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► **To cite this version:**

Kilian Caillaud, Catherine Ladaviere. Water Soluble (Poly)acylhydrazones: Syntheses and Applications. Macromolecular Chemistry and Physics, In press, 10.1002/macp.202200064 . hal-03672462

HAL Id: hal-03672462

<https://hal.science/hal-03672462v1>

Submitted on 19 May 2022

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Water-soluble (poly)acylhydrazones: Syntheses and Applications

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10 Abstract

Acylhydrazone-based (macro)molecules display an increasingly broad spectrum of applications, from small therapeutic molecules and metal-ions probes to self-healing films and biorelevant dynamic polymers. The acylhydrazone function is indeed of prime interest since its unique design combines hydrogen bonds, pH-dependent reversibility, ability to undergo molecular exchanges, and a possible polyvalency. However, a key parameter of its use in aqueous media is the associated water-solubility of the resulting (macro)molecule. This property can indeed become a limitation for applications requiring high concentrations or no organic cosolvent. This review firstly focuses on describing the acylhydrazone function, its properties, and its relevancy in dynamic combinatorial chemistry (DCC). Then it details the synthesis strategies (molecular design, cosolvent, reaction conditions) reported in literature to ensure a sufficient solubilization of both single-bond and multiple-bond acylhydrazone (macro)molecules, together with their various applications.

25 Keywords

Acylhydrazone; polyacylhydrazone; dynamic combinatorial chemistry; dynamer; water-soluble polymers

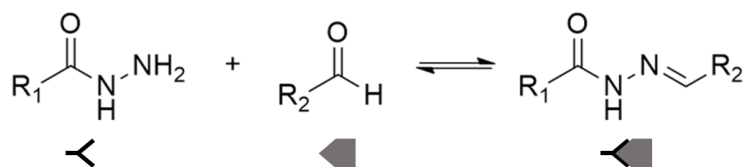
Introduction

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In the past three decades, the increasing development of dynamic combinatorial chemistry (DCC) has led to new synthetic methodologies, to create even more diverse molecules in a minimum of steps.¹⁻⁴ Among the eligible covalent bonds required for DCC, the hydrazone ones (and by extension the acylhydrazone ones) attracted attention thanks to their reversibility and their biocompatibility^{5,6} More, polymers made of acylhydrazone bonds have been found to have very interesting properties, by being able to undergo monomer exchanges through adaptation to the medium according to DCC. These properties can lead to relevant therapeutic molecules, ion probes, self-healing films, or dynamic polymers.^{5,7,8}

40 However, one of the major limitations for the elaboration of acylhydrazone-based (macro)molecules is their poor water-solubility. To circumvent this limitation, many synthesis strategies have been developed. They are different whether the (macro)molecule bears a single acylhydrazone bond or multiple bonds (Figure 1). This distinction will be explained in further parts, in which synthesis strategies of single-bond and multiple-bond acylhydrazone (also named polyacylhydrazones) will be discussed, as well as their associated applications. 45 But before exploring this, an introduction about this particular acylhydrazone function appears to us as pertinent. To this end, a description about its formation, its dynamic behavior, the catalysis of its formation, and a portfolio of (macro)molecular structures implying acylhydrazone bonds from organic solvent will be presented.

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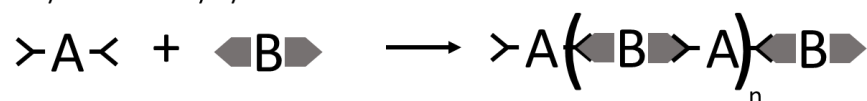


1) Single-bond acylhydrazone



2) Multiple-bond acylhydrazone (polyacylhydrazones)

Polymers with acylhydrazone functions in the main chain



Polymers with acylhydrazone functions in the side chains

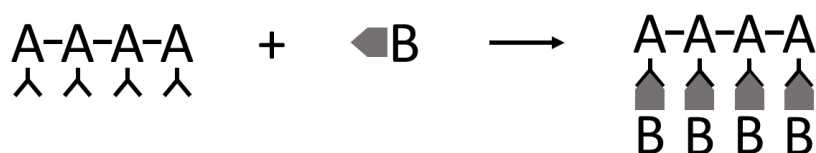
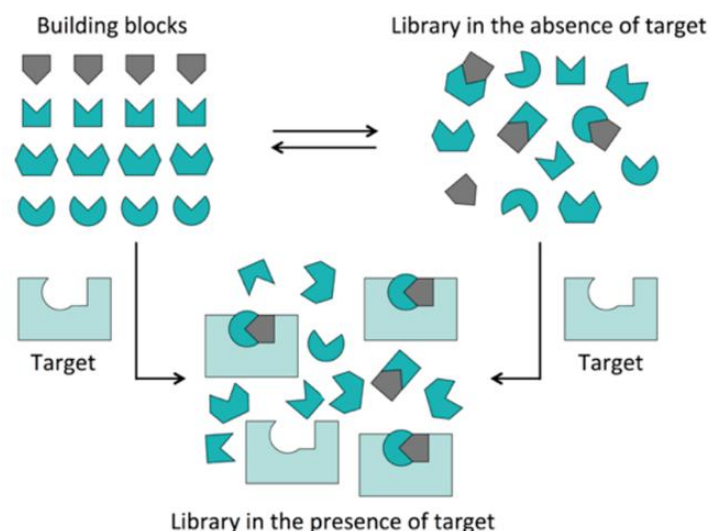


Figure 1 - Schematic representation of the acylhydrazone function synthesis from hydrazide and aldehyde building blocks, leading to 1) single-bond or 2) multiple-bond acylhydrazones.

55 1. Acylhydrazone function

1.1. Description of the acylhydrazone function

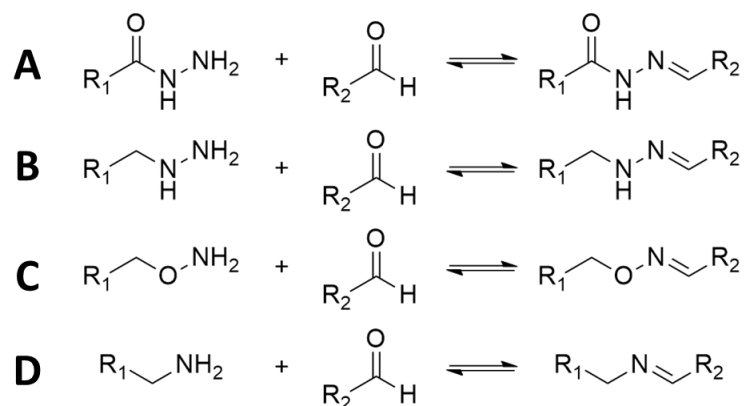
Dynamic bonds (covalent or non-covalent) are known to continuously form and break, depending on the conditions of the medium. Perpetrated by reversible bonds, a dynamic library of molecules can be created by a thermodynamic equilibrium between building blocks, where the most stable constituents are in larger quantities. This equilibrium can then be shifted through the addition of external *stimuli* (e.g. pH, temperature, external molecule). Hence, the amplification of some species informs about the products having the higher affinity toward the stimuli (Figure 2). The use of these reversible-bond building blocks to create polymers has also been exploited. The resulting dynamic polymers, whose length, constitution, and supramolecular arrangement strongly depend on the medium, are called *dynamers*. DCC of molecular or macromolecular species is of prime interest in many fields such as the drug discovery area.^{4,6,9-11}



70

Figure 2 - Schematic representation of a DCC process and the reversible equilibrations of a building block library, depending on the presence of the target. From Frei *et al.*⁹

Among the eligible dynamic functions to be used in DCC (which can be turned into
 75 supramolecular or covalent bonds), the aim of this review is to focus on the very interesting
 and well-known acylhydrazone family. Acylhydrazones can be synthesized through
 condensation between hydrazide and aldehyde moieties, displaying reversibility under mild
 conditions with acid catalysis. The condensation reaction to get acylhydrazones is presented
 on Figure 3, together with other imine bond types.^{4,11-15}



80

Figure 3 - Condensation leading to different imine-type bonds. A) Hydrazide and aldehyde giving acylhydrazone. B) Hydrazine and aldehyde giving hydrazone. C) Aminoxy and aldehyde giving oxime. D) Amine and aldehyde giving imine.

85 Compared to other C=N imine-type dynamic bonds, such as imines, oximes or
 hydrazones, acylhydrazone functionality displays many attractive features, having the
 advantages of both imine and amide groups.^{5,6} Indeed, hydrogen bonds can be performed

thanks to C=O and NH groups, while C=N group provides reversibility. Furthermore, contrary to other types of imines, hydrazones are stable enough thanks to an enhanced electronic density on the carbon to analyze the resulting products.^{9,16} Hydrazones are also less affected by hydrolysis due to their extra nitrogen (enhancing the stability in water by making the “imine” nitrogen less able to be protonized).¹⁷ Finally, an additional electron-withdrawing group such as an acyl group (compared to oximes and hydrazones) makes the resulting acylhydrazones not too stable to be interestingly used in dynamic libraries. It also increases the possibility of hydrolysis, which can be an asset in some situations.^{2,14,18}

The understanding of acylhydrazone hydrolysis is very important since it leads to the reversibility of the reaction. The mechanism of hydrazone hydrolysis was thoroughly studied by Yildiz¹⁹ through DFT calculations. The resulting conclusions are that the hydrazone (and by extension acylhydrazone) undergoes a water nucleophilic addition to give carbinolamine as intermediate species. Then, this carbinolamine is decomposed into hydrazide and carbonyl without any intermolecular proton transfer (Figure 4). The rate-determining step is the dissociation of the intermediate, but it was also demonstrated that the rate of hydrolysis depends on the ease of protonation of the “imine” nitrogen.¹⁶

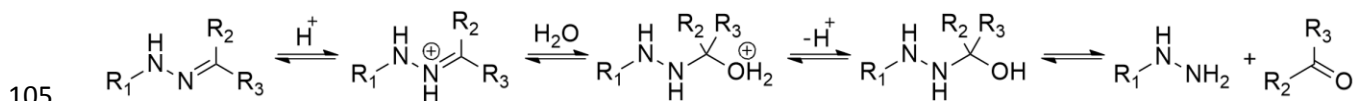


Figure 4 - Hydrolysis mechanism of hydrazone, proposed by Yildiz.¹⁹

Note that another synthetic pathway was explored by Haugen *et al.*²⁰, which implies a ketone linked to an electron-withdrawing group, and diazene compounds, catalyzed by a phosphine (Figure 5). The yields were found to be correct to excellent, depending on the reactants. Because the reaction conditions are harsh (THF, -78 °C to rt in 1h), this way of synthesis will not be explored in this review.

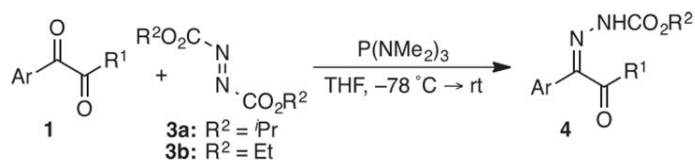


Figure 5 - Alternative acylhydrazone synthesis pathway, from Haugen *et al.*²⁰

Another remarkable property of the hydrazone bond (and by extension acylhydrazone) is its photosensitivity. Indeed, the configuration of its C=N double bond can be reversibly

switched from (*E*)-isomer to (*Z*)-isomer upon UV irradiation (365 nm).²¹⁻²⁴ More, it was showed that UV irradiation can also cleave the acylhydrazone bond, leading to photodegradable polymers.²⁵

The “classic” synthesis of acylhydrazones, from aldehyde and hydrazide, is well known to be very slow in neutral medium. Therefore, many works have been focused on the ways to increase this reaction rate and will be presented in the next part.

