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# Acylhydrazone-based dynamic combinatorial libraries: study of the thermodynamic/kinetic evolution, configurational and coordination dynamics

## Abstract

The kinetic and thermodynamic selectivity of acylhydrazone formation in dynamic combinatorial libraries (DCL) is described. Competition reactions were generated from hydrazides: isoniazid, 4-nitro-benzohydrazide, 4-dimethylamino-benzohydrazide, and nicotinic hydrazide as well as the aldehyde derivatives: benzaldehyde and 2-pyridine-carboxaldehyde. The obtained species and the distribution of the DCLs were monitored by $^{1}$H-NMR spectroscopy finding that those acylhydrazones containing the 4-dimethylamino-benzohydrazide moiety are both the kinetic and thermodynamic product of their respective libraries. Configurational and coordination dynamics for some of these libraries were also investigated. The obtained results allowed the study of the redistribution of components and the amplification of one or more products using light and metal ions as physical and chemical templates, respectively.

**Keywords:** dynamic combinatorial chemistry, acylhydrazones, coordination and configurational dynamics.

# Librerías combinatorias dinámicas basadas en acil-hidrazona: estudio del desarrollo termodinámico/cinético, dinámicas de configuración y de coordinación

## Resumen

Se describe la selectividad cinética y termodinámica de la formación de acil-hidrazona en bibliotecas combinatorias dinámicas (DCL). Se generaron reacciones competitivas a partir de hidrazidas: isoniazida, 4-nitro-benzohidrazida, 4-dimetilamino-benzohidrazida y hidrazida nicotínica; así como a partir de los derivados de aldehído: benzaldehído y 2-piridin-carboxaldehido. Las especies obtenidas y la distribución de los DCLs fueron monitoreados mediante espectroscopia $^{1}$H-NMR, encontrándose que las acil-hidrazonas que contenían la 4-dimetilamino-benzohidrazida son tanto el producto cinético, como el termodinámico de sus respectivas bibliotecas. También se investigaron las dinámicas de configuración y de coordinación para algunas de estas bibliotecas. Los resultados obtenidos permitieron estudiar la redistribución de los componentes y la amplificación de uno o más productos usando luz e iones metálicos como plantillas físicas y químicas, respectivamente.

**Palabras clave:** bibliotecas combinatorias dinámicas, acil-hidrazonas, dinámicas de coordinación y configuración.

# Livrarias combinatórias dinâmicas baseadas na acil-hidrazona: estudo da evolução cinética/termodinâmica, dinâmicas de configuração e da coordenação

## Resumo

É descrita a seletividade cinética e termodinâmica da formação de acil-hidrazonas em livrarias combinatórias dinâmicas (DLC). Foram geradas reações competitivas a partir das hidrazidas: isoniazida, 4-nitro-benzohidrazida, 4-dimetilamino-benzohidrazida e hidrazida nicotínica; além dos derivados de aldeído: benzaldeído e 2-piridin-carboxaldeído. As espécies obtidas e a distribuição dos DLCs foram monitorados mediante espectroscopia $^{1}$H-NMR, foi encontrado que as acil-hidrazonas que continham à 4-dimetilamino-benzohidrazida são tanto o produto cinético como o termodinâmico de suas respectivas livrarias. Também investigaram-se as dinâmicas de configuração e coordenação para algumas destas livrarias. Os resultados obtidos permitem estudar a redistribuicao dos componentes e a amplificação de um ou mais produtos usando luz e íons metálicos como modelos físicos e químicos, respectivamente.

**Palavras-Chave:** livrarias combinatórias dinâmicas, acil-hidrazonas, dinâmicas de coordenação e configuração.

## Introduction

Dynamic combinatorial chemistry (DCC) is a powerful tool to study and create complex chemical systems in a relative simple manner. DCC was defined by Sanders as molecular or supramolecular combinatorial chemistry under thermodynamic control (*1*). When a system is formed by molecular fragments that can react with each other, combining them, a mixture of many compounds that interconverts constantly, will be obtained, i.e. building blocks are connected together by reversible bonds which are continuously forming and breaking in the reaction medium (Figure 1). This product mixture is known as Dynamic Combinatorial Library (DCL). The system is reversible and it is in equilibrium, thus, any external effect could shift this equilibrium. A clean-cut example of these systems and relating Emil Fisher's concept is placing a template in the system, which fits precisely with one member of the library, and subsequently amplify or shift the equilibrium towards the formation of a product (*2*).

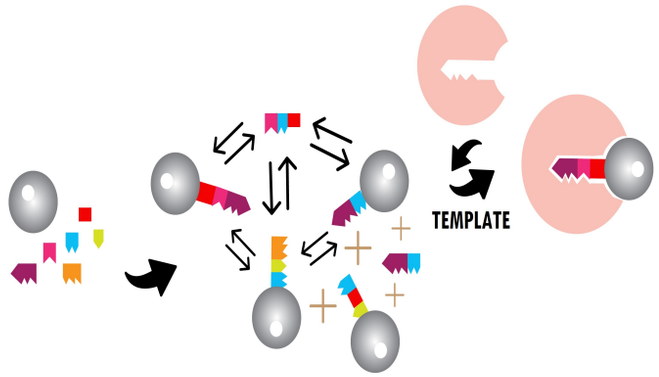


**Figure 1.** Formation of a DCL from "building blocks" and the addition of a template, which causes amplification of the member forming the more stable complex.

DCC and DCL's have been widely used for the synthesis and identification of small molecular receptors (*3-7*). These tools have also helped to generate effective ligands for biomacromolecules and biosensors (*8-10*), synthesis of catalysts (*11-13*), crosslinked materials (*14-16*), capsules and cages (*17-19*), self-replication (*20*), nanomachines (*21*), among others.

Based on previous work done by Lehn's group (*22-24*), we have chosen a set of four hydrazides and two aldehydes as building blocks in order to generate several dynamic combinatorial libraries. These building blocks were selected since the acylhydrazones, which can be formed, have a number of characteristics that make them attractive for DCL's formation: i) unlike the hydrazones, the acylhydrazones have a much weaker double bond making them favorable to perform exchange reactions; ii) these compounds have an imino double bond, which has been widely investigated in our research group and it is known that is sensitive to light (*25-26*); iii) some of these compounds have coordination sites in their chemical structure that serve as tridentate ligand to coordinate to cation metals. Having in mind these characteristics, we have analyzed how the distributions of the formed libraries vary by the presence of the metals and UV light as external stimuli. For this purpose, nuclear magnetic resonance technique was used as a tool for monitoring the evolution of the dynamic library.

## Materials and methods

All starting materials, reagents and solvents, were purchased from Sigma-Aldrich and Alfa Aesar. The hydrazides were used without any further purification. The benzaldehyde was distilled under reduced pressure. $^{1}$H and $^{13}$C-NMR spectra were taken in a 400 MHz Bruker UltraShield spectrometer. UV-Vis spectra were recorded in a Shimadzu UV-1700 PharmaSpec spectrophotometer.

### Standard procedure for the preparation of acylhydrazones

One eq of aldehyde **A-B** was added to an ethanol solution (5.0 mL) of the corresponding hidrazide **1-4** (1 eq) with 5.0 µL of glacial acetic acid. The mixture was heated under reflux of ethanol for 3 to 6 h. The resulting precipitate was collected by vacuum filtration and recrystallized from cold ethanol to afford the pure acylhydrazones in their *E*-configuration.

