402
1	17.123227	4.634305	-4.792578
1	15.915411	8.482265	-3.886691
1	17.109659	7.631087	-2.879746
1	17.624134	8.441596	-4.374992

C'

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Synthesis of 4,5-dicyanobenzenedithiol

Supplementary Material

This experiment proposal was developed by the author in his research work and adapted for second year students of advanced organic chemistry due mainly to its hard experimental difficulty level[1]. No more than 8 students working in pairs have done this work in the same classroom. The utilization of dangerous and foul odour reagents require careful and following cleaning protocols to prevent smell propagation. The 2 steps require long stirring periods but without need of surveillance until next session. The main purpose of this work is illustrating a nucleophilic aromatic substitution and study the reactivity of the aromatic ring and the orientation effects of the substituents as well C-S bond cleavage. In addition, the experiment exposes the student cohort to a wide range of laboratory skills like handling inert gas atmosphere, water sensitive reagents, dry solvents, temperature control, recrystallization, vacuum filtration and acid/base extraction as a method of purification.

Additional notes on the preparation of 1,2-bis(S-benzylthio)-4,5-dicyanobenzene:

Recent sodium hydride should be used. Sometimes the sodium hydride suspension does not remain clear and the addition of 1.0 mL more of benzylmercaptan is require. That way an excess of the thiol is used and all sodium hydride is consumed, so no concerns need to be taken in relation to residual sodium hydride. Reaction set apparatus for 1,2-bis(S-benzylthio)-4,5-dicyanobenzene can be seen in the **Figure SM 6.4.1**.

Figure SM 6.4.1 – Reaction set apparatus for 1,2-bis(S-benzylthio)-4,5-dicyanobenzene

Copper funnel used for hot filtration in thioether recrystallization can be seen in **Figure SM 6.4.2**.

Figure SM 6.4.2 – Copper funnel (previously heated by flame) for hot filtration in thioether recrystallization.

Additional product can be collected from mother liquor of recrystallization stored in the refrigerator. Yield around 65-75 %; mp 188-189ºC (189-190ºC[1,2]). **Figure SM 6.4.3** shows vacuum filtration set-up.

Figure SM 6.4.3 – Vacuum filtration set-up.

Additional notes on the preparation of 4,5-dicyanobenzenedithiol

Due to quick decomposition of aluminium chloride with air, special attention should be done to the weighing and addition otherwise a decrease of yield is observed. Ice and water is added to quench

[1] C. A. M. Afonso, D. P. Simão, L. P. Ferreira, M. S. Serra, M. M. M. Raposo, *100 Experiências de Química Orgânica*, IST Press, 2011, 181.
[2] D Simão *et al*., *Eur. J. Inorg. Chem*., 2001, **12**, 3119-3126.

aluminium compounds present in solution which is exothermic. The gravity filtration after water addition took long time. This aromatic thiol decompose slowly in contact with air and must be stored in a well closed flask, nevertheless it could be purified by acid/base treatment. Yield 45-50 %; mp 100ºC (dec.) (100ºC [1,2]).

Figure SM 6.4.4 – Reaction set apparatus for 4,5-dicyanobenzenedithiol

IR spectra:

Students easily identify in the **Figure SM 6.4.5** a strong band at 2220 cm^{-1} due to the CN group and at 1110 cm^{-1} the absorption band of Ar-S. In the **Figure SM 6.4.6** the absorption band at 2540 cm^{-1} identify the thiol.

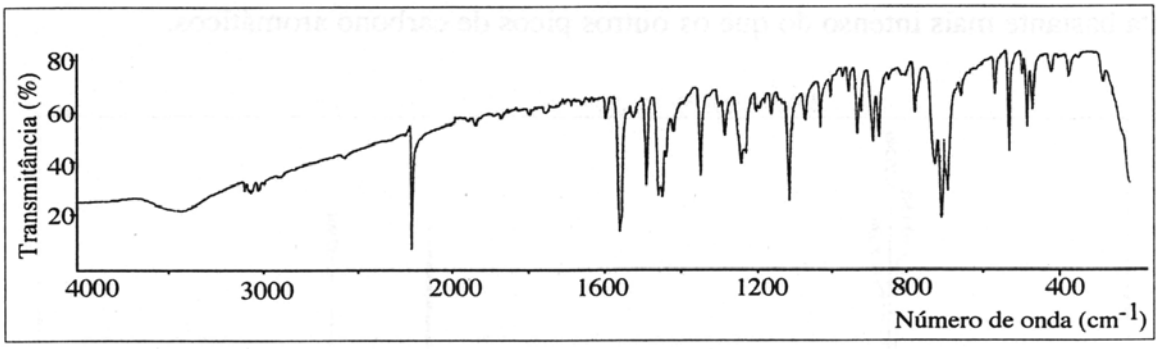

Figure SM 6.4.5 - IR (KBr) of 1,2-bis(S-benzylthio)-4,5-dicyanobenzene

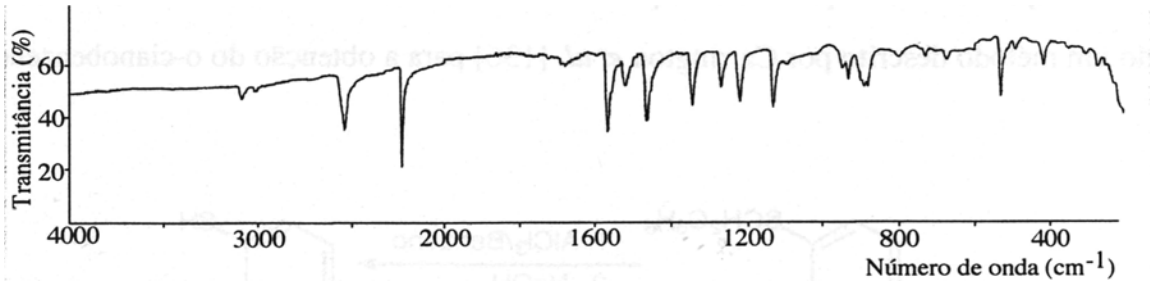

Figure SM 6.4.6 - IR (KBr) of 4,5-dicyanobenzenedithiol

NMR spectra:

The students easily assign the ^{1}H NMR spectra once only singlets are present. For 1,2-bis(S-benzylthio)-4,5-dicyanobenzene in the **Figure SM 6.4.7**, peak solvent (dichloromethane) at 5.32 ppm and water from solvent at 1.5 ppm can be observed as well in ^{13}C NMR at 54.0 ppm (**Figure SM 6.4.9**). For 4,5-dicyanobenzenedithiol in the figure **Figure SM 6.4.8**, peak solvent (acetone) at 2.05 ppm and some impurities can be observed.