1.2. Catalysis of the acylhydrazone formation

Acylhydrazone function displays a reversibility property under acid catalysis, at mild conditions.^{2,5} This reversibility (also reachable by heating^{5,26}), allows hydrazide or aldehyde exchange (Figure 6B). Depending on the chemical nature of the molecule, the pH range of this reversibility state can vary from 3 to 7.²⁷ The rate of this exchange is indeed significantly decreased at $\text{pH} > 7$,²⁷ making the acylhydrazone a convenient and easily switchable bond through pH. This acid-sensitive property includes the possibility of developing pH-sensitive systems, usable for drug or fragrance delivery through the breaking of this acylhydrazone bond and the release of the drug under specific conditions.^{25,28-30} The mechanism of the hydrazone formation explaining this pH-catalysis is shown on Figure 6A. Fulton’s group focused on the mechanistic difference between the acylhydrazone formation (from aldehyde and hydrazide) and the exchange (between two different acylhydrazones), because the rate-determining step is, respectively, the β -elimination of the water and the nucleophilic attack on the protonated hydrazone.³¹⁻³⁵

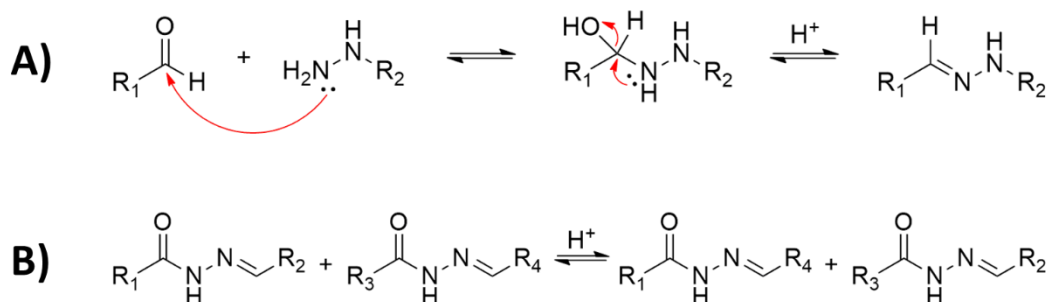


Figure 6 - A) Mechanism of the hydrazone formation, according to Jencks and Kool.^{31,32}
B) Exchange properties of acylhydrazone in acidic medium.

Consequently, for dynamic biomedical applications that imply building blocks exchange, acylhydrazones are hardly usable in neutral biological buffers ($\text{pH}=7.4$) without

catalysts, because of their low reversibility at this pH value. To enhance the acylhydrazone exchange velocity, the use of a trans-amination catalyst was developed, by using aniline (Figure 7).³⁶⁻³⁸ The observed second-order rate constant at biological pH (7.4) was found to be around 14-fold higher for aniline than for uncatalyzed reaction.³⁹

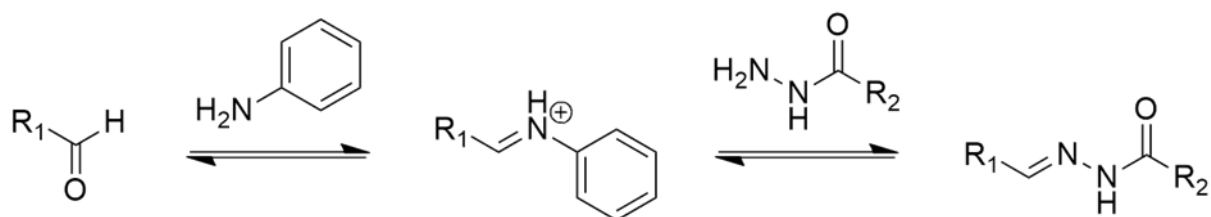


Figure 7 – Mechanism of the formation of acylhydrazone using aniline as a catalyst.³⁶

Domaille and Cha used DNA-anchored aniline to catalyze a DNA-templated acylhydrazone reaction hydrazide and aldehyde, in physiological conditions (Figure 8).⁴⁰

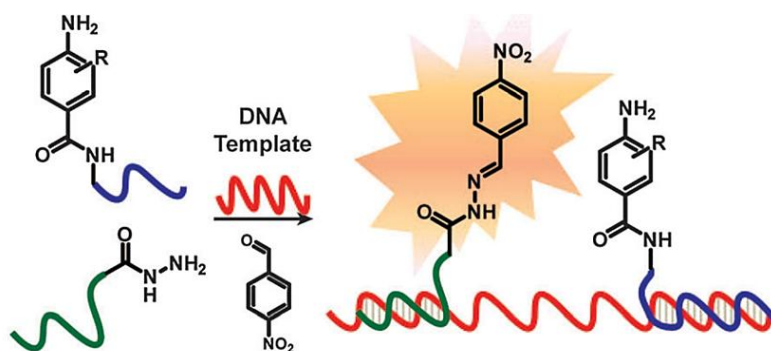


Figure 8 - DNA-anchored aniline for the catalysis of the condensation between DNA-termination hydrazide and aldehyde.⁴⁰

Nevertheless, the use of aniline leads to many problems. Firstly, accelerating the reaction to reasonable time periods requires large amounts of catalyst (up to 10 000 eq). Secondly, aniline is toxic towards cells, which can be a problem if the targeted system is living.^{36,39} Finally, it was shown that the catalytic activity of aniline depends on the reactants, and therefore can become a bias for isoenergetic dynamic libraries.⁴¹

Based on these observations, Blanden *et al.*⁴² found that 4-aminophenylalanine (Figure 9B) was almost as effective as aniline, but with a superior compatibility with biological systems. Moreover, the reaction temperature can be at 0°C, which can be an asset in some biological experiments. Kool's group^{39,43} then tried to improve the catalyst design based on aniline, for hydrazone formation and exchange instead of acylhydrazone, and have

demonstrated that anthranilic acids can be a new class of catalysts for these functions. Particularly, 5-methoxyanthranilic acid (Figure 9C) and 5-methyl-2-aminobenzene phosphonic acid (Figure 9D) showed a second-order rate constant over 6-fold and 8-fold, respectively, higher than the aniline's one. This new class of catalysts is likely to be less toxic than aniline.

Other types of catalysts have been studied at neutral pH, such as an artificial enzyme featuring an aniline residue.⁴⁴ More, an indoline-based catalyst, instead of an aniline-based one, has shown encouraging results, and the most performant one showed a 15-fold rate increase *versus* aniline (Figure 9E).⁴⁵ Morales *et al.*⁴⁶ used pyrrolidine (10 mol%) as a catalyst in water, obtaining a high yield, and therefore paving the way for more green-chemistry-compatible catalysts (Figure 9F).

Larsen *et al.*⁴⁷ developed a series of aqueous buffers that can also play the role of catalysts. The most performant molecules bear several amine groups, and reaction rates more than 200-fold rates of phosphate buffer alone were demonstrated. Furthermore, these catalyzing buffers showed a low toxicity, and therefore can be used for biological reactions of acylhydrazones.

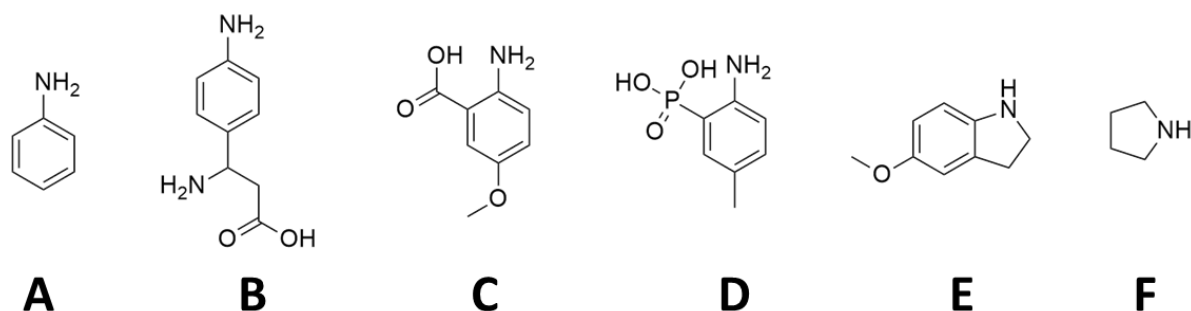


Figure 9 - Different generations of catalysts to increase the reaction rate in neutral conditions.

A) Aniline³⁶, B) 4-aminophenylalanine⁴², C) 5-methoxyanthranilic acid³⁹, D) 5-methyl-2-aminobenzene phosphonic acid⁴³, E) 5-methoxyindoline⁴⁵, F) Pyrrolidine⁴⁶.

Since the use of an additional molecule as a catalyst can add complexity to the acylhydrazone reaction, Kool's group has developed a system of autocatalysis. Indeed, in order to accelerate the reaction rate at neutral pH, the design of hydrazines and aldehydes was tuned. It was shown that when these molecules bear a neighboring acid/base group, the formation of hydrazones is significantly accelerated at biological pH, if the molecule's pK_A is close to the solution's pH. Furthermore, electron-deficient hydrazines, such as hydrazides, react more slowly than electron-rich ones, explaining their enhanced reversibility.^{17,31,48}

200 Beside, Higgs *et al.*³⁵ concluded that the judicious position of hydrogen bond acceptors near the
carbonyl-derived moiety also increases the reaction-rate of the acylhydrazone formation. This
was also demonstrated by both Gois' and Gao's groups⁴⁹⁻⁵², who showed that 2-
formylphenylboronic acid (2-FPBA, Figure 10) promptly reacts with hydrazides, and allows a
205 rapid exchange of hydrazones at pH 7.0 which can be reversibly paused by raising the pH up
to 12.

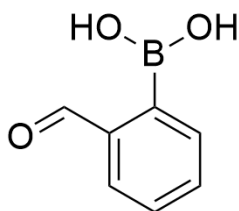


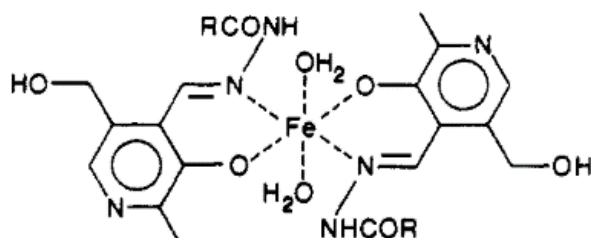
Figure 10 - Chemical formula of 2-formylphenylboronic acid, reacting with hydrazides with
a high rate (thanks to its ortho boronic acid moiety).

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1.3. Portfolio of (supra)molecular structures implying acylhydrazone bonds, from organic-solvent syntheses

Using acylhydrazone bindings' reversibility and supramolecular properties, the
215 elaboration and valorization of various innovative structures were described in literature.
These different structures can be either single- or multiple- acylhydrazone molecule. In this
part, the (supra)molecular structures prepared in organic solvent and insoluble in water will be
described, in order to show a portfolio of the applications of acylhydrazone bonds outside
aqueous environment.