### Standard procedure for the preparation of DCLs

The DCLs were prepared by mixing in a sealed NMR tube (under inert atmosphere of $N_2$, done in a Aldrich AtmosBag) 1 eq of the corresponding aldehydes (225 µL) and acylhydrazines (225 µL) in DMSO-$d_6$ or $CD_3OD$ at 25 °C. The starting time of reaction (t = 0) was considered as the time in which the solution of aldehydes was poured into the NMR tube and entered in contact with the hydrazines solution. $^{1}$H-NMR spectroscopy was used as a tool to monitor the evolution of the library. For those experiments involving UV radiation, a 250 W mercury lamp was used as an UV source. The NMR tube was irradiated while pouring the reagents; in a second experiment the NMR tube with the compounds was allowed to equillibrate during 24 h. Afterwards, the tube was irradiated during 1 h. For the addition of metal ions, solutions of the corresponding $M^{2+}$ ion were standarized by atomic absorption spectroscopy calculating the concentration in a calibration curve.

## Results and discussion

When mixing aldehydes and hydrazines redacción: a large number of $^{1}$H-NMR signals are obtained making difficult to identify the products. Therefore, each possible acylhydrazone, as part of the library, was synthesized from each corresponding hydrazide and aldehyde derivatives, according to a methodology reported previously (*22*) (Figure 2). The reactions were monitored by thin layer chromatography (TLC), and the spectroscopic data were consistent with the proposed structures (*E* configuration) of compounds **A-1** to **B-4** (Figure 3). Details of the synthesis were described in the Materials and methods section.

The synthesis was performed with the aim to identify characteristic signals in the $^{1}$H-NMR spectra of each acylhydrazone. Signals found in the region between 11.5 and 12.5 ppm, which correspond to the N-H protons (as determined by 2D NMR techniques), were chosen to determine the distribution of the products on the libraries (see *Characterization data for acylhydrazones*) and further confirmed by DOSY experiments to corroborate the asignment of the N-H proton signals.

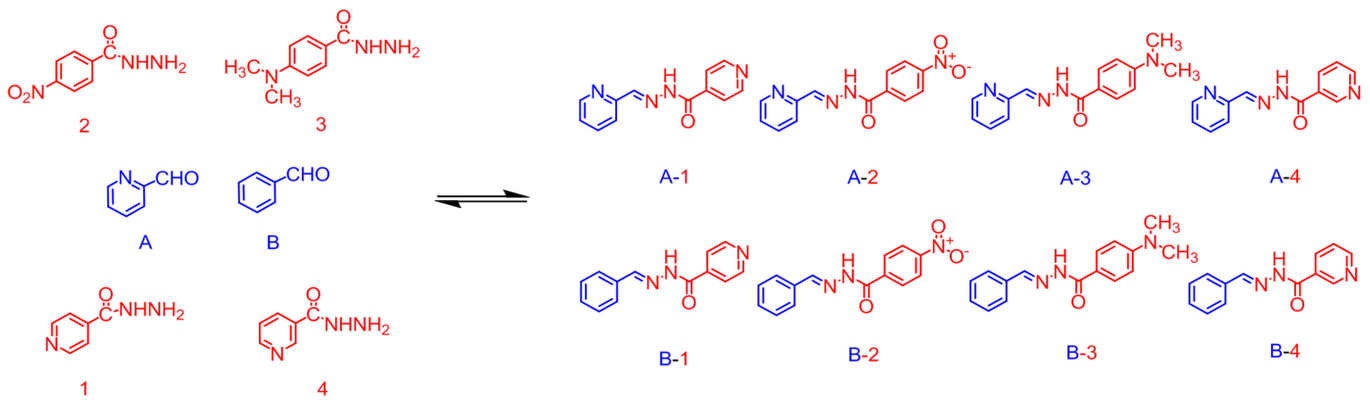


**Figure 2.** Building blocks (hydrazides and aldehydes) and constituent products of hydrazones-based libraries.

The fact that the signals of the N-H protons of each acylhydrazone appear at different chemical shifts, results from the type of substituent, which is present in every one of them, since they contain either electron-withdrawing groups, electron-releasing groups or an electronegative nitrogen at different positions on the ring.

## Characterization data for acylhydrazones

2-pyridinecarboxaldehyde isonicotinoyl hydrazone (**A-1**): Using the method described above, the compound was synthesized and obtained in a 86% yield. M.p.: 166-167°C. Elemental analysis calcd. (%) for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76; found: C, 62.37; H, 4.23; N, 23.82. FT-IR (ATR) ν/cm$^{-1}$ 3292 (N-H), 1665 (C=O), 1539 (C=N). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 12.28 (s, 1H), 8.84–8.78 (m, 2H), 8.64 (d, J 4.29 Hz, 1 H), 8.48 (s, 1 H), 8.03–7.97 (m, 1 H), 7.91 (td, J 7.71, 1.56 Hz, 1 H), 7.86–7.82 (m, 2 H), 7.45 (ddd, J 7.27, 4.93, 1.07 Hz, 1 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 161.93, 152.96, 150.44, 149.65, 149.23, 140.26, 137.04, 124.74, 121.61, 120.13.

2-pyridinecarboxaldehyde p-nitrobenzoyl hydrazone (**A-2**): Using the method described above, the compound was synthesized and obtained in a 96% yield. M.p.:227-228°C. Elemental analysis calcd. (%) for $C_{13}H_{10}N_4O_3$: C, 57.78; H, 3.73; N, 20.73; found : C, 57.86; H, 3.72; N, 20.62. FT-IR (ATR) ν/cm$^{-1}$ 3221 (N-H), 1659 (C=O), 1595(C=N). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 12.31 (s, 1 H), 8.64 (d, J 4.29 Hz, 1 H), 8.49 (s, 1 H), 8.39 (d, J 8.59 Hz, 2 H), 8.17 (d, J 8.59 Hz, 2 H), 8.01 (d, J 7.80 Hz, 1 H) 7.94–7.87 (m, 1 H) 7.47–7.42 (m, 1 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 161.76, 152.96, 149.59, 149.38, 149.12, 138.80, 136.95, 129.26, 124.65, 123.69, 120.08.

2-pyridinecarboxaldehyde p-dimethylamino-benzoyl hydrazone (**A-3**): Using the method described above, the compound was synthesized and obtained in a 87% yield. M.p.: 224-225°C. Elemental analysis calcd. (%) for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.05; N, 20.88; found: C, 67.19; H, 6.07; N, 20.78. FT-IR (ATR) ν/cm$^{-1}$ 3244 (N-H), 1611(C=O), 1516(C=N). ). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 11.77 (s, 1 H), 8.60 (d, J 4.49 Hz, 1 H), 8.45 (s, 1 H), 7.95 (d, J 7.80 Hz, 1 H), 7.88 (dd, J 7.61, 1.56 Hz, 1 H), 7.83 (d, J 8.98 Hz, 2 H), 7.39 (dd, J 7.41, 4.88 Hz, 1 H), 6.76 (d, J 8.98 Hz, 2 H), 3.00 (s, 6 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 163.09, 153.66, 152.61, 149.48, 146.32, 136.83, 129.24, 124.12, 119.67, 119.08, 110.84, 39.51.