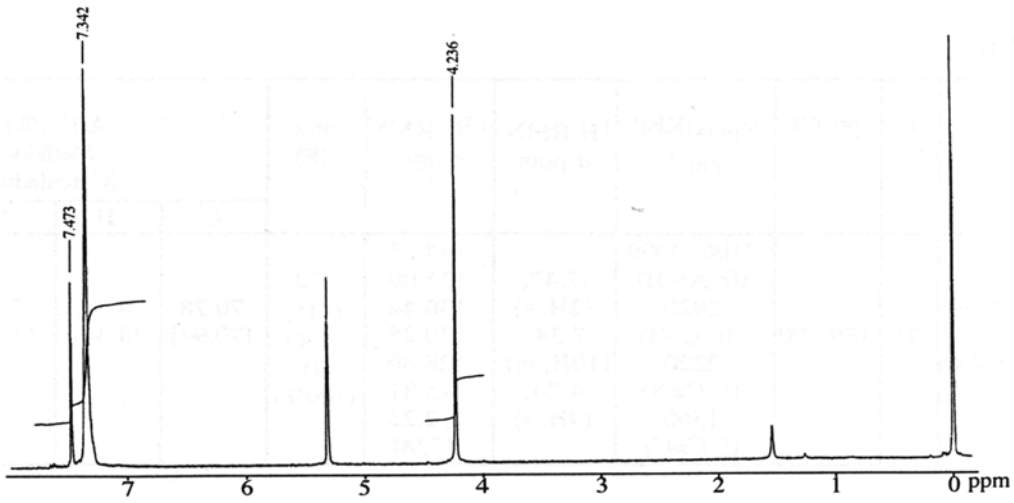

Figure SM 6.4.7 - ^{1}H NMR of 1,2-bis(S-benzylthio)-4,5-dicyanobenzene (300 MHz, CD_2Cl_2)

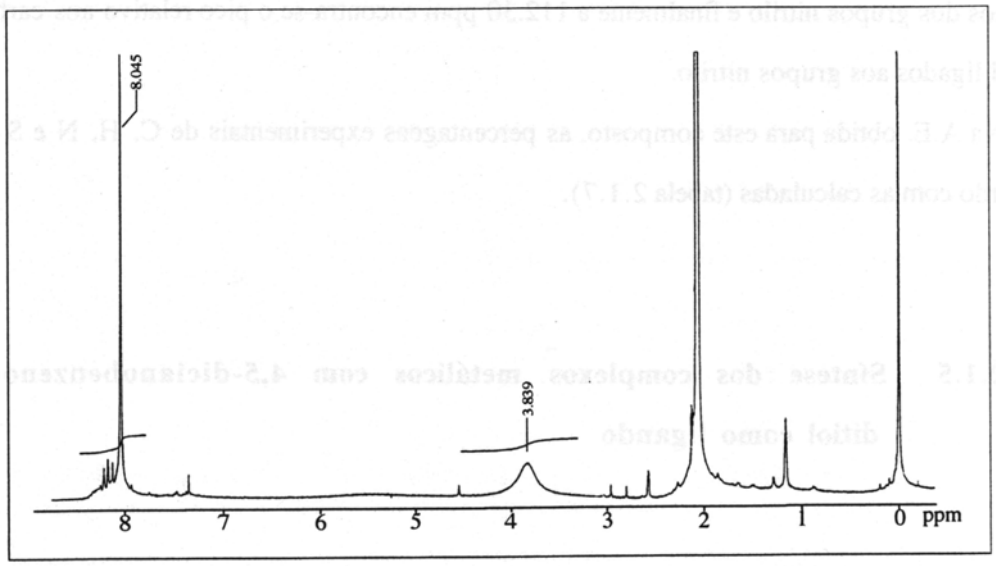

Figure SM 6.4.8 - ^{1}H NMR of 4,5-dicyanobenzenedithiol (300 MHz, $(CD_3)_2CO$)

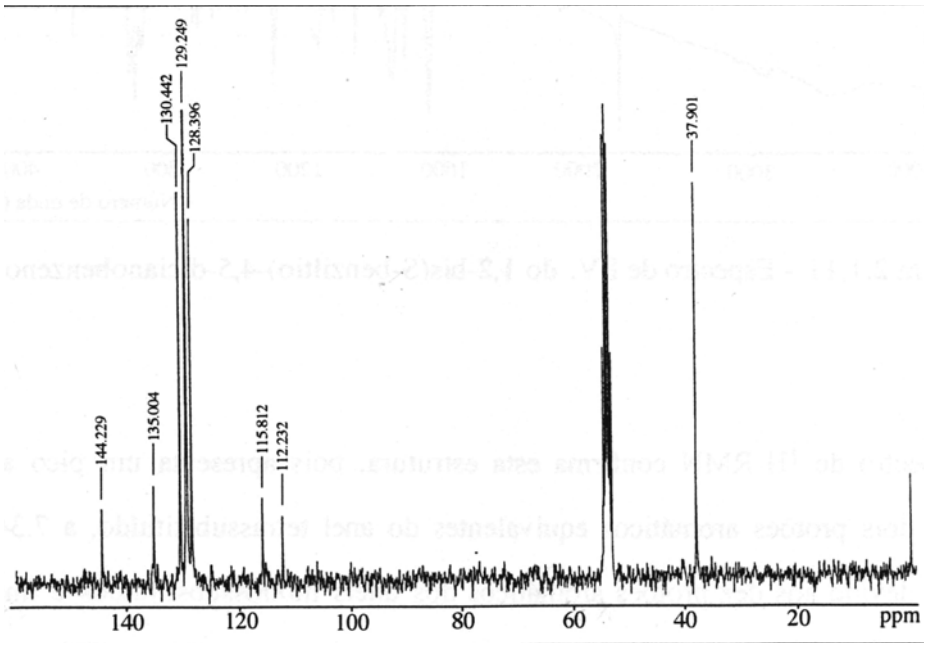

Figure SM 6.4.9 – ^{13}C NMR of 1,2-bis(S-benzylthio)-4,5-dicyanobenzene (300 MHz, CD_2Cl_2)

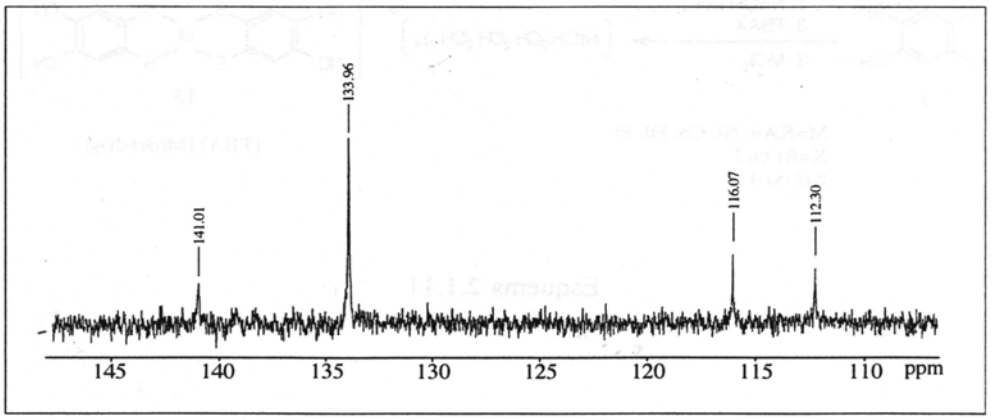

Figure SM 6.4.10 – ^{13}C NMR of 4,5-dicyanobenzenedithiol (300 MHz, $(CD_3)_2CO$)

IR, NMR data and more information concerning mass spectra and elemental analysis can be found in the author paper[2].

Nucleophilic Aromatic Substitution Reactions in 3,6-bis-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine

Supplementary Material

The goal of this experiment is the synthesis of four 1,2,4,5-tetrazine derivatives by S_NAr reactions starting from 3,6-Bis-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine. From a pedagogical point of view, these experiments provide the students the possibility of observing experimentally the different reactivity of N-, O- and C-nucleophiles in S_NAr reactions involving tetrazine rings, and thus establish a trend in the nucleophilicity strength of the different reagents used.