220 A common valorization of acylhydrazone puts forward its ability to bind with metal
ions, as a Schiff base. These ligands are synthesized in organic solvents, and usable in
aqueous solvents. Acylhydrazone derivatives have been firstly used as iron-chelating
compounds.⁵³ Later, the same group synthesized acylhydrazones from three aldehydes and
fourteen hydrazides in order to find the most suitable ligands to eliminate iron from the body,
225 a consequence of long-term transfusion therapy. The structures of the complexes
[Fe(L)₂·2H₂O] were determined, and it was shown that the acylhydrazone plays a key role in
the complexation (Figure 11).⁵⁴



230 **Figure 11** - Example of an iron complex structure reached with acylhydrazone ligands, from Edward *et al.*⁵⁴

Ligands able to complex with Al(III)^{55,56}, Cu(II)⁵⁷⁻⁵⁹, Fe(II)⁵⁴, Zn(II)⁶⁰⁻⁶², Cd(II)⁶⁰, Co(III)⁶³ or Ag(I)⁶⁴ have been developed by several groups, for metal ions extraction or the
 235 formation of therapeutic complexes. Probes for the detection of anions have also been explored through acylhydrazone derivatives. For instance, a highly sensitive and highly specific probe have been developed to detect cyanide ions in water (Figure 12),⁶⁵ or to hypochlorite ions in water.⁶⁶ These molecular probes were synthesized in ethanol or DMSO medium and used in an aqueous solvent (with non negligible amount of organic co-solvent
 240 to ensure good solubility).

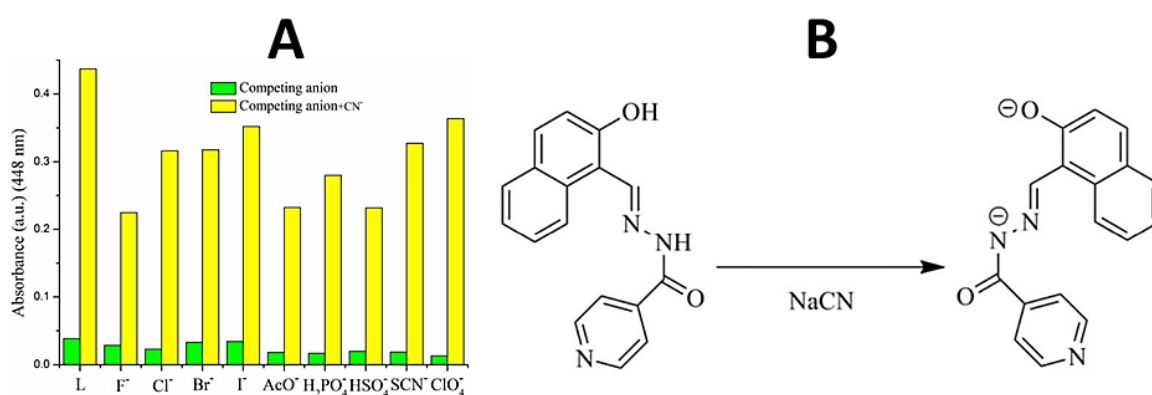


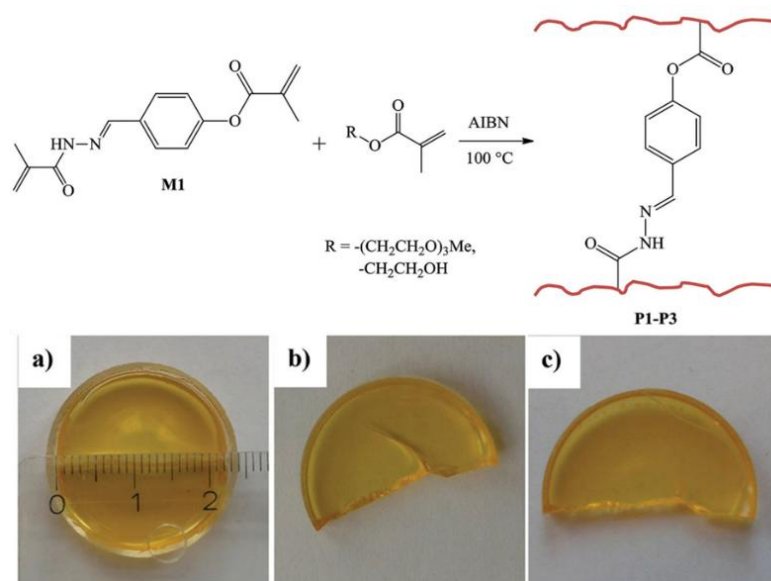
Figure 12 - (A) Absorbance in presence of CN⁻ and competing anions (L being the probe alone), and (B) the possible mechanism of the CN⁻ sensing. Adapted from Hu *et al.*⁶⁵

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An original application of acylhydrazones was proposed by Qi *et al.*⁶⁷, by tuning the fluorescent properties of a naphthyl-based acylhydrazone through fumigation by TFA or NEt₃. Such a property could have a possible outcome as a security ink.

As stated in the first part, one of the most common uses of acylhydrazone is in the
 250 field of polymers, and particularly in dynamic polymers, but other structures were obtained by

employing the acylhydrazone function, such as dendrimers⁶⁸, [2]-catenanes⁶⁹, organogels⁷⁰, and hydrogels⁷¹. Finally, acylhydrazone polymers can also be valorized as films, with self-healing properties (Figure 13),⁷² or tunable Young modulus and T_g .⁷³



255 **Figure 13** – Cross-linked film with self-healing properties. a) Polymer film, b) Film after
 260 scratch, c) Healed scratch after heating at 100°C for 64h. Adapted from Kuhl *et al.*⁷²

Copolymers adaptable to their environment thanks to their reversibility properties,^{11,26,74} or “optodynamers”, which are films displaying color and fluorescence by stacking with
 260 another one (Figure 14), were elaborated too.⁷⁵

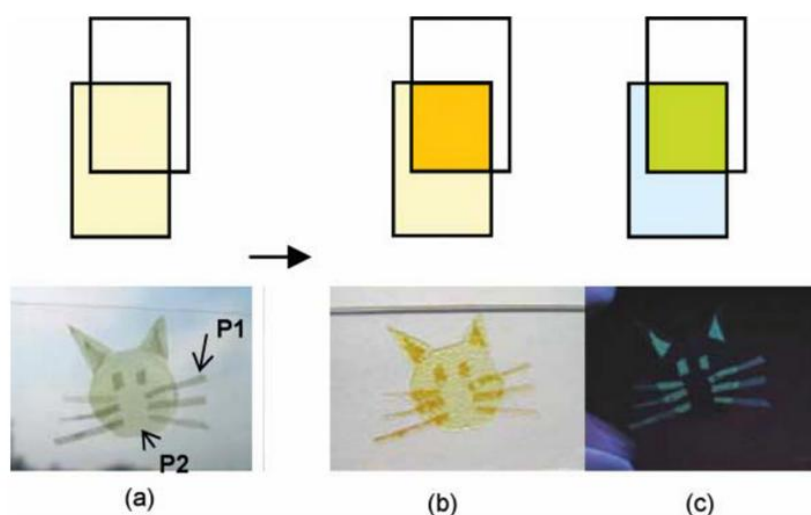


Figure 14 - Visual color and fluorescence change after heating superimposed polymer films (P1 and P2). a) color before heating, b) color after heating, c) fluorescence after heating (at 365 nm).⁷⁵

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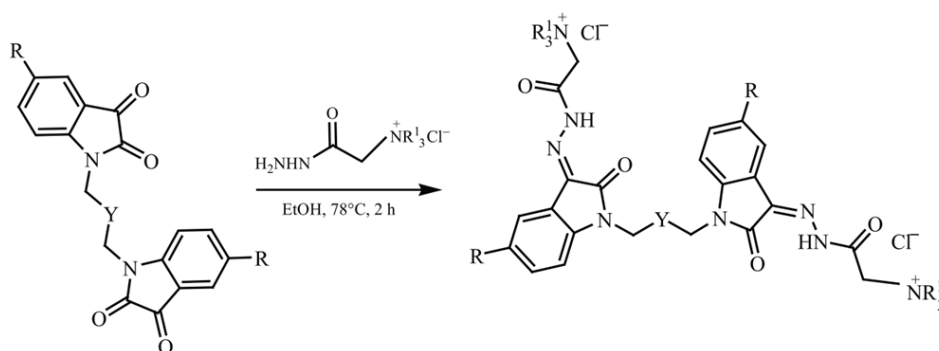
In the next two parts of this paper, different strategies to design i) water-soluble single-bond acylhydrazone dimers/oligomers, or ii) polyacylhydrazones will be presented, such as using appended moieties to help their solubilization, synthesizing the targeted
270 molecules in organic solvents (but with a water-solubility application), or using a mixed water/organic solvent.

2. Single-bond acylhydrazone synthesis strategies

As previously written, dimers of acylhydrazone (and by extension small oligomers)
275 are different from polymers not only because of their molar mass, but also their number of acylhydrazone bonds per species. Throughout the multiple syntheses and uses of water-soluble single acylhydrazone bonds, two approaches stand out. The first one is the synthesis in organic solvents, most often in ethanol or DMF, with a water-soluble purpose for the resulting species, such as metal complexation or therapeutic action. The second one is the
280 synthesis directly in water, or an organic/water solvent mixture, with the same goals.

2.1. Synthesis strategies in organic solvents with a water-soluble purpose

Despite being much less popular than other imines bonds (such as oximes) for bioconjugation, acylhydrazone have been used in this area in multiple ways by several
285 groups.¹⁷ Combining 1H-indole-2,3-dione (isatin) derivative and Girard's reagent, Bogdanov *et al.*^{76,77} successfully obtained water-soluble acylhydrazone from a reaction in refluxed ethanol. Later, they combined two isatin derivatives and performed the same reaction (Figure 15). Antimicrobial activity tests were carried out, and some compounds showed a high activity toward *S. aureus* and *B. cereus*, compared to a control drug. Hemolytic activity
290 experiments were also performed, and the same compounds were found to be less toxic than control drugs.



295 **Figure 15** - Reaction conditions of the acylhydrazone synthesis by the combination of two isatin derivatives. Y = alkyl chains of different lengths. R = H or Br. $\text{NR}_3^1 = \text{NMe}_3$ or Py.

From Bogdanov *et al.*⁷⁷

A good strategy to make biologically active acylhydrazone compounds is to dilute a DMSO solution of acylhydrazone compounds in water. It allows an easy synthesis in ethanol, while being then able to be active in water. Dos Santos Filho's group⁷⁸ synthesized an acylhydrazone by this way, having activity against the malaria parasite which is resistant to chloroquine, but with a $\log P > 2$. They studied the mechanism of action of the most active compounds. Similarly, some acylhydrazone derived from N-(arylsulfonyl)methionine showed a strong activity against Gram (+) bacteria, and some compounds particularly on *S. aureus*, *E. faecalis*, *L. monocytogenes*, and *B. cereus*.⁷⁹ Furthermore, an antifungal potential drug candidate was found against *C. neoformans* by tuning BIBM ((E)-N'-(3-bromo-6-hydroxybenzylidene)-2-methylbenzohydrazide), a previously-reported active acylhydrazone.⁸⁰ Finally, some acylhydrazones also led to antiviral compounds, as demonstrated by Hu *et al.*⁸¹ against tobacco mosaic virus, which led to more efficient compounds than the classic ribavirin. It is clear to say that the molecular structure (with the acylhydrazone in the central position) and the antimicrobial activity of all these compounds are more or less similar. Based on these results, another series of acylhydrazones (Figure 16) was synthesized in ethanol. It showed activity against many bacteria, virus and fungi, while having a moderate to low cytotoxicity.