2-pyridinecarboxaldehyde nicotinoyl hydrazone (**A-4**): Using the method described above, the compound was synthesized and obtained in a 91% yield. M.p.: 148-150°C. Elemental analysis calcd. (%) for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76; found: C, 58.63; H, 4.74; N, 22.81. FT-IR (ATR) ν/cm$^{-1}$ 3474 (N-H), 1668(C=O), 1593 (C=N). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 12.24 (s, 1 H), 9.08 (d, J = 1.56 Hz, 1 H), 8.82–8.75 (m, 1 H), 8.63 (d, J = 4.68 Hz, 1 H), 8.46 (s, 1 H), 8.28 (d, J = 7.80 Hz, 1 H), 8.00 (d, J = 7.80 Hz, 1 H), 7.94–7.86 (m, 1 H), 7.59 (dd, J = 7.80, 4.88 Hz, 1 H), 7.48–7.40 (m, 1 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 162.12, 153.10, 152.61, 149.70, 148.75, 148.73, 137.13, 135.73, 129.05, 124.76, 123.83, 120.20.

Benzaldehyde isonicotinoyl hydrazone (**B-1**): Using the method described above, the compound was synthesized and obtained in a 71% yield. M.p.: 198-199°C. Elemental analysis calcd. (%) for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66; found: C, 68.73; H, 4.89; N, 18.38. FT-IR (KBr) ν/cm$^{-1}$ 3455 (N-H), 1692 (C=O), 1566 (C=N). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 12.11 (s, 1 H), 8.79 (d, J 4.10 Hz, 2 H), 8.46 (s, 1 H), 7.83 (d, J 3.90 Hz, 2 H), 7.78–7.74 (m, 2 H), 7.47 (br. s., 3 H). $^{13}$C-NMR (100.60 MHz, DMSO-d6) δ 161.65, 150.33, 149.07, 140.47, 134.01, 130.41, 128.90, 127.27, 121.53.

Benzaldehyde p-nitrobenzoyl hydrazone (**B-2**): Using the method described above, the compound B-2 was obtained with a 79% yield. M.p.: 260-262°C. Elemental analysis calcd. (%) for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61; found: C, 61.65; H, 4.15; N, 15.35. FT-IR (KBr) ν/cm$^{-1}$ 3450 (N-H), 1656 (C=O), 1554 (C=N). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 12.17 (s, 1 H), 8.47 (s, 1 H), 8.37 (d, J 8.59 Hz, 2 H), 8.15 (d, J 8.78 Hz, 2 H), 7.78–7.73 (m, 2 H), 7.49–7.45 (m, 3 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 161.61, 149.31, 149.03, 139.11, 134.06, 130.44, 129.21, 128.93, 127.31, 123.69.

Benzaldehyde p-dimethylamino-benzoyl hydrazone (**B-3**): Using the method described above, the compound was synthesized and obtained in a 82% yield. M.p.: 283-285°C. Elemental analysis calcd. (%) for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72; found: C,71.48; H, 6.50; N, 15.82. FT-IR (KBr) ν/cm$^{-1}$ 3223 (N-H), 1614 (C=O), 1524 (C=N). $^1$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 11.57 (br. s., 1 H), 8.42 (br. s., 1 H), 7.82 (d, J 8.78 Hz, 2 H) 7.70 (d, J 6.63 Hz, 2 H), 7.48–7.40 (m, 3 H), 6.76 (d, J 8.98 Hz, 2 H), 3.00 (s, 6 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 163.07, 152.48, 145.96, 134.68, 129.69, 128.79, 126.84, 119.44, 110.81.

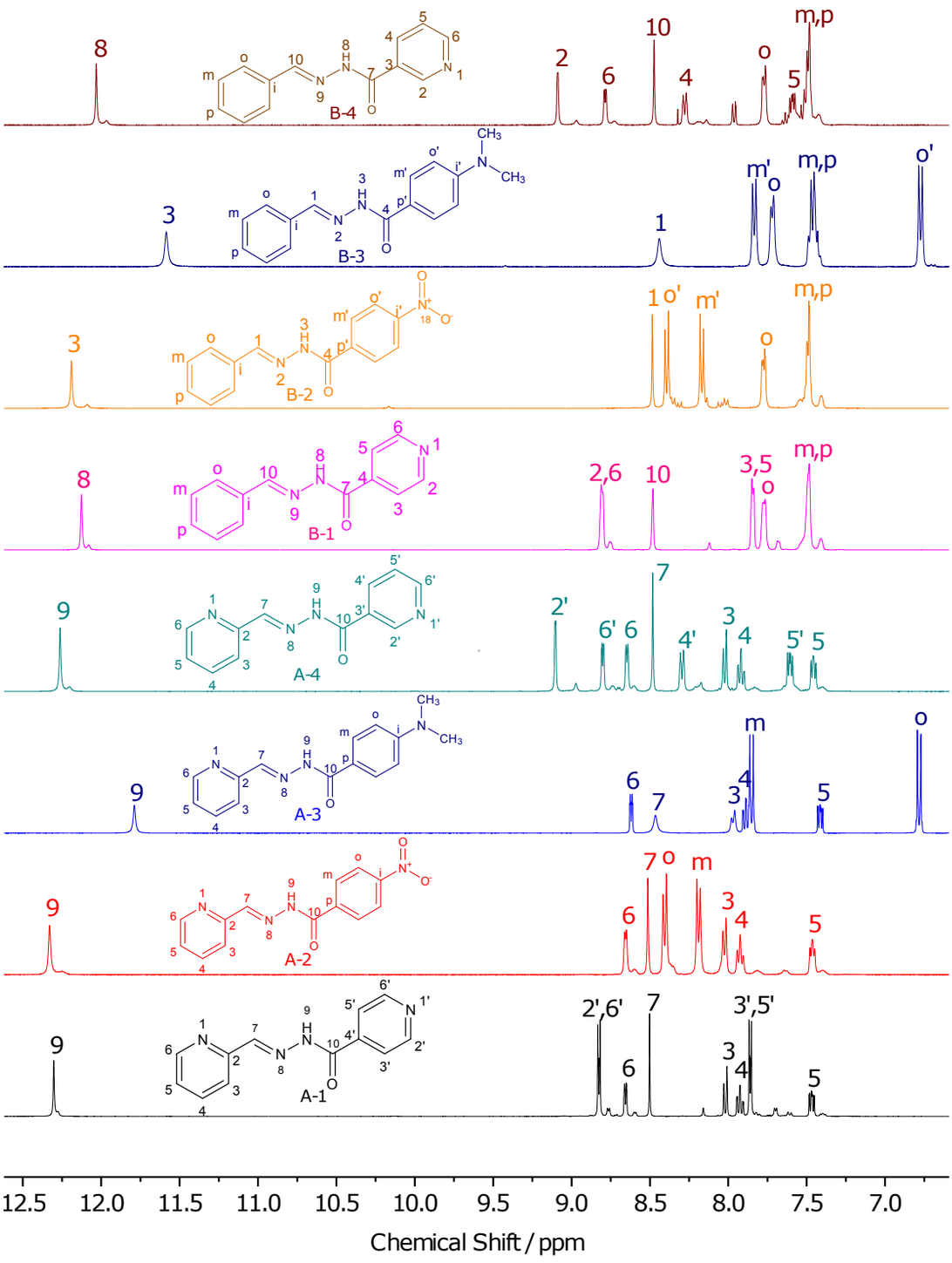


**Figure 3.** $^{1}$H-NMR (400 MHz, DMSO-*d6*) spectra of the products of dynamical combinatorial libraries.

Benzaldehyde nicotinoyl hydrazone (**B-4**): Using the method described above, the compound was synthesized and obtained in a 70% yield. M.p.: 129-130°C. Elemental analysis calcd. (%) for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66; found: C, 67.47; H, 4.94; N, 17.68. FT-IR (KBr) ν/cm$^{-1}$ 3270 (N-H), 1653 (C=O), 1550 (C=N). $^{1}$H-NMR (400 MHz, DMSO-*d6*) $\delta$ 12.01 (s, 1 H), 9.07 (d, J 1.17 Hz, 1 H), 8.77 (d, J 3.90 Hz, 1 H), 8.46 (s, 1 H), 8.26 (d, J 7.80 Hz, 1 H), 7.79–7.71 (m, 2 H), 7.57 (dd, J 7.80, 4.88 Hz, 1 H), 7.50–7.45 (m, 3 H). $^{13}$C-NMR (100.60 MHz, DMSO-*d6*) $\delta$ 161.67, 152.26, 148.54, 135.41, 134.10, 130.24, 129.22, 128.84, 128.52, 127.17, 123.58.