This experiment is suitable for students with previous laboratory experience in organic chemistry that feel comfortable with unitary operations, and previous experience on the preparation of a chromatography column is highly desirable. The experiment procedure is divided into 3 sessions with 4 hours each. Given the duration of the reactions involved (namely that of Reaction 2), this protocol is suitable for laboratory projects taken at an advanced level that do not have to comply with the usual time restriction of 3 to 4h available for laboratory classes in most institutions. Nevertheless this protocol can be reorganized to fit into a more restrict laboratory session depending on the timetable available for classes: for example, if only two 4h sessions are available, Reaction 2 can be eliminated, Reactions 1 and 3 can be condensed into a single session and Reaction 4 executed in the second session.

One MSc. student and one PhD. student from *Faculdade de Ciências,* ULisboa (Portugal) assessed the reproducibility of all experiments. The ranges of experiment yields obtained are listed in **Table SM 6.5.1**. NMR spectra were acquired by the students in a 400 MHz Brucker Avance spectrometer using $CDCl_3$, DMSO-d^6 or $(CD_3)_2CO$ as solvent (cf. captions of figures **SM 6.5.3 – SM 6.5.11**).

Table X.1 – Yield range of each synthetic step.

Product	Yield Range (%)
2-(6-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazin-3-yl)malononitrile (**2**)	51 - 65
6-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(pyridin-4-yl)-1,2,4,5-tetrazine-3-amine (**3**)	70 - 83
3,6-dimethoxy-1,2,4,5-tetrazine (**4**)	32 - 54
3-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methoxy-1,2,4,5-tetrazine (**5**)	15 - 27

Notes for the synthesis of 2-(6-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine-3-yl) malononitrile (2) (Reaction 1, 3 h)

The synthesis of 2-(6-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine-3-yl)malononitrile is straightforward. After stirring the reaction mixture for 2 hours, it is mandatory that the solvent is evaporated prior to the washing with the hydrochloride solution. Filtration can be performed with a Büchner filtration apparatus, but a sintered glass filter allows a greater recovery of the product. This step also allows obtaining a more pure product since the

secondary products that may form, like the di-malononitrile dissubstituted product, are water-soluble. The product is rather insoluble in most chlorinated solvents. Acetone was found to be the best solvent for isolating the product, albeit a significant amount of acetone is needed (~250 mL). Moreover, dissolving the product in acetone followed by drying with anhydrous magnesium sulphate allows the removal of most of the water from the washing steps, and promotes an easier and faster drying of the solid.

^{1}H NMR spectra can be performed in deuterated acetone, but ^{13}C spectra are rather difficult to obtain in this solvent due to the low solubility of the product. Being so, students are advised to dissolve the sample in deuterated DMSO. Note that the acidic proton of the sp^3 carbon of the malononitrile group is not observed in the ^{1}H-NMR spectra, in accordance with reference [8].

Notes for the synthesis of 6-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(pyridin-4-yl)-1,2,4,5-tetrazin-3-amine (3) (Reaction 2, pre-session of 45 min plus 1h15 min for work-up)

The synthesis of 6-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(pyridin-4-yl)-1,2,4,5-tetrazin-3-amine is also quite straightforward and no major concerns are needed, the only remark being the reaction time. Since most teaching institutions do not offer long laboratory sessions, but foremost by the fact that laboratory sessions are seldom scheduled to the following day, reaction 1 is the most difficult to apply in the classroom context. As an alternative, this reaction can be initiated in a 30-45 min pre-session the day before the lab session, and the isolation of the product carried out during the 4 h laboratory practice.

In the chromatography step the elution with dichloromethane:ethanol (9:1) takes *ca.* 50 min to complete.

Notes for the synthesis of 3,6-dimethoxy-1,2,4,5-tetrazine (4) (Reaction 3, 1.5h)

This reaction is the simplest of this experiment although the work-up can be challenging. The reaction takes about 1 h for the starting material to be fully consumed. Heating the reaction to 30 – 35 ºC can speed up reaction. It is usually a very clean reaction with little secondary products formed, and hence the chromatographic step can be avoided if no secondary products are present in the TLC analysis of the reaction crude. **Figure SM 6.5.1** is an example of a reaction that does not need a chromatographic step.

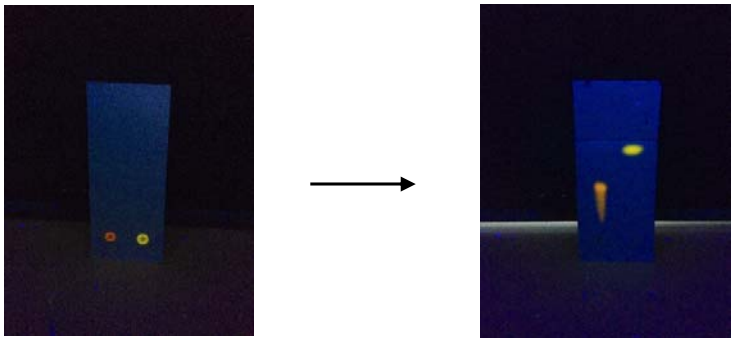

Figure SM 6.5.1 – TLC plates before (A) and after elution (B) of Reaction 3 crude with hexane/ethyl acetate (2:3). Left spot: starting material; right spot: reaction crude.

Although this reaction is very simple to perform, the isolation of the product may be troublesome: the desired compound is highly soluble in water, and it is also very volatile. It is mandatory that no heating is used during the work-up of the reaction. The solvent removal after the chromatographic step should be made preferably by placing the flask in an ice-cold water recipient and using a vacuum pump. This is in fact the most time consuming step of the experiment. Also, the product should be kept in a well-capped vessel after isolation.

Notes for the synthesis of 3-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methoxy-1,2,4,5-tetrazine (5) (Reaction 4, 4h)

Reaction of a catalytic amount of sodium methoxide and 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine is the most difficult reaction of this experiment, in the sense that students must constantly monitor the reaction by TLC in order to avoid the formation of 3,6-dimethoxy-1,2,4,5-tetrazine. The reaction temperature should be kept at 25ºC to make the control easier to achieve. Lower reaction times are possible by addition of a second portion of sodium methoxide, but this speeding of the reaction will lead to an increase of the formation of the 3,6-dimethoxy-1,2,4,5-tetrazine side product.

Separation is easily performed by preparative TLC (PTLC). For this procedure, students are recommended to charge no more than 50 mg of crude in the PTLC plate in order to get a good separation. An example of an overload of the PTLC is shown in **Figure SM X.2**. It is clear from this figure that at least two side products eluted with the desired product. The reaction yield is fairly low (15 to 27 %) due to some degree of adsorption of the product in silica gel, but enough product is obtained to ensure its characterization.