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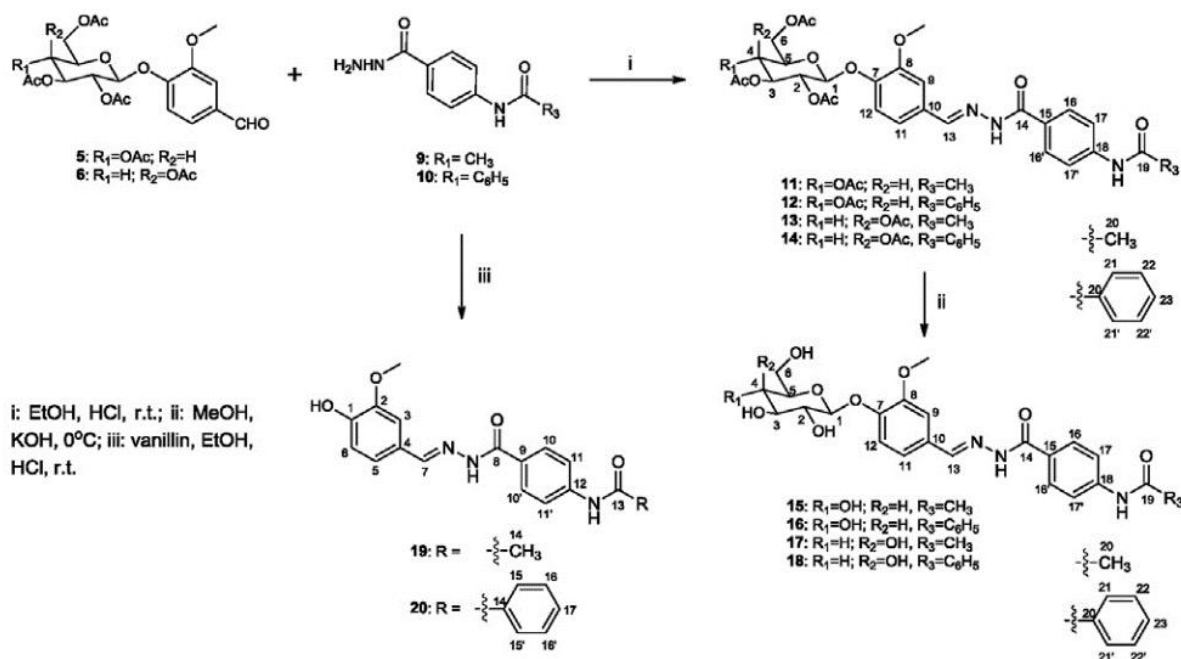


Figure 16 - Synthesis procedure of the biologically active acylhydrazone, from Guilherme *et al.*⁸²

320 Fraga's group^{83,84} designed active acylhydrazone based on the structure of preexisting drugs, giving birth to new active compounds, such as analgesics, or more recently, water-soluble potential inhibitors for cancer (based on trichostatin A). Similarly, Congiu and Onnis⁸⁵ designed an acylhydrazone derivative from two existing active compounds (Figure 17). The obtained molecule showed cytotoxicity against several cancer cells, while some
 325 compounds showed no sign of acute toxicity in mice. Interestingly, by tuning aryl decoration, a correlation was found between log P and the average activity parameter. Gupta *et al.*⁸⁶ also contributed to the rationalization of the acylhydrazone activity relation to the molecule design, by showing that by tuning the imine aryl decoration, the authors could increase the antimicrobial activity toward the considered bacteria. Indeed, if the substituted groups are
 330 electron-releasing, the activity increases towards *B. subtilis*, *E. coli*, and *A. niger*, whereas if they are electron-withdrawing, the activity increases against *S. aureus*.

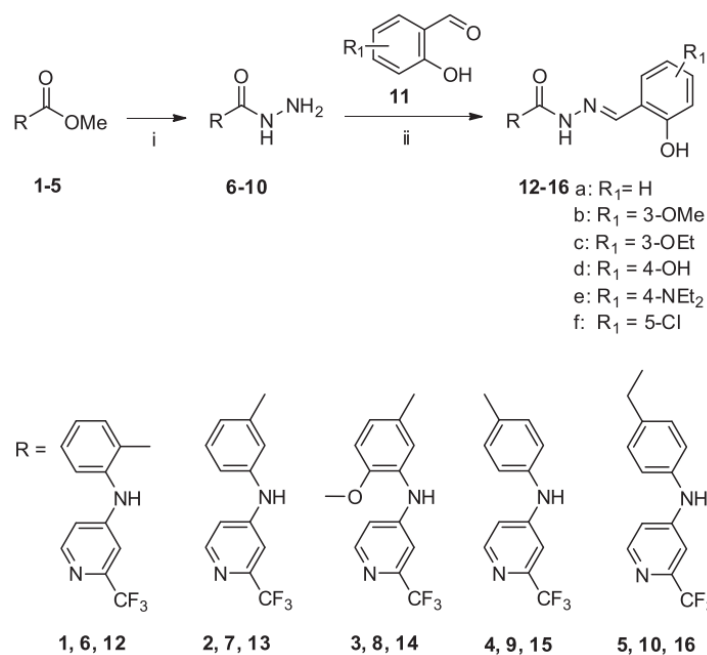


Figure 17 - Synthesis procedure of the tested compounds against several cancer cells, from
 335 Congiu & Onnis. Reaction conditions : (i) hydrazine hydrate, EtOH, reflux, 6 h ; (ii) EtOH,
 AcOH (cat), reflux, 2 h.⁸⁵

2.2. Synthesis strategies in aqueous medium

340 As for the syntheses in organic solvents, water-based syntheses can be used for the
 preparation of metallic complexes, leading to original DCLs. Goral *et al.*⁶³ elaborated
 compounds being “doubly-dynamic” due to their metal-ligand interactions and their
 acylhydrazone bonds. Exchange kinetic parameters (ligand or acylhydrazone) were measured
 at different conditions (pH, temperature, oxidation) in 30 % acetonitrile in 0.01 M aqueous
 NH₄OAc, at pH = 7. Later, a triple-DCL was created from guanine-based components,
 345 through metal-ion binding, hydrogen bonds, and acylhydrazone bonds (Figure 18). The latter
 was used as a gelation factor, allowing the formation of a quartet assembly of guanines
 complexed by the metal ion, and consecutively, a gel. Thus, the gelation had interestingly the
 ability to redirect the acylhydrazone distribution, and macroscopic properties depending on
 the cation and the acylhydrazone structure. These reactions were performed in sodium acetate
 350 buffer.⁸⁷⁻⁸⁹

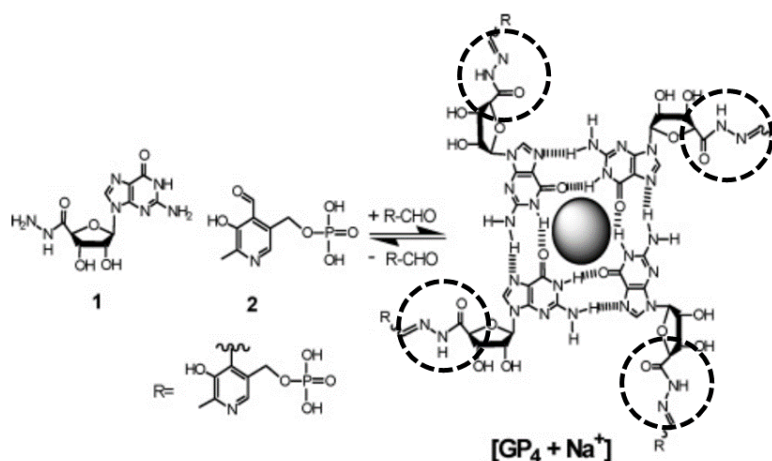


Figure 18 - Reversible synthesis of acylhydrazone bonds (highlighted in dashed circles),
 355 allowing a quartet assembly of the guanosine hydrazides thanks to the addition of a metal ion
 (represented by the gray sphere). Adapted from Bulher *et al.*⁸⁹

The synthesis of acylhydrazone bonds in water can also lead to biologically active
 compounds. Recently, Dascalu *et al.*⁹⁰ prepared hydrazone- and acylhydrazone-based species
 360 using only water as a solvent, from aldehydes, hydrazines, and hydrazides (Figure 19). Many
 of all the hydrazone compounds showed an antifungal activity against a lot of the tested
 strains. Interestingly, switching to acylhydrazone compounds enhanced the activity of some
 of them, comparably to marked drugs, but also narrowed the spectrum of activity to the tested
 strains, while showing no cytotoxicity.

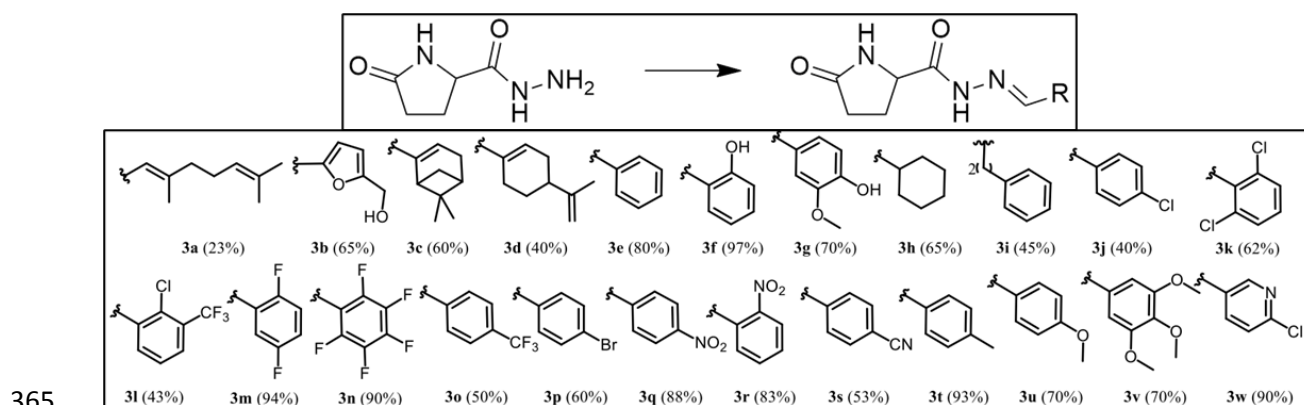
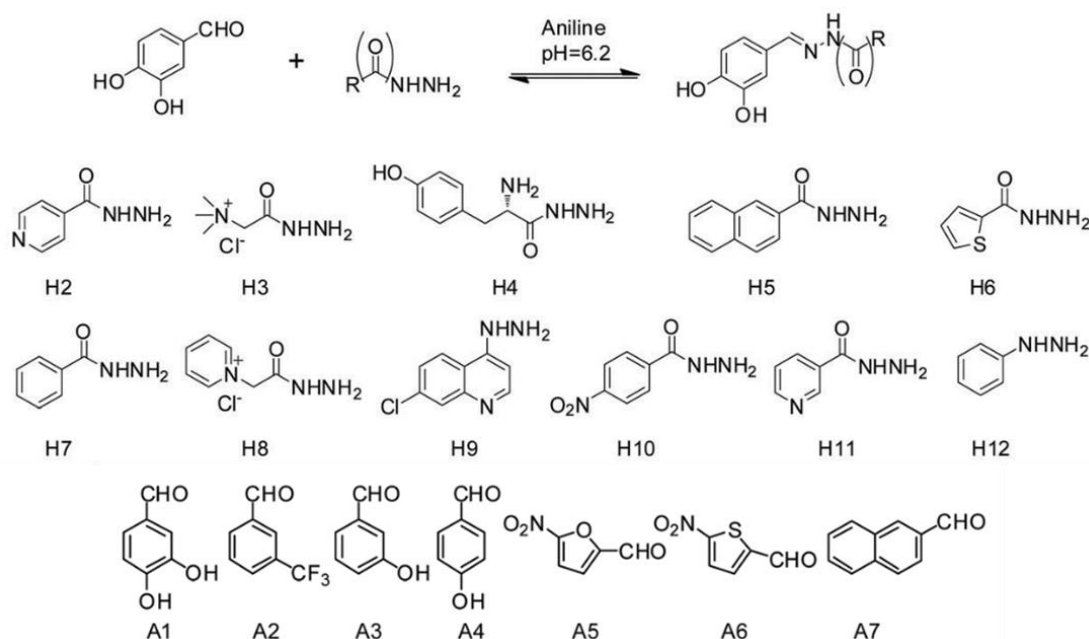