## Competitive reactions of acylhydrazines 1-4 with aldehydes A and B

Two competitive reactions, named DCL-1 and DCL-2, were carried out from the hydrazides 1-4 and aldehyde **A** (DCL-1) or **B** (DCL-2) by mixing equimolar amounts of the respective building blocks in a NMR tube and using a deuterated solvent (Figure 2). The libraries with **A** and **B** were monitored for 320 min by $^{1}$H-NMR spectroscopy, further time did not showed any changes in the relative concentration of the DCL. The relative amount of acylhydrazone formed was calculated from the relative intensities of the corresponding signals and compared to an internal standart (1,4-dioxane). As shown in Figure 4 for DCL-1 and Figure 5 for DCL-2, the appearance of four new signals in the aforementioned region shows the formation of the four corresponding acylhydrazones (**A-1**, **A-2**, **A-3** and **A-4**).

From the NMR data, kinetic traces for acylhydrazones formation were ploted. Aditionally, equillibrium distributions of the different acylhydrazones are shown in Figure 6. From these results, it is observed that **A-3** and **B-3** are the acylhydrazones kinetically and thermodynamically favored in their respective DCLs. The latter is understood based on the greater nucleophilicity of acylhydrazine **3**.

Likewise, the highest rate of formation and stability of **A-3** and **B-3** may also be explained if we consider that the precursors of the other acylhydrazones have in their structure either one electron withdrawing group or an electronegative nitrogen in the aromatic ring, which by both inductive and resonance effects generate an electronic deficiency in the molecule, making it less reactive towards the nucleophilic attack of the nitrogen to the carbonyl group of the aldehyde (*2*).

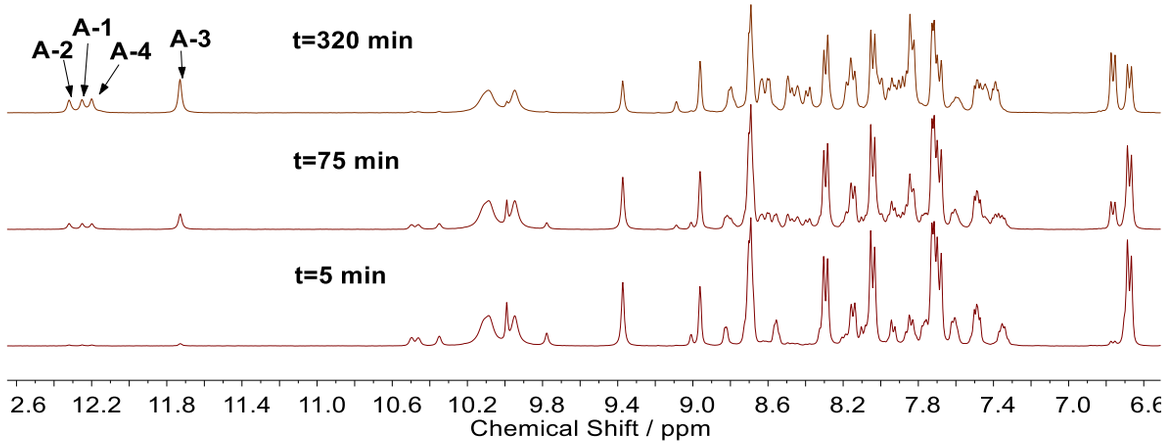


**Figure 4.** $^{1}$H-NMR spectra at three selcted (aleatorially) times of the DCL-1 formed by **1-4** and **A** in DMSO-$d_6$.

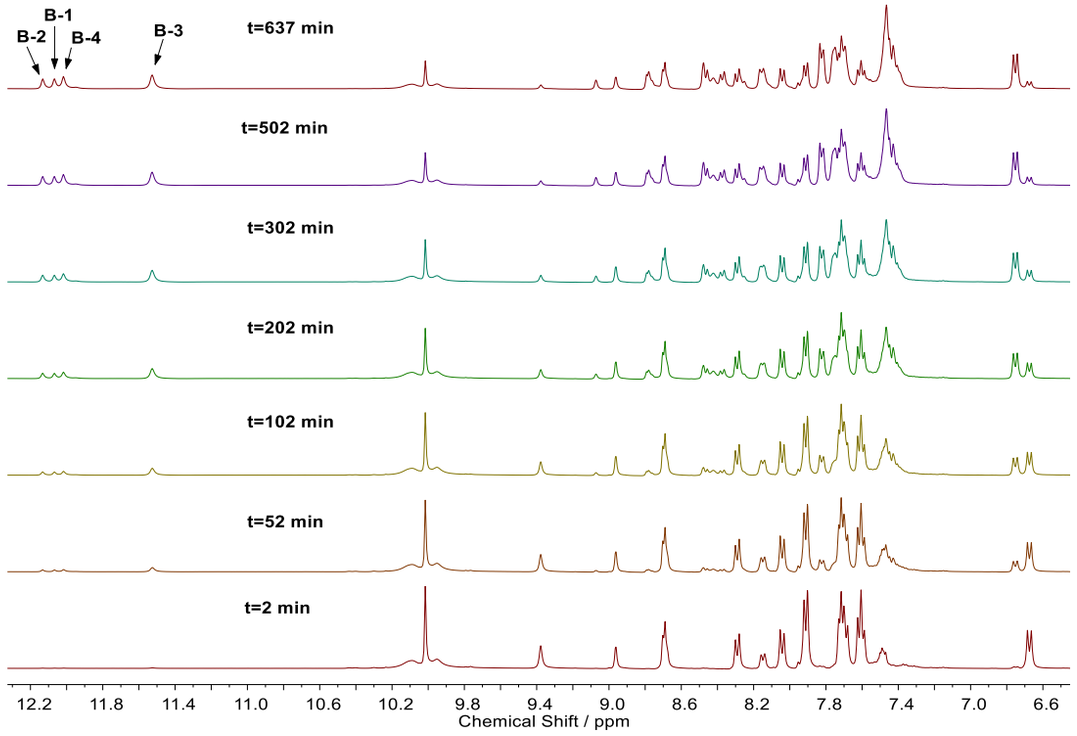


**Figure 5.** $^{1}$ H-NMR (400 MHz, DMSO-d6) spectra at different times of the library formed by **1-4** and **B** (DCL-2).