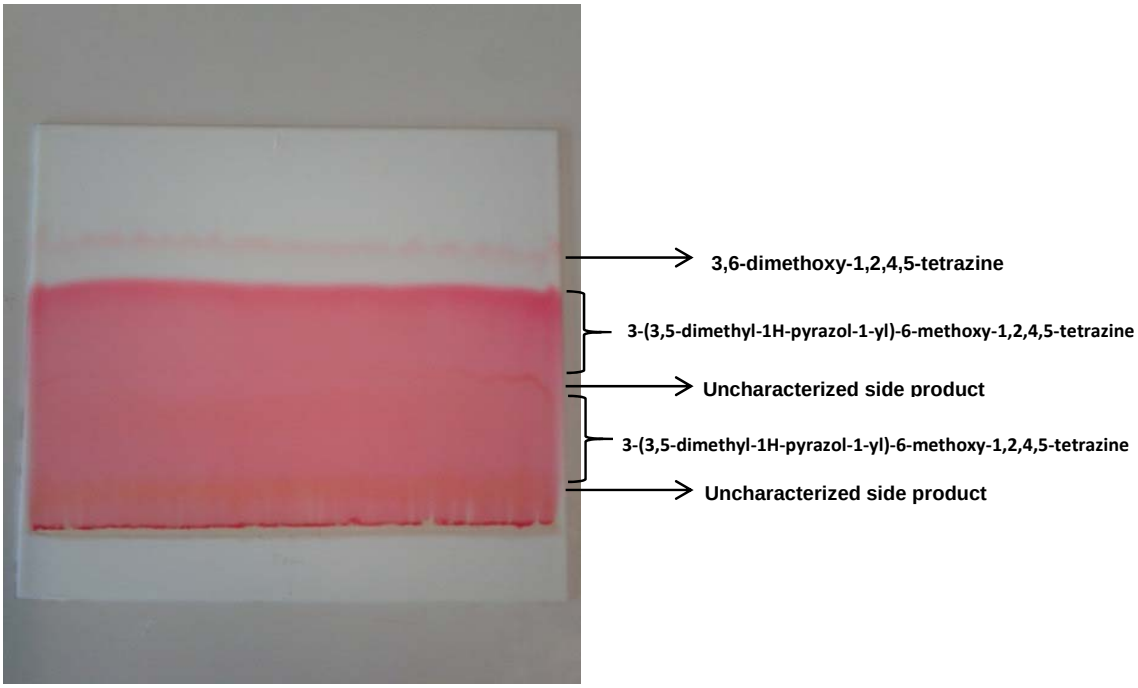

Figure SM 6.5.2 – Example of an overloading PTLC plate in the separation of 3-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methoxy-1,2,4,5-tetrazine (**5**).

NMR Spectra

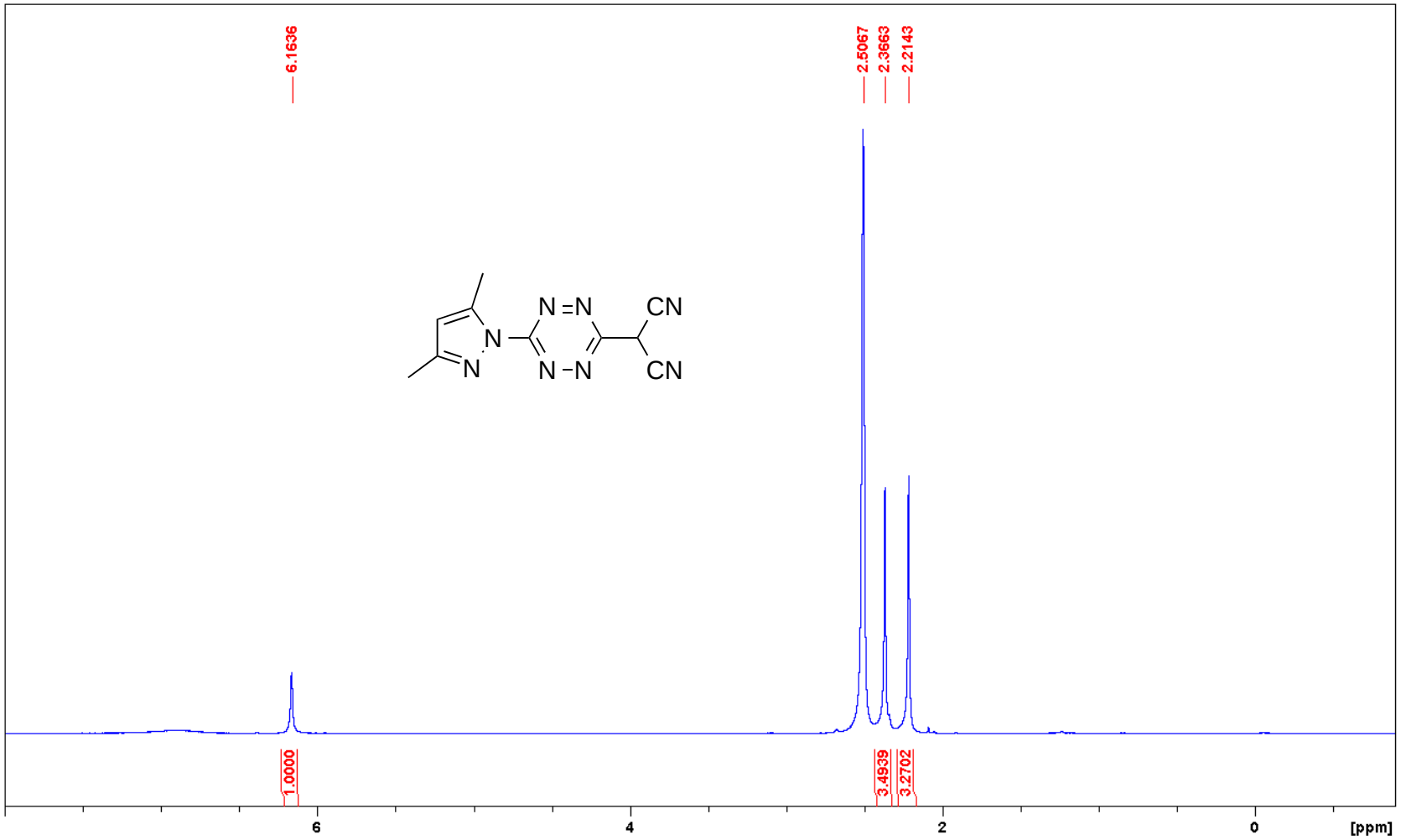

Figure SM 6.5.3 – ^{1}H NMR in DMSO-d^{6} for 2-(6-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine-3-yl)malononitrile (**2**).