Figure 19 - Top: Synthesis of the acylhydrazone-based molecules, reaction steps and
 conditions: i) $\text{CH}_3\text{SO}_3\text{H}$, $\text{MeOH}/\text{CHCl}_3$, reflux, ii) hydrazine monohydrate, iii) aldehyde 1
 equiv., H_2O , rt, 1h. Bottom: R groups with reaction yields. Adapted from Dascalu *et al.*⁹⁰

370 Using the same components (aldehydes, hydrazines, and hydrazides) to create a DCL
 (Figure 20), Fu *et al.*⁹¹ screened the resulting acylhydrazones to inhibit the UDP-

galactopyranose mutase (UGM), an enzyme involved in mycobacterial cell wall biosynthesis. The reaction was carried out in an aqueous buffer (pH = 6.2), with aniline as a catalyst.



375

Figure 20 - Dynamic combinatorial library components for the identification of UDP-galactopyranose mutase inhibitors. The generation of the library was performed in an aqueous buffer, with aniline as a catalyst. Adapted from Fu *et al.*⁹¹

380

Using DCC, Hirsch's group^{92,93} synthesized libraries from five hydrazides and five aldehydes to determine an inhibitor of the endiothiapepsin enzyme, in ammonium acetate buffer (pH = 4.6). Some potent molecules were identified, also showing that the protease selectively binds to the E isomers of acylhydrazone-based compounds.

385

Using water as the major solvent in their syntheses, Vicini *et al.*⁹⁴ prepared bioactive acylhydrazone molecules, with an anti-HIV-1 activity. The molecules were synthesized from hydrophobic aldehydes and water-soluble hydrazides in a water/ethanol (7:3 v:v) solvent, precipitated, filtered and washed with water. However, note that for the bioactivity studies, they were finally dissolved in DMSO and diluted in water.

390

Lehn's group^{95,96} prepared DCLs of building blocks (hydrazides, monoaldehydes, and dialdehydes), obtaining water-soluble species. The synthesis solvent was a sodium acetate buffer (pH = 4) with the addition of CH₃CN or DMSO to increase the solubility of some compounds. They were prepared from bioactive building blocks, and led to potent enzyme inhibitors, after the templating of the dynamic equilibrium (for acetylcholinesterase and HPr kinase enzymes). They later used the same approach, with DCLs prepared from sugar-based

395 aldehydes, hydrazides, dihydrazides, and trihydrazides for the screening and the identification
of active compounds able to bind to a carbohydrate-binding protein, Concanavalin A (Figure
21). Because of the sugar-based structure of the aldehydes, the building blocks and the
resulting acylhydrazone species could easily be solubilized in an ammonium formate buffer
400 solvent.^{27,97}

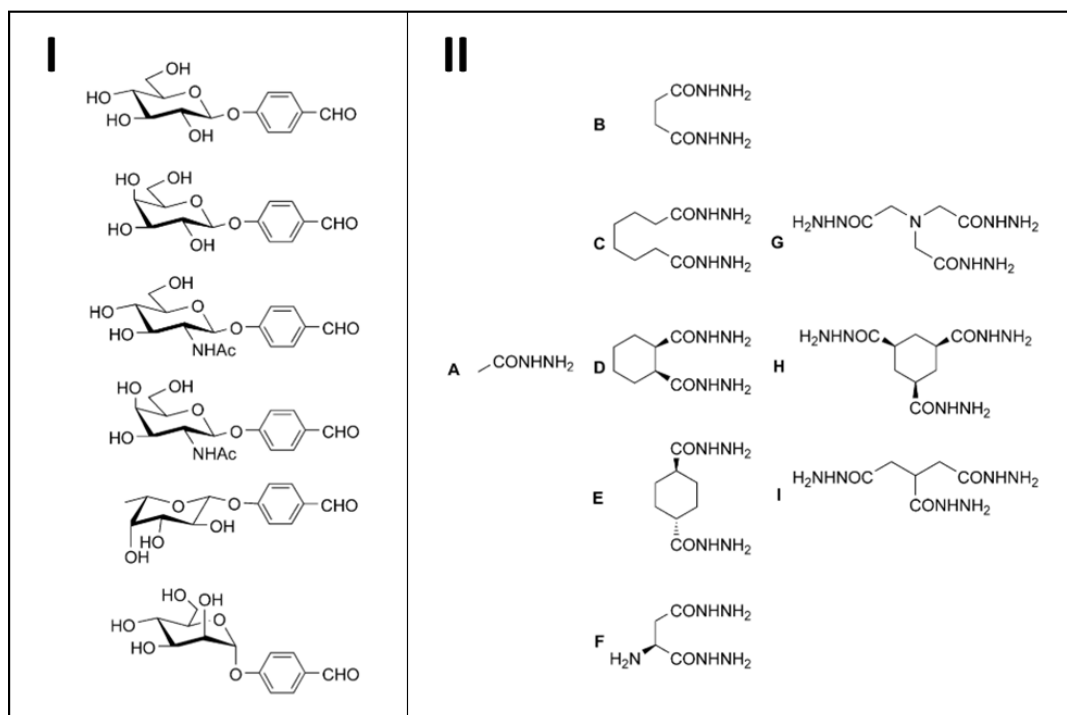


Figure 21 - Building blocks of the DCL generated in an ammonium formate buffer (pH = 4) without the use of an organic solvent. I - Sugar-based aldehydes. II - Mono-, di- and trihydrazides. Adapted from Ramström *et al.*²⁷

405

Greaney *et al.*^{98,99} prepared a DCL of acylhydrazones thanks to aldehydes and dihydrazides, in order to identify a novel enzyme inhibitor. The synthesis was performed in a mixture of DMSO/ammonium acetate buffer (pH = 6.4, 15/85 v/v), and a potent species was amplified after the introduction of the enzyme in the dynamic equilibrium. Similarly, Poulsen¹⁰⁰ generated a DCL from different aldehydes and hydrazides compounds, either *in situ* with the enzyme as a template in NH₄OAc buffer and 1 v% DMSO, or from a preformed equilibrium established in pure DMSO with acidic resin, and diluted to reach the same final conditions as the *in situ* DCL with the protein. The analysis tool used to characterize the DCL
415 was the mass spectrometry (LC-MS) coupled to NMR, providing a faster screening time.

Through their studies on the catalysis of the acylhydrazone formation, Kool and Crisalli^{17,31,39,43,48} explored their synthesis from aldehydes and hydrazides. The main motivation of their work was to successfully prepare acylhydrazones in a neutral aqueous medium. So, they used a phosphate buffer saline (PBS, pH = 7.4) in all their experiments, with the addition of 10 v% of DMF to ensure a good solubilization of reactants and products.

Similarly, the other groups working on improving the catalysis of the acylhydrazone formation in water used water-soluble building blocks, and syntheses were made at room temperature in aqueous buffers. The pH of these buffers (phosphate or acetate) varies from 4.5 to 7.4. The addition of 10 and 20 v% DMF was necessary for Zhou *et al.* and Trausel *et al.*, respectively, to improve the solubilization of both building blocks, and resulting compounds.

The use of acylhydrazone as a tool for time-controlled release of fragrance was explored by Levrant *et al.*^{11,30} by binding a water-soluble hydrazide to a highly volatile aldehyde (Figure 22). The synthesis was carried out in a water/ethanol mixture (2/1, v/v), the aqueous phase being phosphate (pH = 2.47) or citrate (pH = 4.48) buffers.

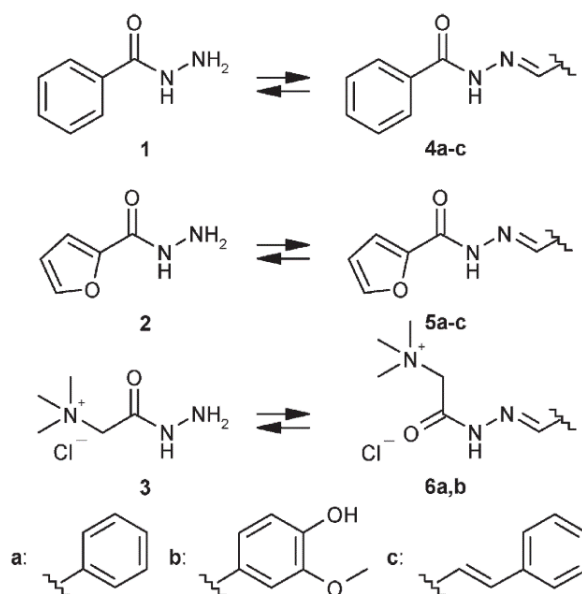


Figure 22 - Structure of acylhydrazones obtained by the reaction of hydrazides 1-3 with volatile aldehydes a-c. The reactions were carried out in a water/ethanol mixture (2/1, v/v) at acidic pH. Adapted from Levrant *et al.*³⁰

435

3. Multiple-bond acylhydrazones synthesis strategies

Similarly to single-acylhydrazone molecules, polymers constituted of several acylhydrazone bonds display reversibility in their initial reactants in acidic conditions, and kinetical freeze under basic conditions. This property allows these kinds of polymers to be dynamic, giving “dynamers”. The features of polyacylhydrazones are i) high yields in mild conditions, ii) dynamic covalent polymers, and iii) many hydrogen bond interactions. Lehn^{2,101} describes such polymers as “reversible polyamides”, because of the similarity between the functions, but also because of their poor solubility in classic organic solvents and water, which complicates their synthesis and characterization.