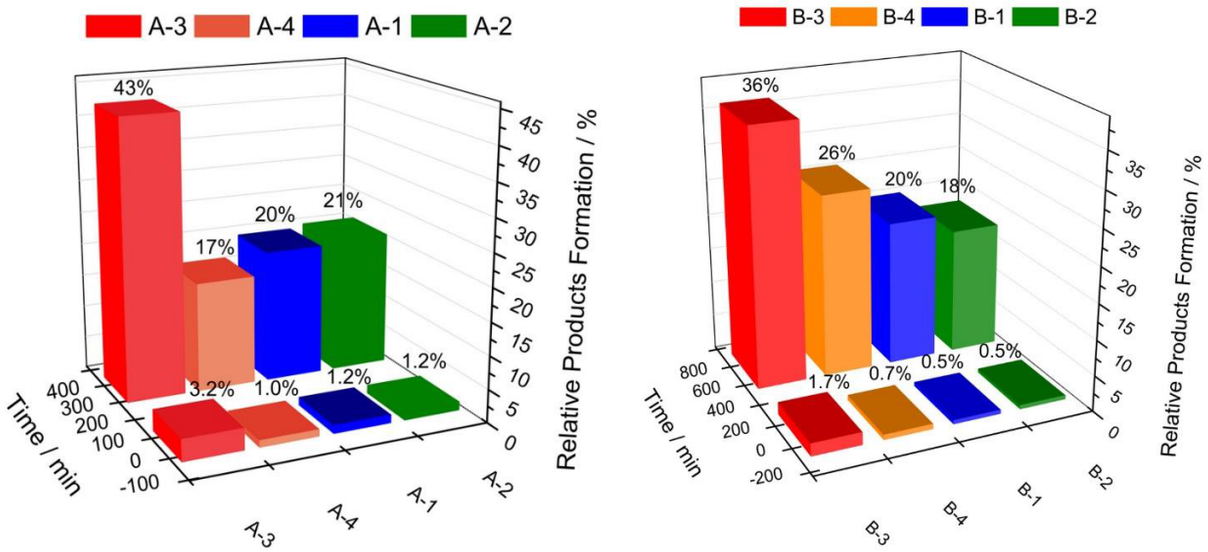


**Figure 6.** Equilibrium distributions of different acylhydrazones **A-1** to **A-4** (DCL-1, left) and **B-1** to **B-4** (DCL-2, right) in the DCL formed from aldehydes **A** and **B** and acylhydrazines **1-4**.

While in **A-3** and **B-3**, the dimethylamine group makes the molecule more electron-rich, thus confering a higher reactivity for the nucleophilic attack, which is reflected in the greater proportion and the greater stability of this acylhydrazones. Although equillibrium was confirmed by a control experiment using different starting concentrations of previously prepared acylhydrazones reaching the same final distributions, it is important to remark that those distributions are reached in longer times which implies a slow amine interchange in the DCL. Despite that amine interchange can be increased by changing the nature of the solvent, this was not considered in this study to avoid issues with the solubility of the reagents.

## DCL-3: hydrazides 1-4 plus aldehydes A and B

Aldehydes **A-B** were added to an equimolar mixture of hydrazides **1-4**. The reaction was monitored for 817 min, resulting in 168 $^{1}$H-NMR spectra in total (some of them are shown in Figure 7).

Figure 8 shows the kinetic trace of the competition DCL reaction. According to the results, **A-3** is the kinetic product, not only for the larger nucleophilicity of acylhydrazine **3** but also for the larger electrophilicity of aldehyde **A**, which plays an important role in the reaction kinetics. Likewise, acylhydrazones formed from **A** were found in larger amounts than the ones formed from **B**.

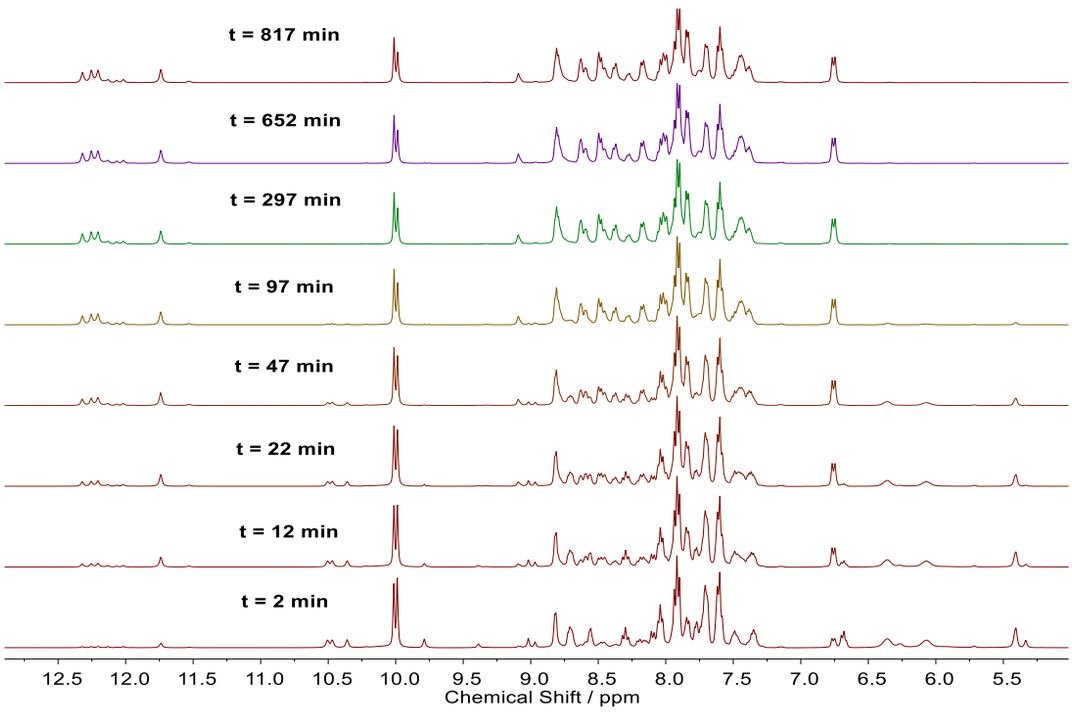


**Figure 7.** $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra at different times of the library formed by **1-4** and **A-B** (DCL-3).

This can be attributed to the electronegative nitrogen present in A, which by inductive effect causes the carbonyl group to be more electron deficient, making it more susceptible to nucleophilic attack. Noteworthy, data fit to a kinetic model is quite difficult for this system, however, within the first 8% of the reaction, the DCL follows a second order reaction with a 4-5% error, this allows to estimate that acylhydrazone **A-3** is generated in around 12-fold faster than its **B-3** counterpart. Interestingly, electrophilicity of the aldehyde is more important than acylhydrazine nucleophilicity in both, kinetic and thermodynamic control of the DCL. Upon equilibrium, A-containing acylhydrazones exhibit similar concentrations, which supports the dynamic character of the DCL.

The evident acylhydrazine interchange is probably due to the conjugation of the hydrazonic nitrogen (-NH-) with the carbonyl group which reduces the conjugation of this one with the imino group (C=N), making the latter a more reactive bond towards nucleophiles such as hydrazides or water (*27*). Therefore, the exchange reaction promotes another product to be formed at the expense of **A-3**, but still, at the end of the experiment (t = 817 min), this acylhydrazone continues to be the one with the highest percentage yield, therefore **A-3** is the thermodynamic product in the library. The difference between the greater proportion of acylhydrazones formed from **A**, as compared with the generated from **B**, confirms the higher reactivity of 2-pyridinecarboxaldehyde over benzaldehyde, due to the presence of an electronegative nitrogen atom in the ring.