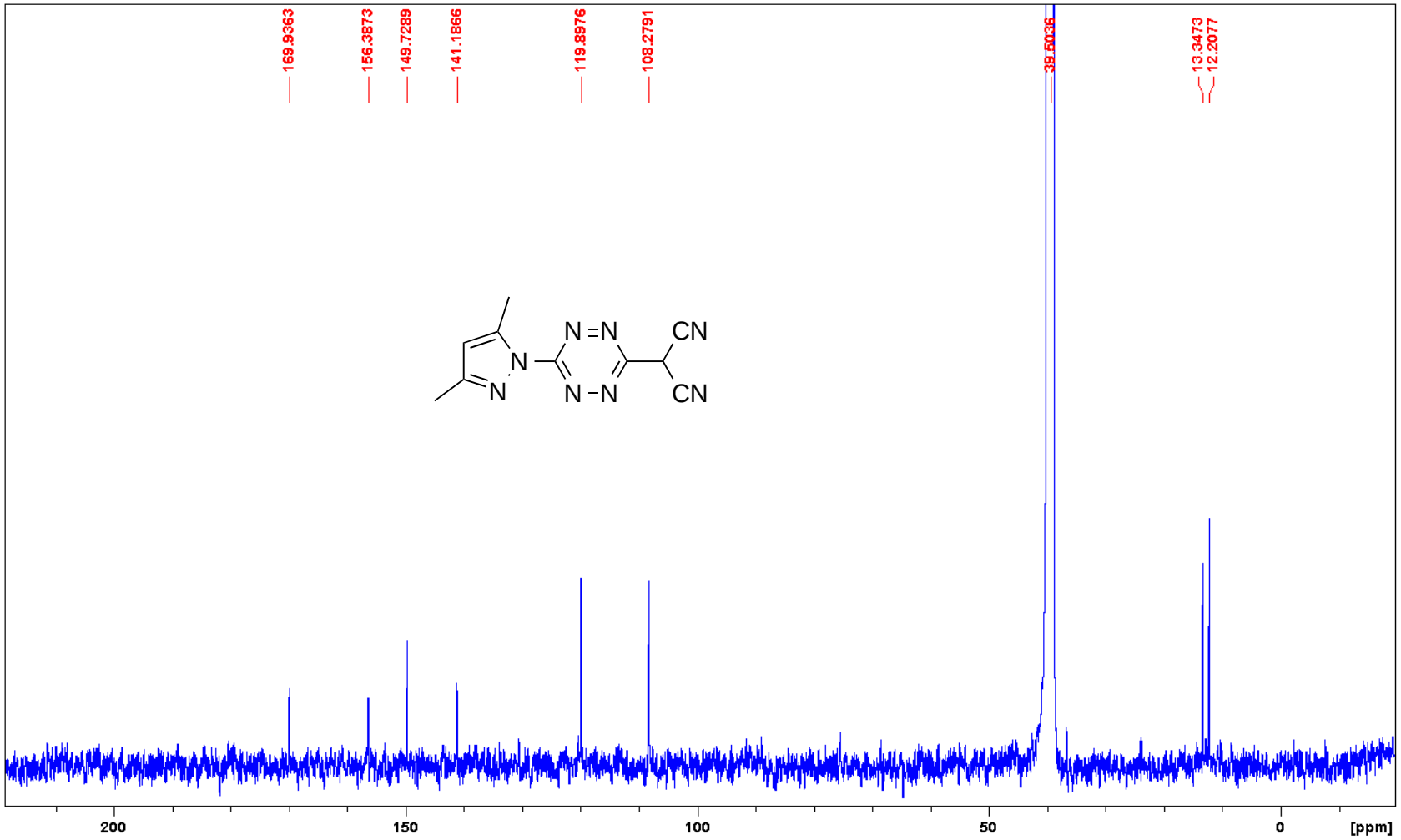

Figure SM 6.5.4 –^{13}C NMR in DMSO-d^6 for 2-(6-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine-3-yl)malononitrile (**2**).

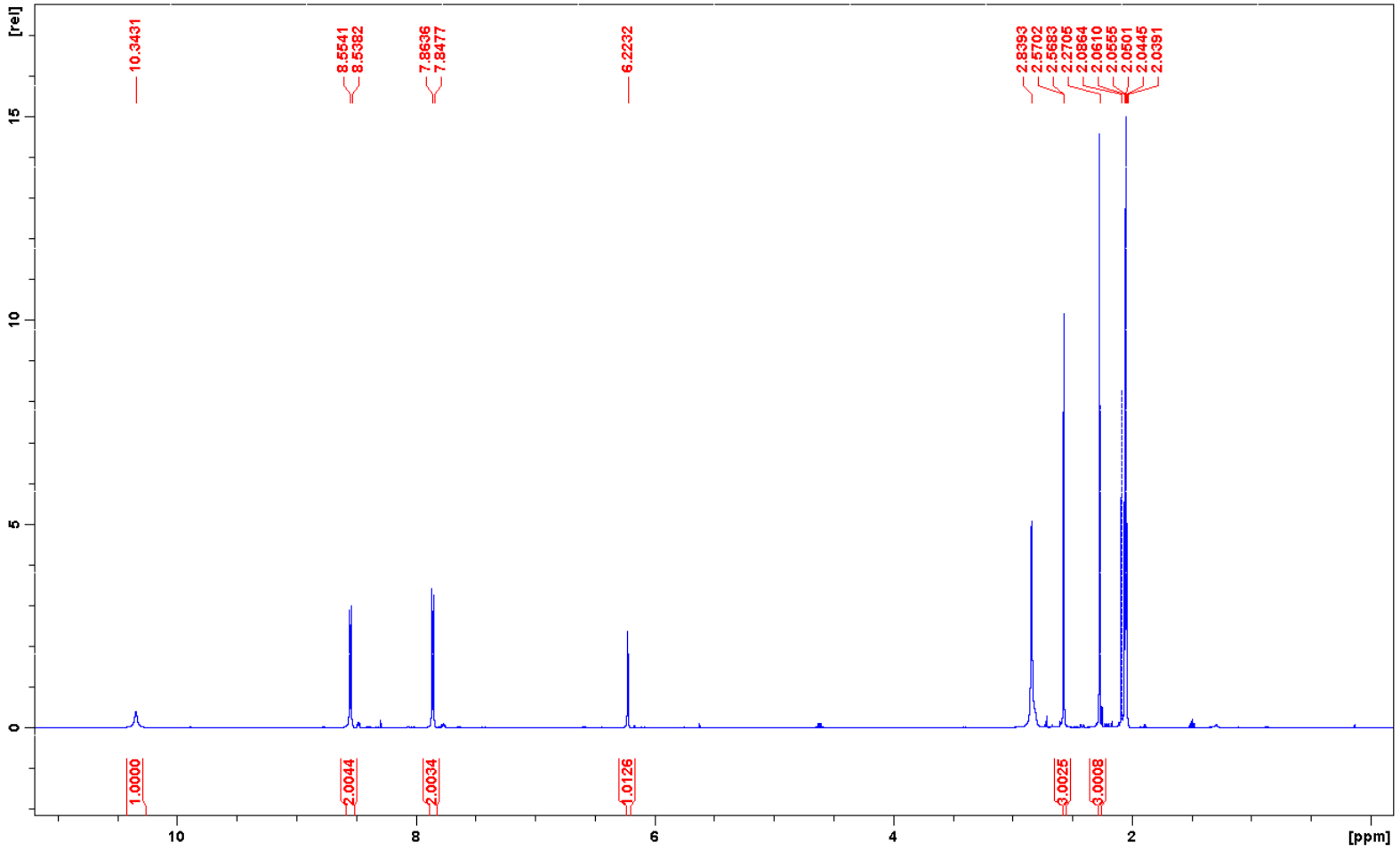

Figure SM 6.5.5 – ^{1}H NMR in $(CD_3)_2CO$ for 6-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(pyridin-4-yl)-1,2,4,5-tetrazine-3-amine (**3**).