According to literature, polyacylhydrazones can be classified in three categories. The first one is based on synthetic polymers with acylhydrazone functions in the main chain, obtained from either dihydrazides and dialdehydes, or monomers carrying both hydrazide and aldehyde moieties. Focusing on this kind of water-soluble polymers, a differentiation of their synthesis solvent (in organic or aqueous solvents) seems to be relevant in this review. Indeed, because of the intrinsic poor water-solubility of such polymers, the employed strategies will be different in those cases.^{102,103}

The second category corresponds to synthetic polymers with acylhydrazone functions in their side-chains. They are basically (water-soluble or not) polymers bearing multiple pending aldehydes or hydrazides moieties, and able to undergo exchange with building blocks decorated with the complementary function, thermodynamically reaching an equilibrium.^{102,103}

A third category might as well be considered, not by its polymeric structure (*i.e.*, the location of acylhydrazone function in the polymer), but by its composition. Indeed, this polymer family can bear the acylhydrazone functions in the main or side chains, but they are composed of water-soluble bio-based components, such as carbohydrates, amino acids, or nuclei acids. Thus, they can have interesting properties and many advantages compared to “classic” synthetic dynamers or biopolymers.^{68,104} These three categories will be successively detailed in the next paragraphs.

3.1. Synthetic polymers with acylhydrazone functions in the main chain

Synthetic polyacylhydrazone with the functions in the main chain have several advantageous intrinsic structural properties compared to polymers whose acylhydrazone functions are located in the side chains. Beyond its dynamic behavior that was described above, it can be a homopolymer with a repeating unit bearing aldehyde and hydrazide

functions, but also a copolymer with different repeating units bearing aldehyde or/and hydrazide functions. An interesting feature can be found when the repeating units are either dialdehydes or dihydrazides, because the resulting polymer is strictly alternating.¹⁰¹

475 These water-soluble polyacylhydrazones can be prepared in an organic solvent for more convenient synthesis and purification, then diluted or dissolved in water (with the aim of using them in bioactivity applications). They can also be directly synthesized in water when the solubilities of both the monomers and the resulting polymer are high enough. Note that some syntheses, as described below, use organic co-solvents to enhance the solubility.^{5,101}

480 3.1.1. Synthesis strategies in organic solvents

The first polyacylhydrazone from dihydrazides and dialdehydes to our knowledge was synthesized by Michel and Murphey in 1963.¹⁰⁵ They reported many successful polymerizations in HMPA, DMF or DMSO, but struggled to re-dissolve the polymers once isolated from the solvent.

485 More recently, Cousins *et al.*¹⁰⁶ designed in 1999 one of the first DCLs based on these types of linkages, by mixing together four species in CH₂Cl₂ (Figure 23). Through ESI-MS characterization, they observed all the combinations possible (more than 130 species) up to undecamers, with mainly cyclic compounds. The catalysis was performed with drops of TFA (also used for *in situ* deprotection of the aldehydes), and NEt₃ was chosen to freeze the
490 equilibrium. Interestingly, some species were found to be amplified by the addition of [18]crown-6, while the original distribution could be regenerated by adding KBr (3eq), which compete with the library components of the crown ether.^{4,107} Later, an equilibration of the same components with templates such as quinine, cinchonine, quinoline or quinuclidine led to surprising results. Indeed, diastereomeric templates such as quinine and quinidine led to a
495 distribution of different oligomer macrocycles, amplifying the tetramer and the dimer respectively.¹⁰⁸ Lam *et al.*⁶⁹ prepared a DCL from the PFm building block, and used acetylcholine as a template. Instead of giving a library made of macrocycles, as stated before, [2]-catenanes were obtained. As a matter of interest, only one diastereomer emerged. These results confirmed that chiral templates can differently modify DCL, and non-chiral templates
500 can lead to chiral supramolecular structures.⁶⁹

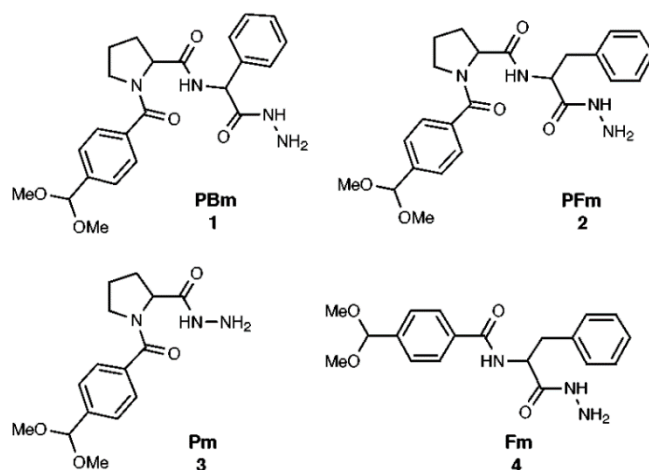


Figure 23 - Monomers used by Sanders' group for the generation of their DCL.¹⁰⁶

505 Similarly, Voshell *et al.*¹⁰⁹ showed that a DCL made of two racemic compounds with the addition of a chiral template, (-)-adenosine, can result in the amplification of only one diastereoisomer species (Figure 24). In this study, the cyclic dimer (S,S) was amplified with an enantiomeric excess of 21% when templated by the (-)-adenosine, while no enantiomeric amplification was observed for the untemplated dynamic library.

510

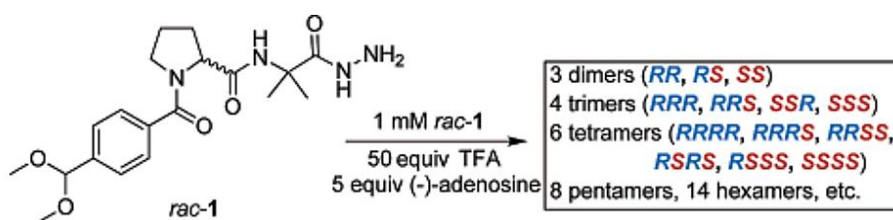


Figure 24 - Racemic DCL, yielding in a distribution of cyclic oligomers. The addition of (-)-adenosine amplified the dimers proportions over the untemplated distribution, and the SS dimer was found with an enantiomeric excess of 21%.¹⁰⁹

515

Lehn's group⁵ synthesized polyacylhydrazones from seven different monomers in DMSO or DMF, using drops of AcOH or TFA as catalyst. They studied the influence of temperature and time on the polymer formation or exchange rate. They also reported their high insolubility in commonly used organic solvents, because of the high number of hydrogen bonds occurring both intra- and inter-molecularly. According to Lehn's group, only high-boiling-point solvents able to break these hydrogen bonds, such as DMSO or DMF, can solubilize them with help of heating.

Ulrich's group prepared polyacylhydrazones from dihydrazides and dialdehydes, carefully designed to be able to complex plasmids, and vectorize them up to cell nuclei (Figure 25). The polycondensation was performed in methanol. It was demonstrated that the smaller macrocycles are preferentially formed at low concentration, whereas the linear polymers can be found at high concentrations. These dynamers effectively complexed dsDNA in aqueous buffers at biological pH.¹¹⁰

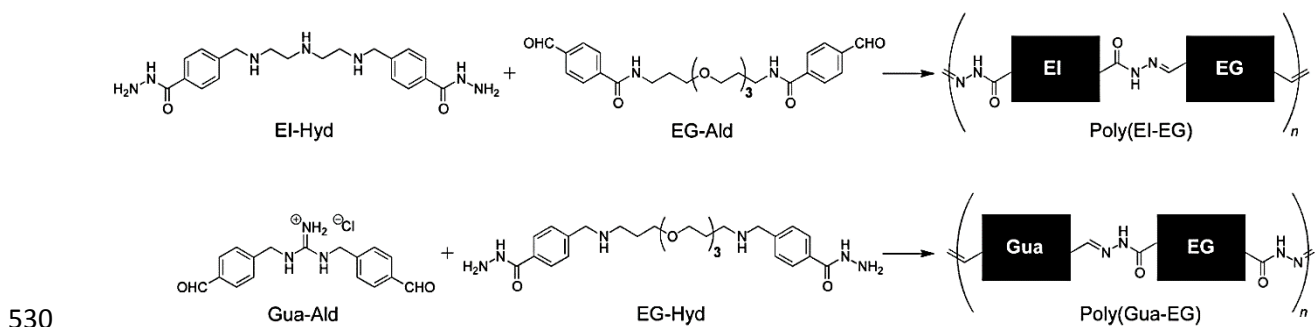


Figure 25 - Monomers synthesized by Ulrich et al¹¹⁰, resulting in dynamers able to complex plasmids and vectorize them up to cell nuclei.

3.1.2. Synthesis strategies in aqueous solvents

535 Furthering Michel and Murphey's work, Roberts and Thomas¹¹¹ synthesized polyacylhydrazones in DMF/H₂O (1/1 v/v) at room temperature, from carbohydrate-based hydrazides and dicarbonyl compounds (Figure 26). The obtained polymer from compounds 1a and 2,3-butanedione was insoluble in common solvents (except DMSO, boiling DMF, or water), but the addition of adipic dihydrazide to form a random copolymer led to water-

540 soluble polymers at room-temperature.

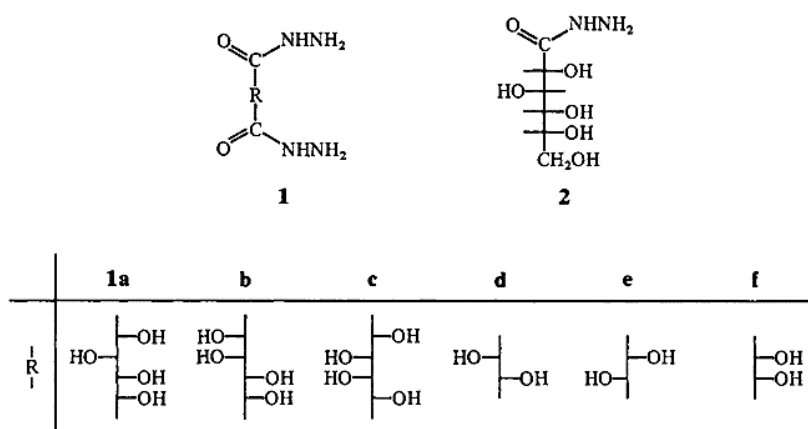


Figure 26 - Carbohydrate-based hydrazides and dihydrazides used for the synthesis of Roberts and Thomas' polyacylhydrazones.¹¹¹

545 Beeren *et al.*¹¹² performed polyacylhydrazone exchange reaction in aqueous buffer (carbonate, pH = 3). They studied the kinetical trap that macrocycles can create over linear species, making monomer exchange very slow when macrocycles are mixed together. Nevertheless, they also demonstrated that the addition of 4-hydroxybenzhydrazide can overcome these potential wells, and lead to an equilibrated final distribution.

550 By using amphiphilic dialdehydes and dihydrazides, Frantz Folmer-Andersen *et al.*¹¹³⁻¹¹⁵ synthesized polyacylhydrazones in water, at pH = 2.0 (Figure 27A). These dynamers have the property to self-assemble into rods, because of hydrophobically-driven folding. The nucleation-growth effect can be observed, as hydrophobic interactions (and π -stacking) favor the addition of a monomer to the end of the polymer over the formation of smaller species. 555 More, the addition of co-solvents such as DMF or ACN prevents the formation of these secondary helical rod-like conformations. Surprisingly, the increase in temperature leads to an increase of the molar mass of the polymers (Figure 27B), and if the temperature is below LCST, the rod-like conformation is conserved. If the temperature is above LCST, the aggregation of polymers becomes too rapid, and large aggregates made of intertwined rods are created. 560 are created.