## Effect of UV light irradiation

Acylhydrazones formed from the aldehyde **A** exhibit, in the *Z* configuration, a thermodynamic stabilization by the formation of an intramolecular hydrogen bond between the amine hydrogen and the pyridine nitrogen upon photochemical isomerization. Meanwhile, the *Z* configuration of acylhydrazones from **B** do not exhibit this thermodynamic stabilization (*25, 26*). With this in mind, it was interesting to observe the effect of UV light irradiation on the acylhydrazone distribution of the DCLs. For this purpose, the library was formed only with hydrazides **1** and **4** as well as the aldehydes **A** and **B**, due to their solubility in MeOH-$d_4$.

The latter was used instead of DMSO-$d_6$ because photoisomerization experiments in DMSO-$d_6$ did not exhibit any appreciable changes, even after 150 min of UV irradiation, contrary to MeOH-$d_4$ (Figure 9). This contrasting result is due to the viscosity of DMSO which slows down the photoisomerization of hydrazone-based compounds (*25-28*).

In a typical procedure, a competition reaction was carried out until equillibrium was reached. Afterwards, the mixture was irradiated with UV light using a mercury lamp of 250 W. The competition reaction was monitored for 228 min. The relative concentrations of each acylhydrazone were calculated only at the end of the experiment and the results were 39/15/29/17% of **A-1**/**A-4**/**B-1**/**B-4**, respectively. The fact that those products containing the hydrazide **1** are in greater proportion, suggests that hydrazide **4** is less nucleophilic by the overall inductive effect that the N of the pyridine ring in position 3 has on the R group. Once the equilibrium was reached, the library was subjected to UV irradiation for 1 h and then was monitored by $^1$H-NMR. It is remarkable the appearance of new signals in the spectra shown in Figures 10-12 which correspond to the *Z* isomers of compounds **A-1** and **A-4**.

The relative percentages shown in Table 1 were calculated by integrating those signals obtained in Figure 11 that are not overlapped and then by the substraction between these and the overlapped ones, the integrals and therefore the percentages of the latters were obtained. From the distribution of acylhydrazones it can be observed that the product which is amplified after 60 min of UV light irradiation is the *Z* isomer of **A-1**, suggesting the adaptation of the library when a stimulus is applied. Vantomme *et al.* (*27*) also observed the same photoselection in a different DCL with similar yields of photoisomerization.

A second DCL was generated from the same building blocks (acylhydrazines **1** and **2** and aldehydes **A** and **B**) in the presence of UV light irradiation. For this purpose, the NMR tubes were irradiated with a mercury vapor lamp during 1 h before the reaction started. Afterwards, the $^1$H-NMR spectra were taken to observe the distribution of the library (Figure 13). The amplified product for this DCL was the acylhydrazone **B-1** (Table 1). The presence of **A-1** and **A-4** *Z* isomers on the library proved that UV light is part of the system, however, this also indicates that whether UV light is added at the beginning or at the end of the reaction, the amplified product will be a different one.

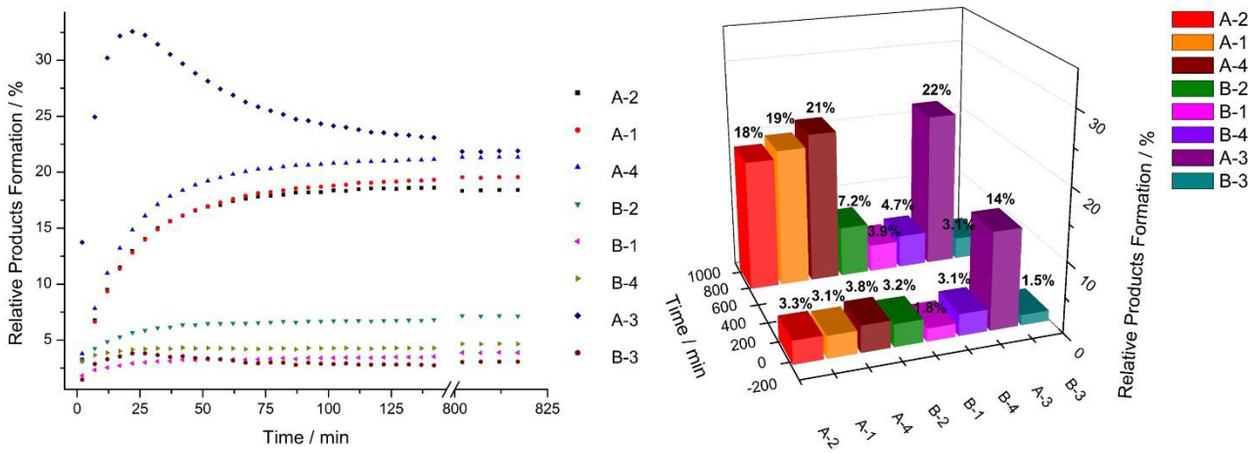


**Figure 8.** Kinetic trace of relative product formation over time of the library DCL-3.

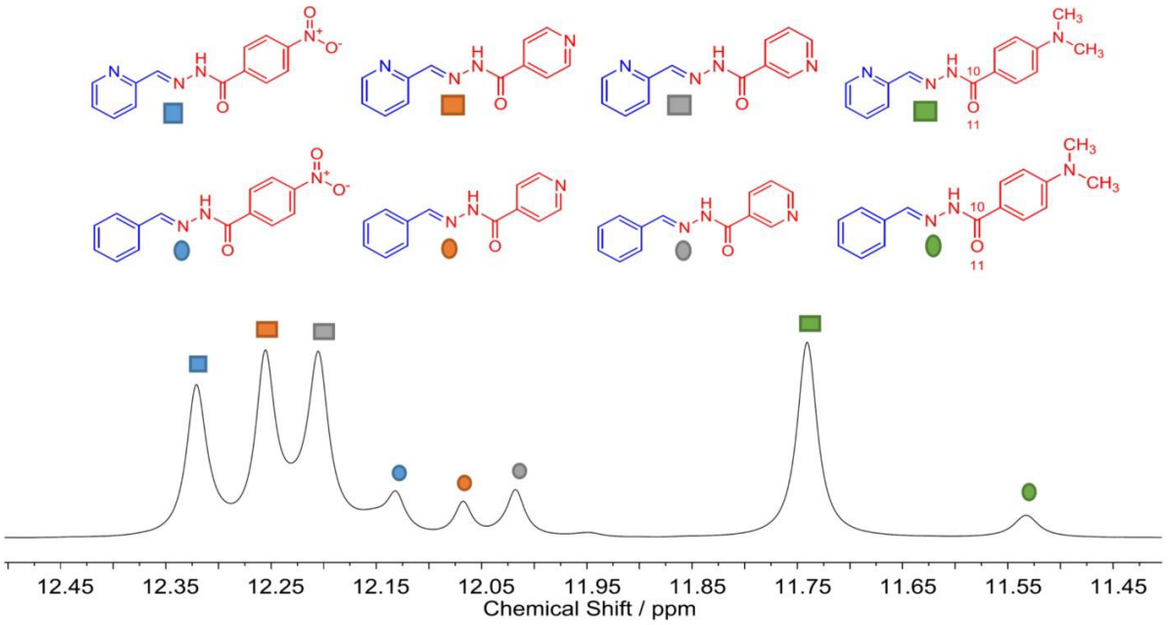


**Figure 9.** A portion of $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectrum at t = 817 min and the assignment of signals.