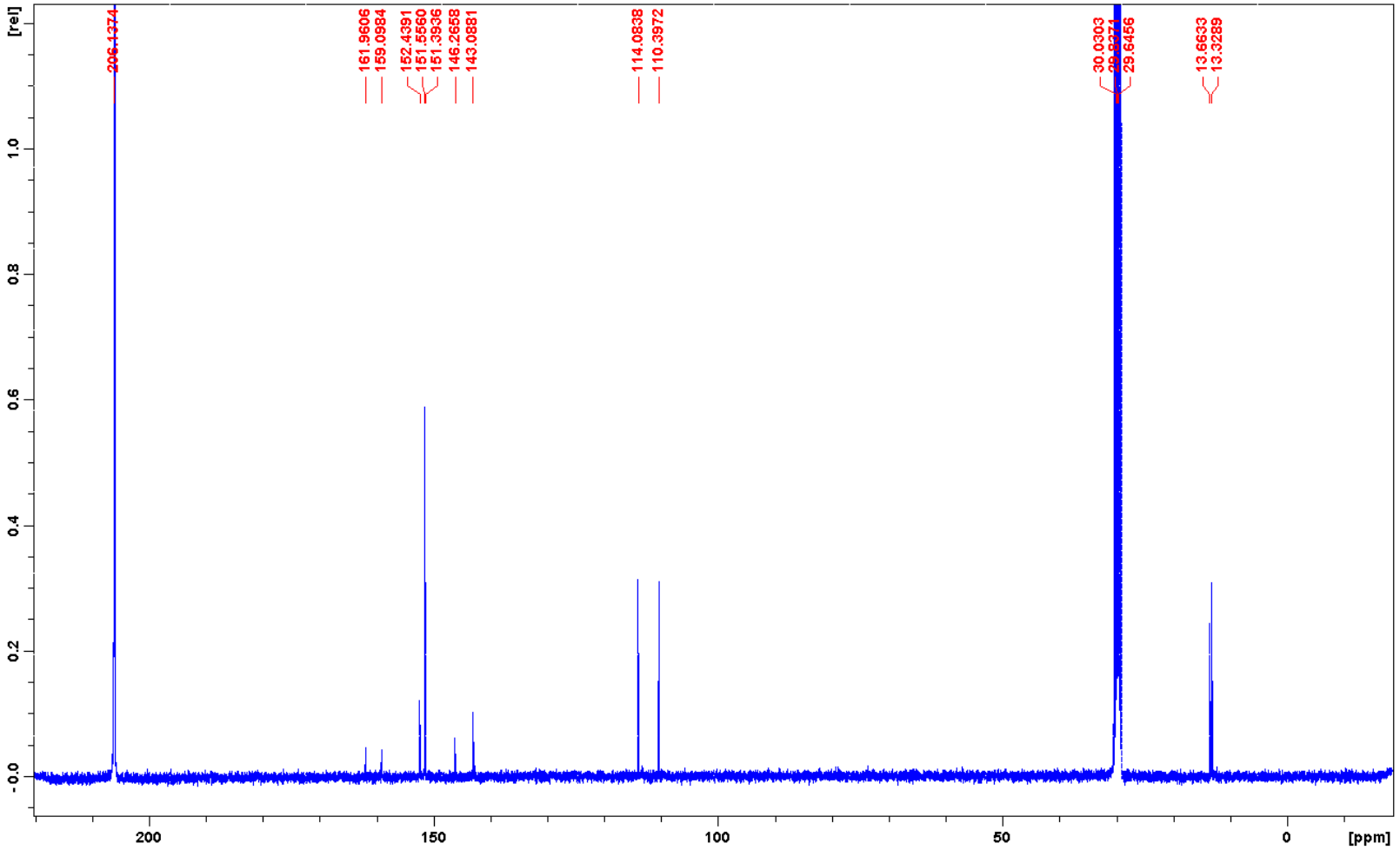

Figure SM 6.5.6 – ^{13}C NMR in $(CD_3)_2CO$ for 6-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(pyridin-4-yl)-1,2,4,5-tetrazine-3-amine (**3**).

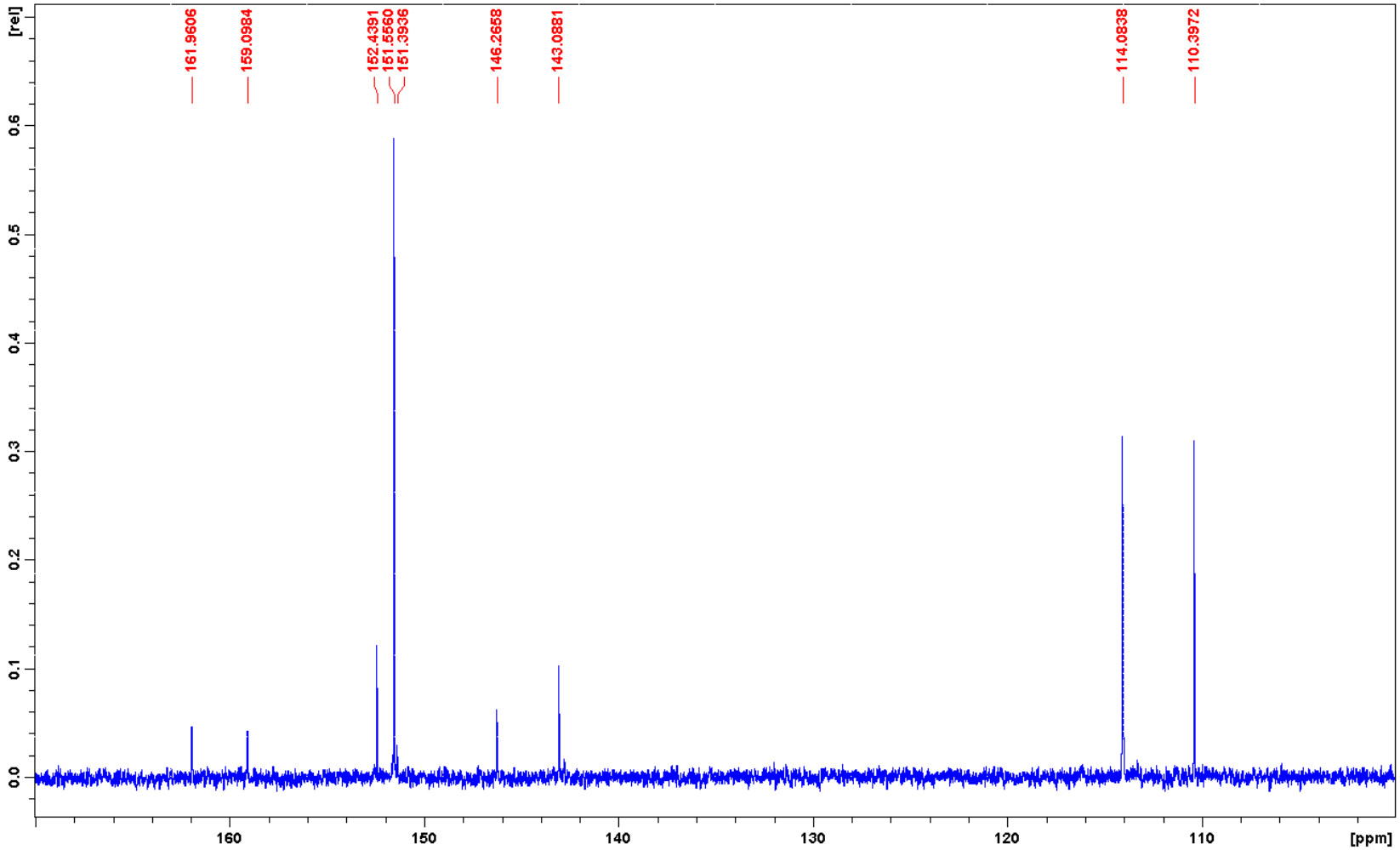

Figure SM 6.5.7 – Expansion of ^{13}C NMR in $(CD_3)_2CO$ for 6-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(pyridin-4-yl)-1,2,4,5-tetrazine-3-amine (**3**).