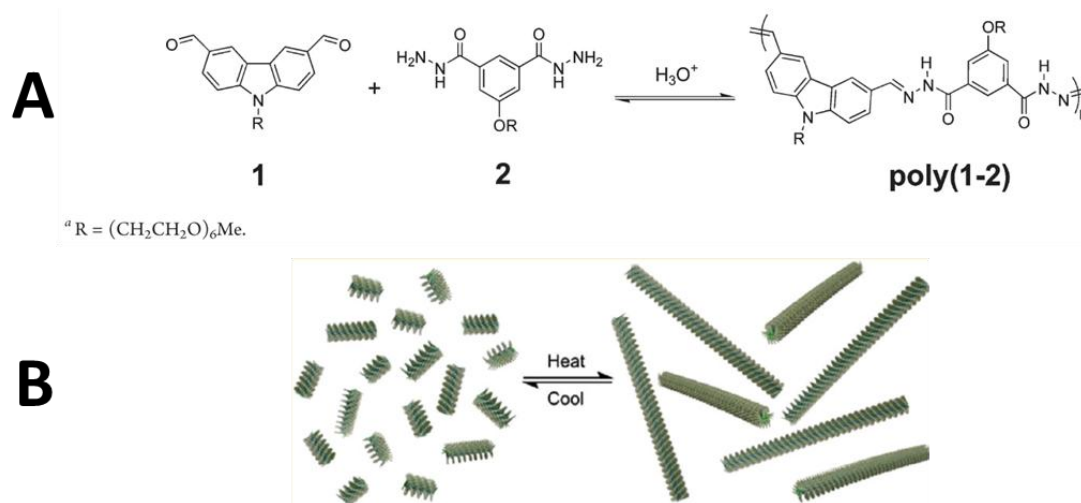


Figure 27 - A) Polymerization of dialdehyde 1 and dihydrazide 2. B) The heating of the rod-like structures (below LCST) reversibly aggregates them bigger rods. Adapted from Folmer-Andersen *et al.*¹¹⁶

565 Very recently, Psarrou *et al.*²⁵ made a water-soluble polyacylhydrazone from adipic dihydrazide and a dialdehyde containing poly(ethylene glycol) segment between phenyl groups (Figure 28A). The synthesis was interestingly performed only in MilliQ water (with

drops of HCl) at 25 °C, yielding in a 28,000 g.mol⁻¹ copolymer. Then, doxorubicin was added and attached to the hydrazide end-group thanks to its carbonyl moiety. These polymers were found to have a self-assembling behavior into spherical nanoparticles, which can be disrupted through the addition of acid (pH = 2.0) or light irradiation (254 nm, 0.1 mW.cm⁻¹). These two *stimuli* can be combined in a synergistic manner to accelerate the disruption even more (Figure 28B). These experiments demonstrated that a hydrophobic drug, such as doxorubicin, can be covalently linked to a water-soluble polymer, and can be released under particular conditions at a useful location, showing the vector role potential of polyacylhydrazones.

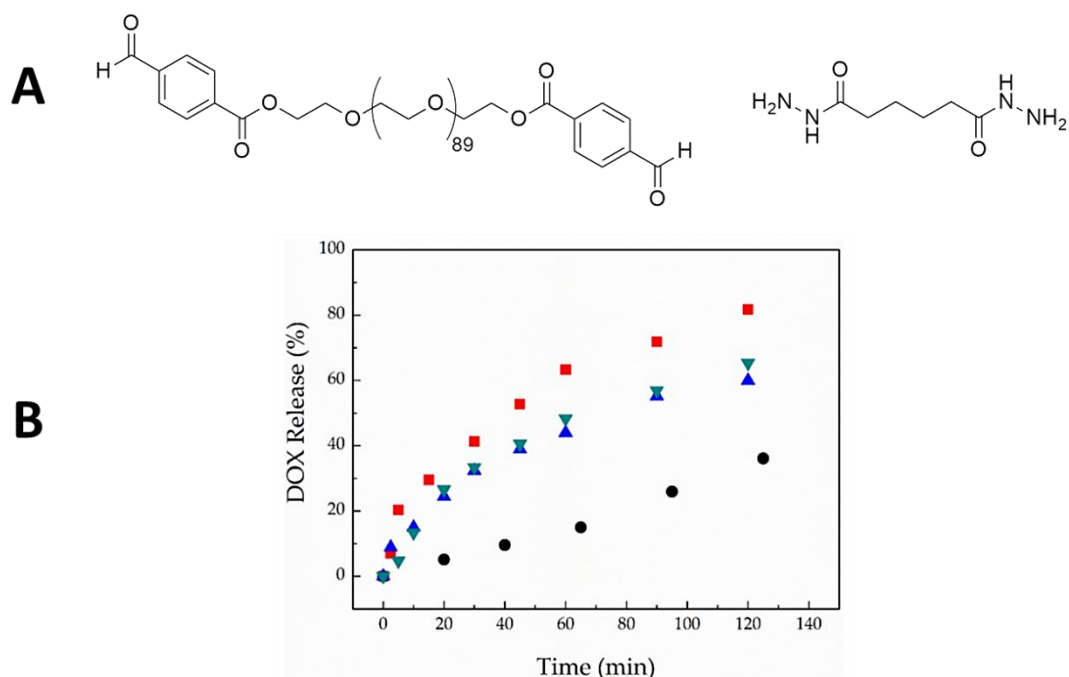
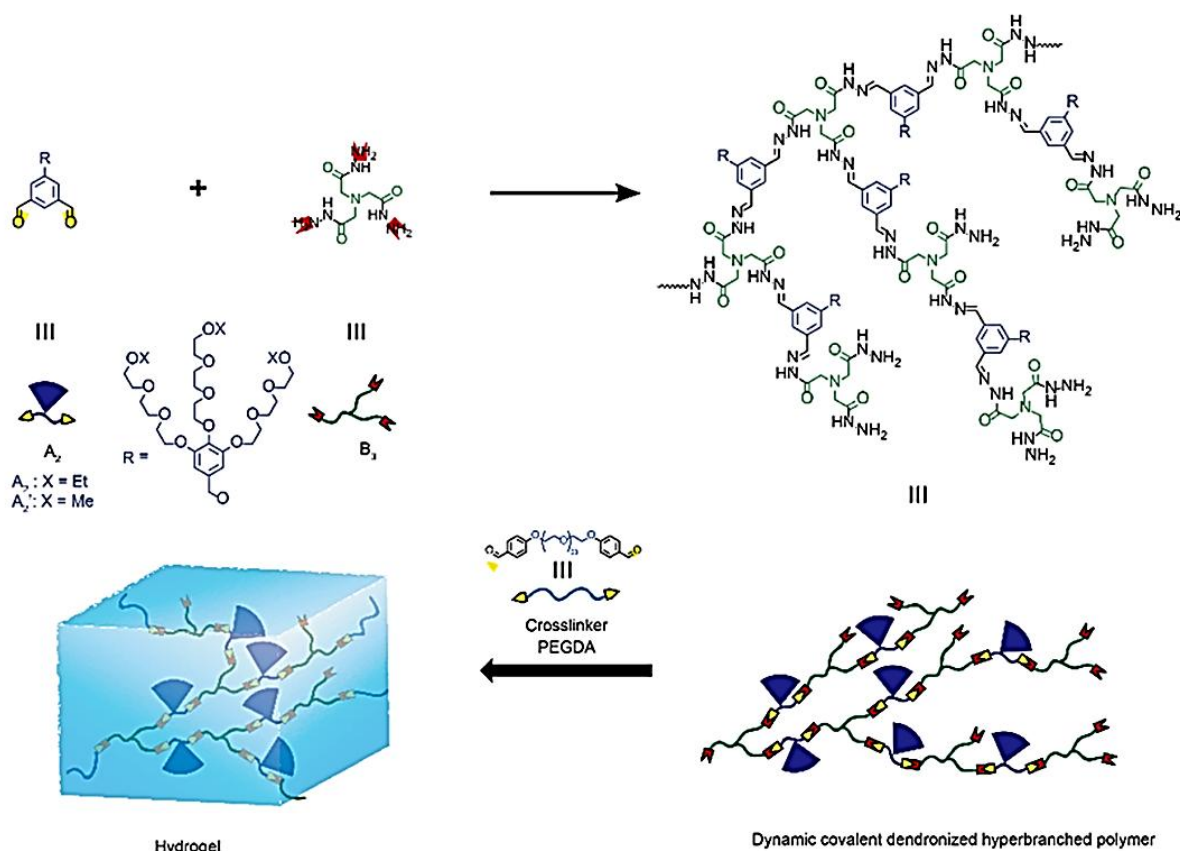


Figure 28 - A) Water-soluble monomers for the synthesis of the polyacylhydrazones by Psarrou *et al.* B) Synergistic effect of the doxorubicin (DOX) release upon irradiation and medium pH (7.4 = blue triangles, 5.2 = green triangles, 2.0 = red squares). The black circles are the samples without irradiation at pH 2.0. Adapted from Psarrou *et al.*²⁵

Hydrogen bonds can also be an asset for these polymers, as Daines *et al.*⁷ demonstrated by using polyacylhydrazones in coatings. The synthesis was performed in water, from bis-functional levulinoyl esters and small water-soluble dihydrazides, yielding in a 50 % (w/v) final concentration, and a clear viscous solution for the case of adipic dihydrazide. Solutions showed linear and macrocycles species with degrees of polymerization around 6.

Water-soluble hyperbranched polymers were also developed. Indeed, Zhang *et al.*¹¹⁷ synthesized them in an aqueous buffer at pH 4.0, then lyophilized. These dendritic

hyperbranched polymers demonstrated precipitation upon heating, and re-solubilization upon cooling. Their cloud point depends on the solution pH. The authors also turned these dendrimers into hydrogels *via* cross-linking with dialdehydes at low pH (Figure 29). These hydrogels showed self-healing properties, no cytotoxicity, and good mechanical properties, making them good candidates for biomedical applications. Montenegro's group^{68,118} also worked on amphiphilic dendrimers made of acylhydrazone linkages, by screening DCLs. They developed dendrons able to transfect into cells thanks to their cationic charges, together with their hydrophobic tails which are able to complex functional DNA or siRNA, self-assembling into polyplexes as a means of vectorization.