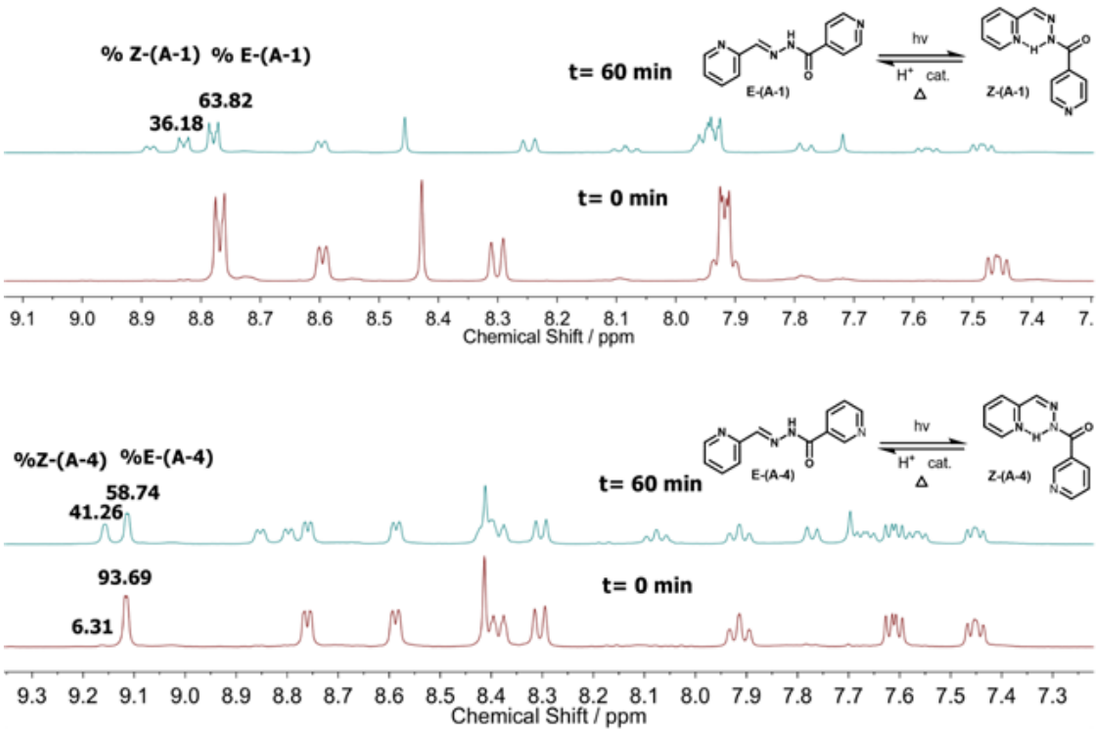


**Figure 10.** A portion of the $^{1}$H-NMR (400 MHz, MeOH-$d_4$) spectra of acylhydrazones **A-1** (top) and **A-4** (bottom) taken at different times under UV irradiation.

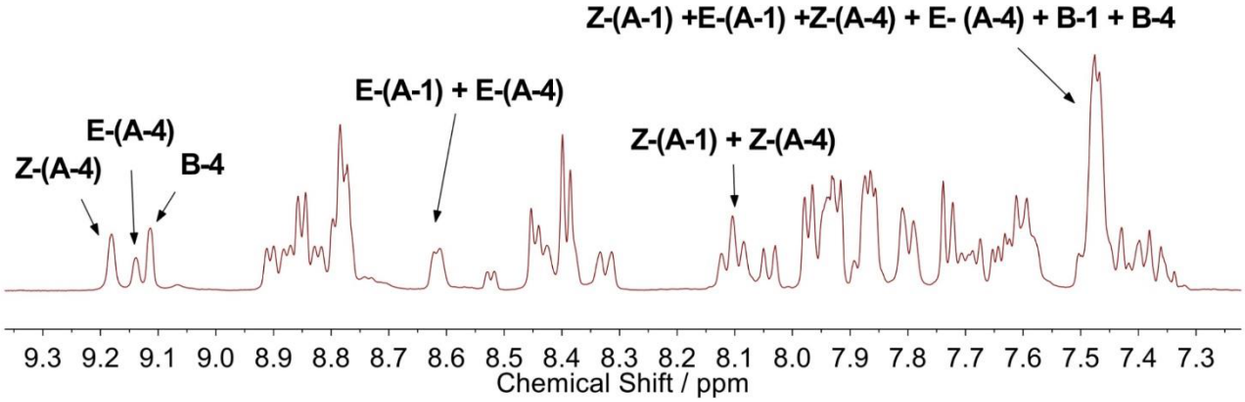


**Figure 11.** A portion of the $^{1}$H-NMR spectra of the DCL-1 after 1h of irradiation in MeOH-$d_4$.

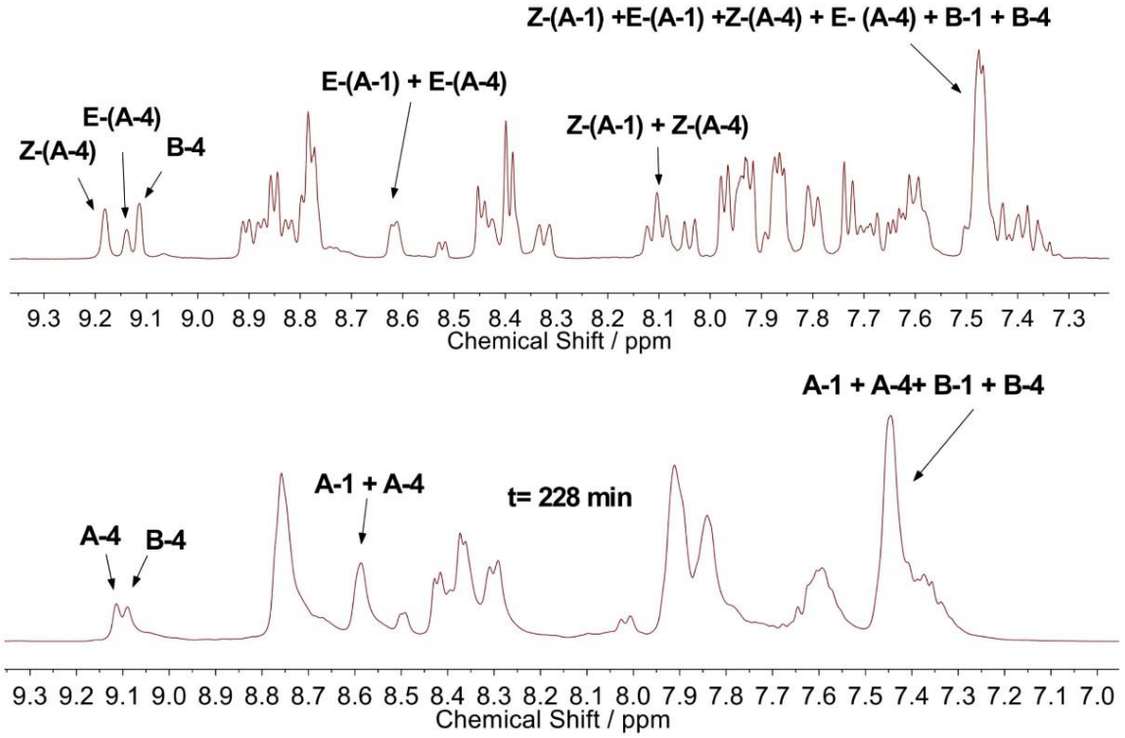


**Figure 12.** A portion of $^{1}$H-NMR (400 MHz, MeOH-$d_4$) spectrum after irradiation with UV light.

**Table 1.** Relative percent of products observed in two DCL´s

| Acylhydrazone | Relative contribution (%) | |
|---|---|---|
| | DCL + UV light after equilibrium[a] | DCL + UV light from t = 0[b] |
| *(E)*-A-1 | 13.5 | 12.8 |
| *(Z)*-A-1 | 28.4 | 20.3 |
| *(E)*-A-4 | 9.6 | 11.7 |
| *(Z)*-A-4 | 18.6 | 12.0 |
| B-1 | 11.5 | 24.7 |
| B-4 | 18.4 | 18.5 |