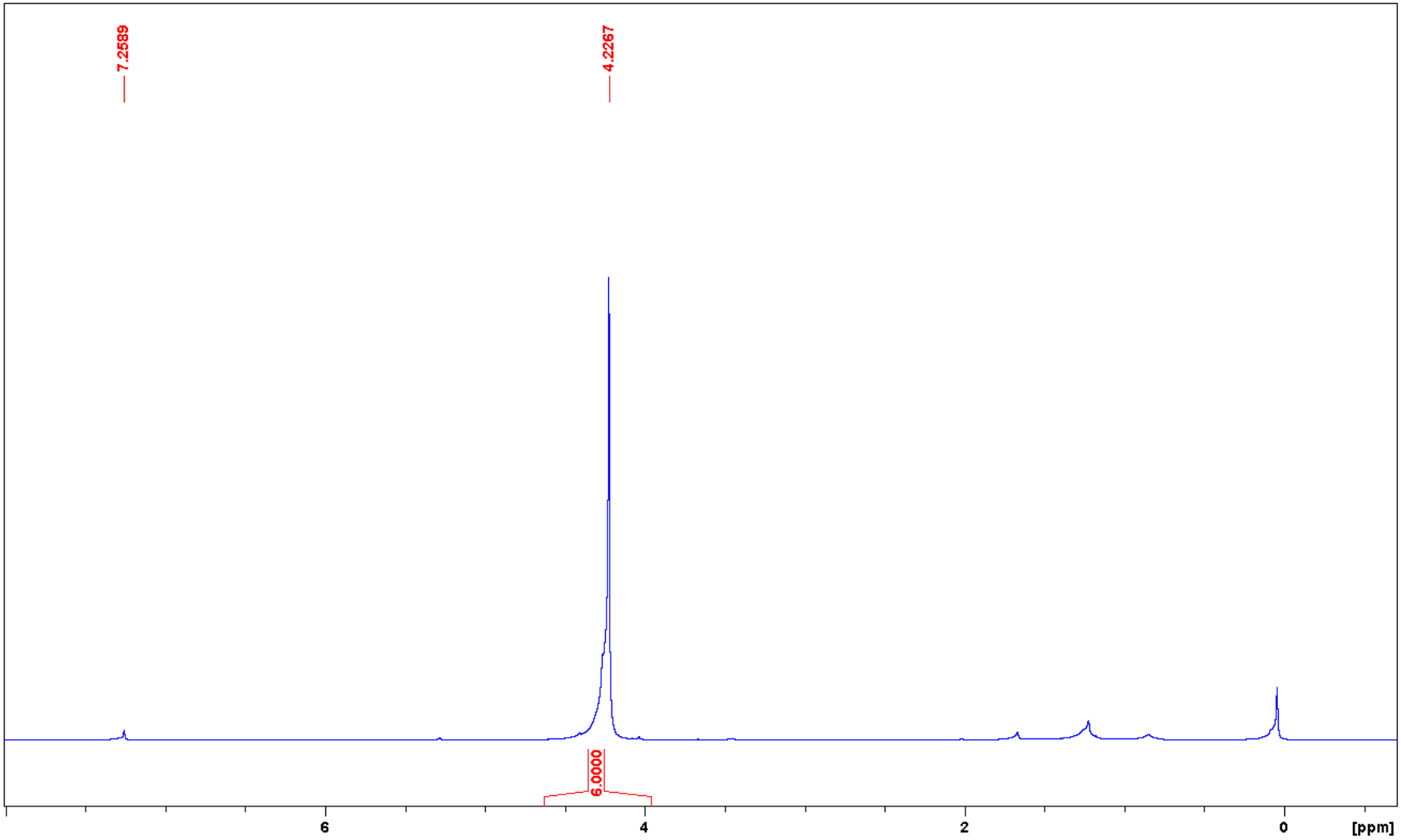

Figure SM 6.5.8 –^{1}H NMR in $CDCl_3$ for 3,6-dimethoxy-1,2,4,5-tetrazine (**4**).

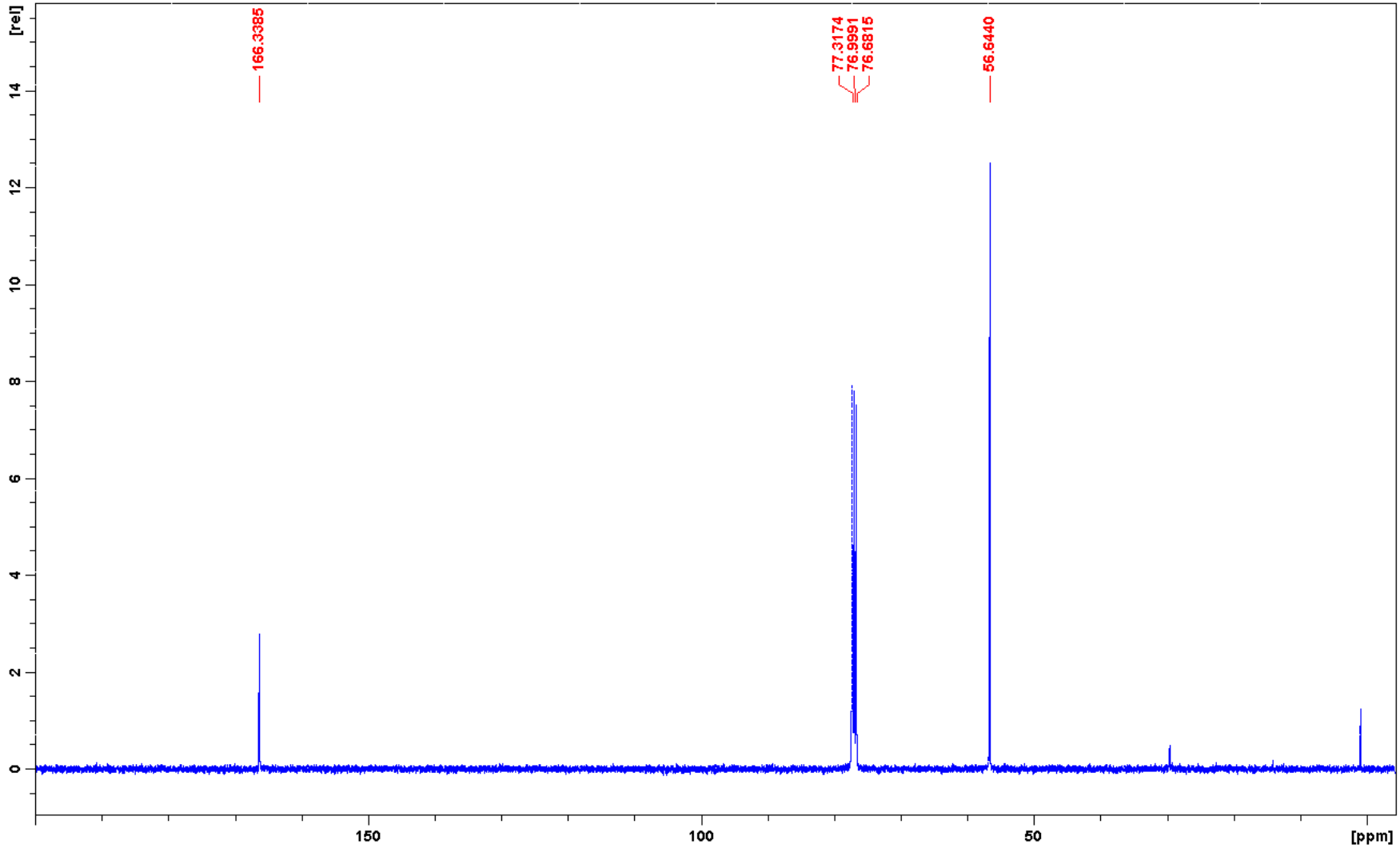

Figure SM 6.5.9 –^{1}H NMR in $CDCl_3$ for 3,6-dimethoxy-1,2,4,5-tetrazine (**4**).

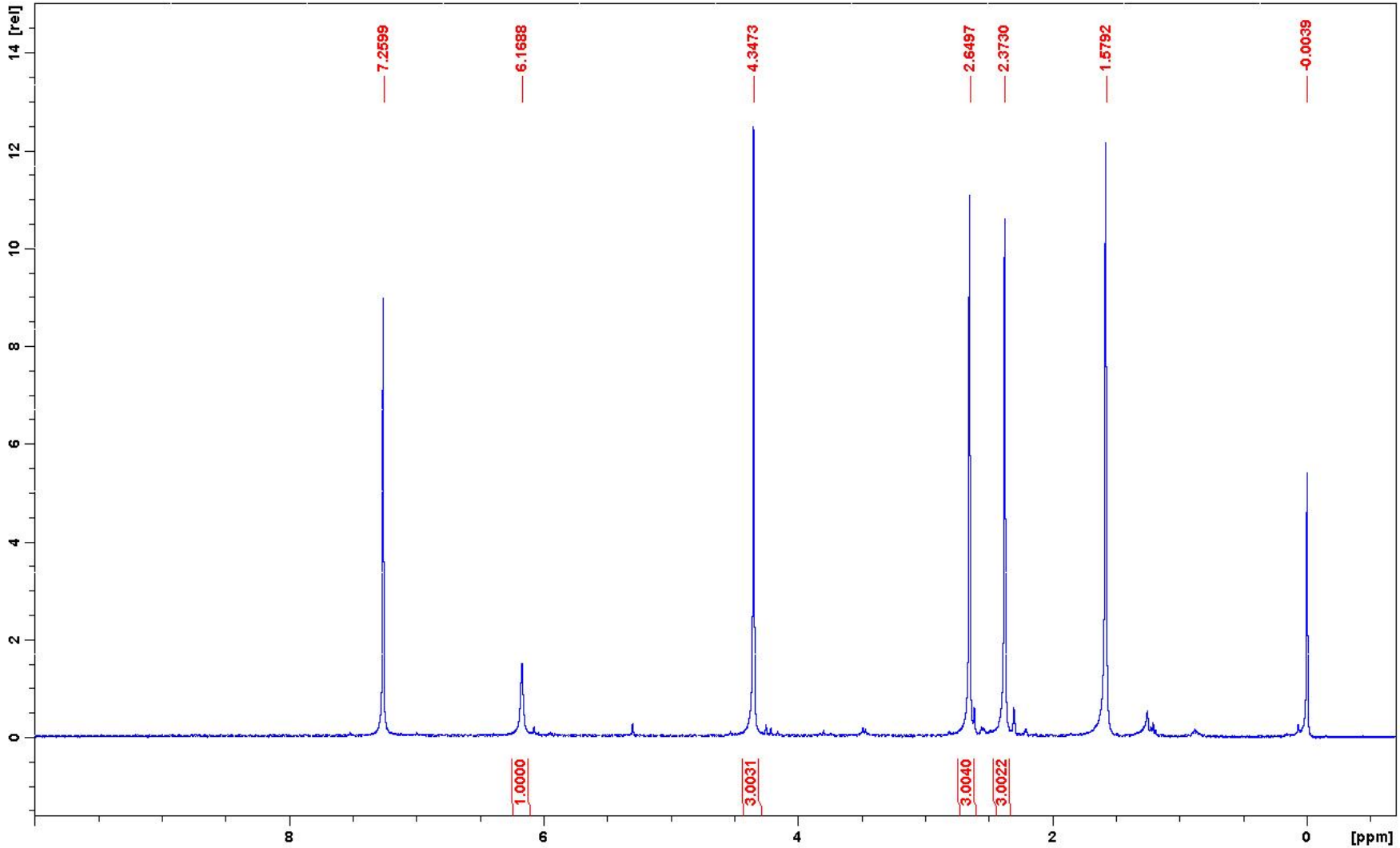

Figure SM 6.5.10 – ^{1}H NMR in $CDCl_3$ for 3-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methoxy-1,2,4,5-tetrazine (**5**).

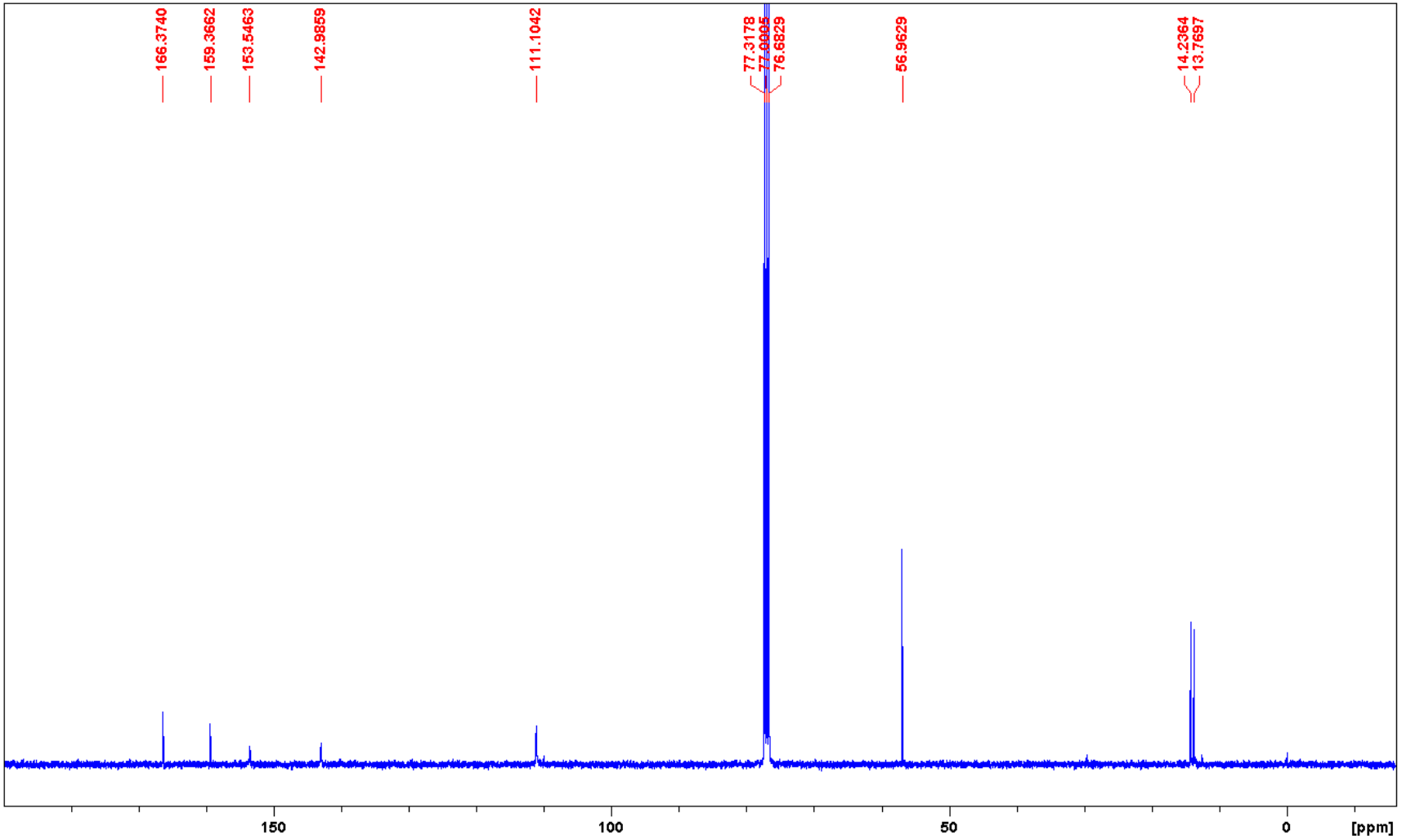

Figure SM 6.5.11 –^{13}C NMR in $CDCl_3$ for 3-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methoxy-1,2,4,5-tetrazine (**5**).