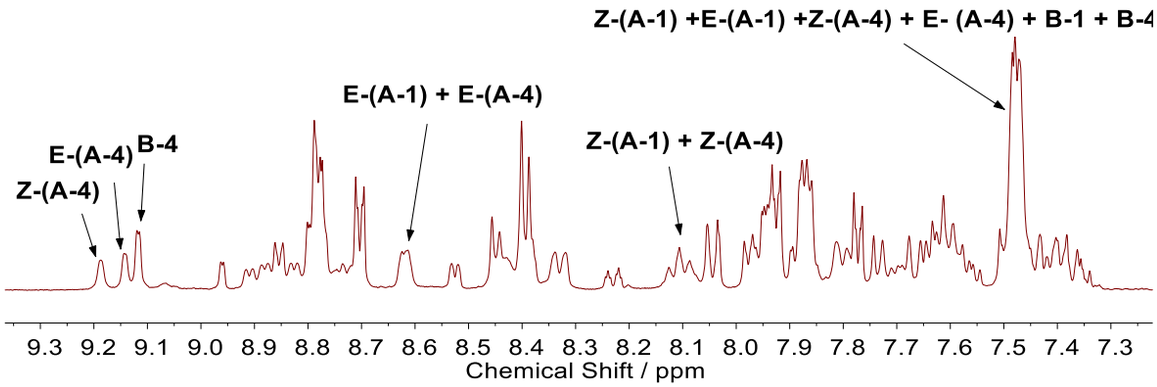


**Figure 13.** A portion of the $^1$H-NMR spectra of the DCL in MeOH-$d_4$ irradiated with UV light at the beginning of reaction.

Noteworthy, in both cases the resulting product distribution is quite difficult to analyze even by the use of 2D NMR experiments, besides, once the UV light is removed the products concentrations do not go back to the previous distributions. These results imply that the system is in a metastable thermodynamic point due to the hydrogen bond formation (*22, 25*). In any case, these experiments proved the use of UV light as an irreversible template in DCL amplification and deserves further exploration.

## Effect of the introduction of metal cations on DCL distributions

Finally, we wanted to explore the role of metal ions on DCL distributions, since it is well known that hydrazones and acylhydrazones derived from 2-pyridinecarboxaldehydes and 2-pyridinehydrazines or acylhydrazines are able to coordinate metal cations in a terpyrdine-like fashion (*22, 25, 26*). In this regard it can be thought that the introduction of metal ions can be used as a template to amplify acylhydrazones derived from aldehyde **A**. Accordingly, hydrazines **1** and **4** and aldehydes **A** y **B** were used to form the DCL; in addition, 0.5 eq of $Zn(OTf)_2$ were added to the mixture, the reaction was monitored for 820 min. Comparing this library with other DCL in the present work (Figure 14) it was observed from the beginning of the reaction the apearence of only four N-H signals instead of eight corresponding to the formation of every possible acylhydrazone.

The ones that disappear, correspond to those acylhydrazones derived from aldehyde **A**, those which have a propitious structure to form a complex with $Zn^{2+}$ by their tridentate NNO coordination site (*28-32*).

These signals disappearance are proof of the formation of $ML_2$ type complexes usually formed with this kind of ligands (*22, 25-27*). When they form a complex, these ligands are deprotonated, either because there is a relatively basic environment or because the enol form of the ligand predominates (*32*). Although it is known that the addition of this template ($M^{2+}$ ions) results in the formation of such complexes, it is not possible to know which component is amplified, because the signals from each product are highly overlapped. Therefore, it is necessary to determine the binding and stability constants to have a clearer idea of what it is inside the solution.

Although DCLs have been studied with some detail over the last years, it is difficult to compare our results with the literature. Since, on one hand, most reports deal with the use of biological chemical templates and only one article introduces UV light to a DCL (based on aldehydes and hydrazines) obtaining similar results (*27*). On the other hand, metal ion selection has been studied for a more simple system (*23*) and similar to the present work a metalloselection was observed.

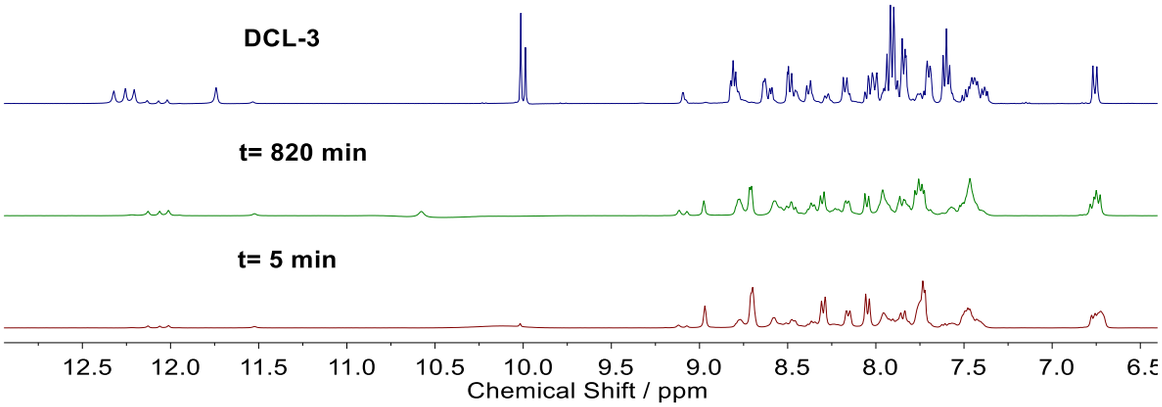


**Figure 14.** A portion of the $^1$H-NMR spectra of the library formed from four hydrazides, both aldehydes and $Zn^{2+}$, in DMSO-$d_6$ and the spectrum of DCL-3.

## Conclusions

Generation of dynamic combinatorial libraries derived from acylhydrazines **1-4** and aldehydes **A** and **B** were monitored by $^1$H-NMR spectroscopy, achieving the calculation of the products distribution in time in most cases. The acylhydrazone **A-3** was both the kinetic and thermodynamic product of two of the formed libraries, confirming the greater nucleophilicity of the corresponding hydrazide due to the electron releasing character of the dimethylamino group and the higher electrophilicity of 2-pyridincarboxaldehyde as compared with benzaldehyde, because of the presence of an electronegative nitrogen atom in its structure.

In competitive DCL reactions acylhydrazone products derived from hydrazide **1** (versus hydrazide **4**) were found in higher yields, suggesting that the reduced nucleophilicity of hydrazide **4** is due to the overall inductive effect that the pyridine ring N in position 3 has on the R group (R=-(CO)-NH-$NH_2$). Exposure to a physical stimulus such as irradiation with UV light, demostrated that DCLs respond or adapt themselves to that stimulus, reorganizing and leading to the formation of a new library. In this particular case, it was also observed that depending on the time when the stimulus is added, the amplified product change, because the formation of the *E* isomer occurs first that the *Z* one, still in the presence of UV light.

Finally, the disappearance of the signals corresponding to the acylhydrazones $NH_2$ fragment derived from 2-pyridinecarboxaldehyde, by adding $Zn^{2+}$, demostrates the formation of $ML_2$ type complexes and its amplification.

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