600

Figure 29 - Dendritic hyperbranched polymers developed by Zhang *et al.*, that can be turned into hydrogels upon addition of dialdehyde cross-linkers.¹¹⁷

3.2. Synthetic polymers with acylhydrazone functions in side chains

605

The introduction of acylhydrazone bonds on the side chain of polymeric structures can lead to complex dynamic polymers, by branching the pending aldehyde or hydrazide to the complementary function borne by grafting entities (Figure 30). A high variety of side-chain species can lead to multifunctional “brush-like” polymeric structures, providing many kinds of interactions, particularly useful for bioactive applications. Furthermore, this type of

610 polymer can easily be converted into hydrogels by binding cross-linkers instead of functional species.^{102,103}

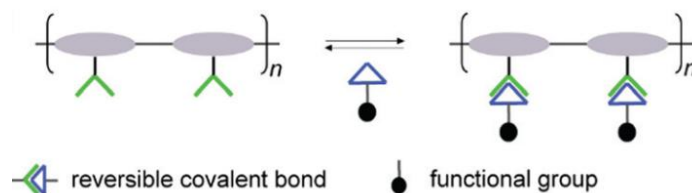


Figure 30 - Polymer-scaffolded dynamic bonds strategy, from Zhang *et al.*¹⁰³

615 Montenegro's group^{119,120} developed a polymer scaffold bearing hydrazide moieties, able to bind with either cationic or hydrophobic aldehydes. Once synthesized in DMSO/H₂O (1/1 v/v), the resulting amphiphilic polymer demonstrated self-assembling properties when combined with plasmid DNA or siRNA, forming polyplexes. These polyplexes demonstrated the ability to undergo transfection into human cells, hence releasing the plasmid in the cell
620 (Figure 31). A higher-DP polymer was required to complex the plasmid DNA (> 4,500 bases) than siRNA (< 25 bases). The same strategy, with identical reaction conditions, was then applied to transfect mRNA.¹²¹ Another approach was to replace the polymer scaffold by a peptidic scaffold instead of a "polyhydrazide" one, with pendant cationic and hydrazone moieties, branching it to hydrophobic aldehydes, for a successful transfection of plasmid
625 DNA in human cells.¹²²

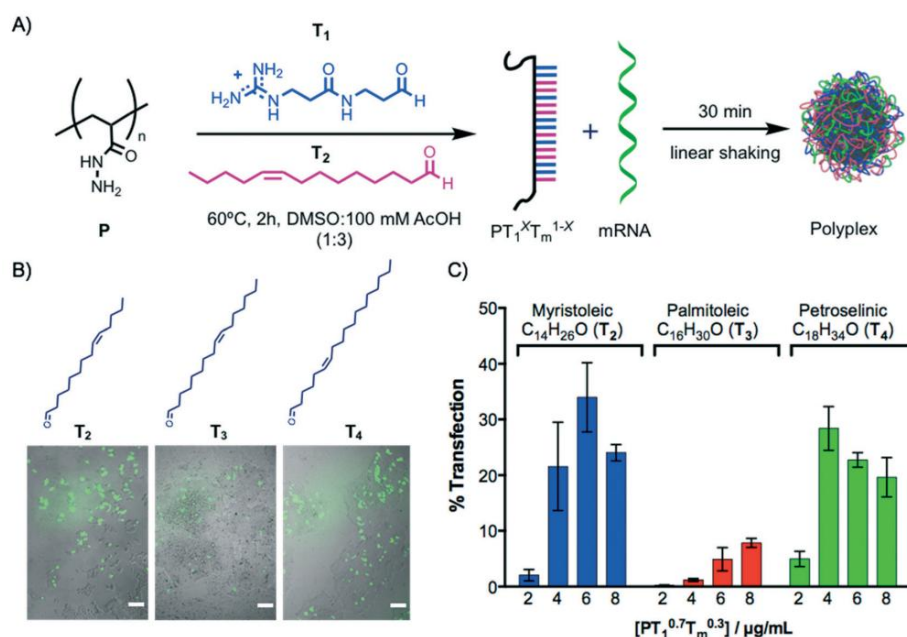
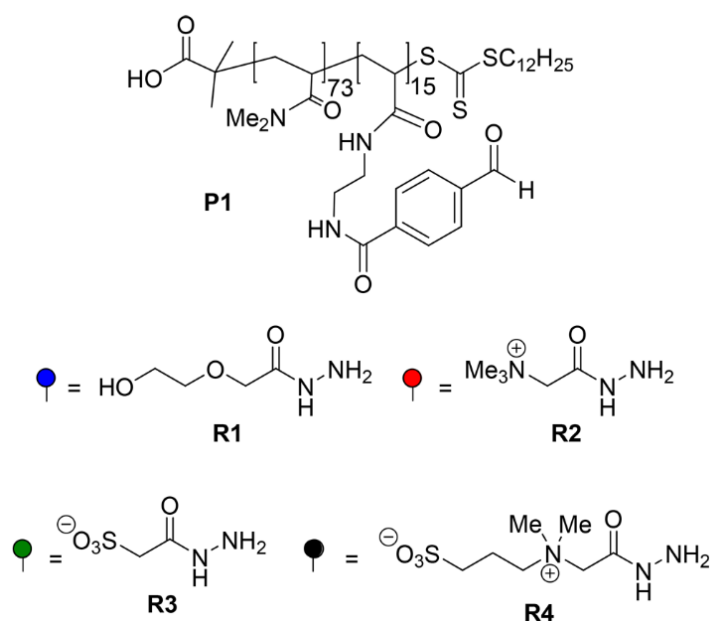


Figure 31 - A) Polyplex formation process, from "polyhydrazide", named P, branched with cationic and hydrophobic aldehydes to complex mRNA. B) Microscopy images of Hek293

630 cells depending on the hydrophobic aldehyde. C) Transfection percentage depending on the hydrophobic aldehyde type.¹²¹

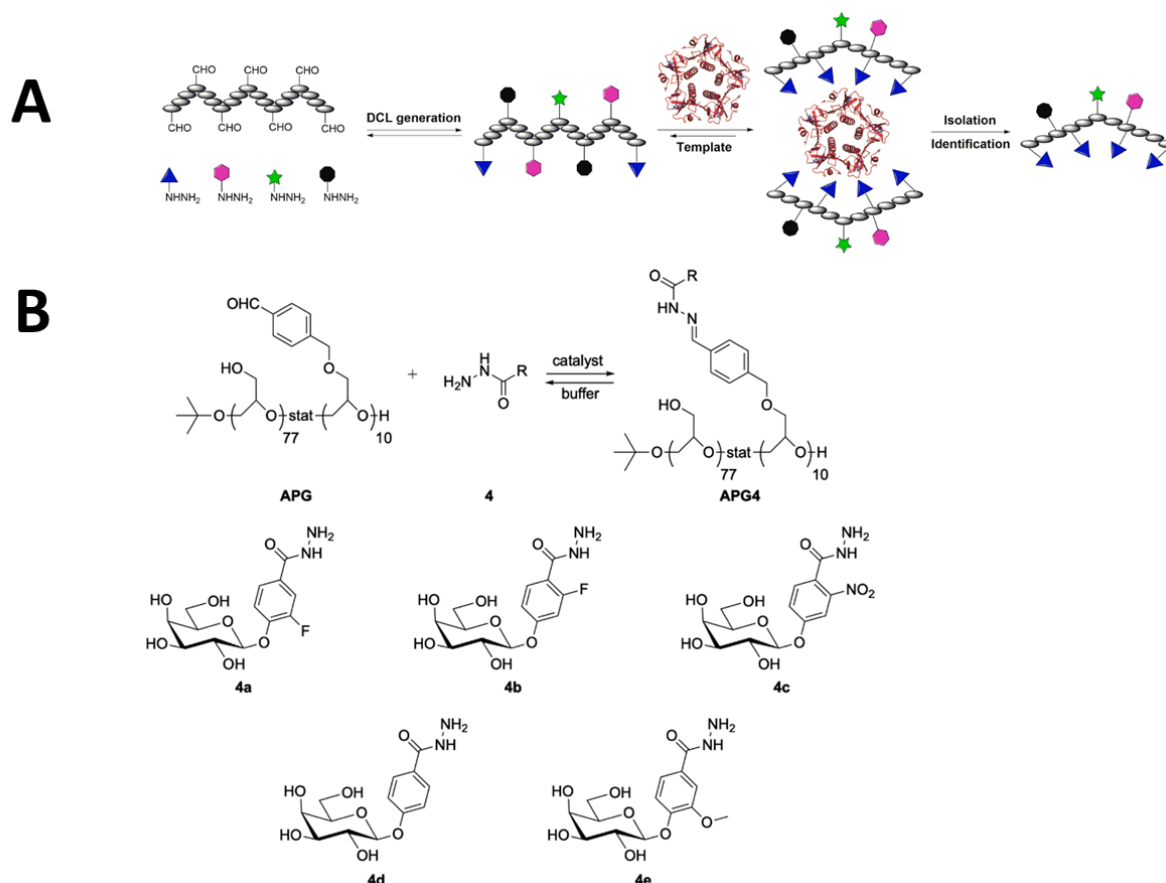
Fulton's group¹²³⁻¹²⁵ also worked on polymer scaffolds, but with aldehyde moieties on pendant chains. Using different hydrazone residues, they developed a DCL in which the diversity point is the functionalization of the polymer scaffold (Figure 32). The resulting DCL
635 demonstrated a high sensitivity to the surface potential of the templates (= biomacromolecules). The equilibrium condition being an aqueous buffer (pD = 4.5 in D₂O), albumins (BSA and HSA), with pI = 5.5 (so positively charged in this buffer), displayed amplifications of anionic components over neutral, cationic or zwitterionic ones. On the other hand, pepsin, a negatively charged protein (pI = 2.2), displayed preference for
640 functionalization with cationic residues. Interestingly, when all components except the cationic one are mixed with pepsin, the anionic one stand out, showing that the driving force of the amplification is more complex than the template surface potential alone.



645 **Figure 32** - Polymer scaffold (P1) with pending aldehyde moieties, and hydrazone residues (from R1 to R4) with various charges.¹²⁵

Similarly, Xu *et al.*¹²⁶ used poly(glycidol) functionalized with aldehydes, and generated a DCL through side-chain condensation with hydrazides. The reaction was
650 performed in PBS buffer at pH 6.2, with 0.5 % DMSO. Using acetylcholinesterase as the template and five different hydrazides as building blocks, a resulting polymer-scaffolded side-

chain distribution demonstrated inhibition of the enzyme, with cytotoxicity and bioactivity above the compared commercial drug. They also used LTB (protein from *E. coli*) as templates with another set of dihydrazides, containing galactose derivatives (Figure 33). The amplified
 655 ligand from this DCL was then branched alone on the polymer scaffold, and the polymer showed 60,000-fold inhibition potency than the ligand alone. More, it demonstrated more than 10 times less cytotoxicity than doxorubicin, the control drug.¹²⁷



660 **Figure 33** - A) DCC strategy to find an inhibitor to LTB protein through polymer-scaffolded library generation. B) Library components with the polymer scaffold (APG) and the hydrazide residues (4a-4e). Adapted from Xu *et al.*¹²⁶

Whitaker *et al.*¹²⁸ used the same polymer scaffolds as previously described, and cross-
 665 linked the aldehyde moieties with succinic dihydrazide, leading to single-chain polymer nanoparticles (SCPNs), in an acetate buffer (pH 4.5) with 0.1 M NaCl. When heated above LCST, these SCPNs become hydrophobic and aggregated, therefore increase the local concentration and favor intermolecular cross-linking, forming hydrogels. Cooling to room temperature has the effect on slowly re-dissolving the gel into SCPNs, at day-level speed,

670 depending on the aldehyde density of the polymers. Moreover, Deng *et al.*^{14,129} synthesized a hydrogel based on cross-linked polyacylhydrazones which showed a pH-responsive sol-gel behavior with self-healing properties.

3.3. Bio-based polymers with acylhydrazone functions in the main and side chains

675 As the previous polymers, bio-based polymers with acylhydrazone functions in the main and side chains (= biodynamers) can undergo a repeating unit exchange and continuous constitutional modifications. Thus, they have the particularity to be dynamic analogs of biopolymers. This statement implies that whereas the previously presented polymers must be based on synthetic repeating units with a hydrophilicity (and often bioactivity and biocompatibility) purpose, the biodynamers are made of biorelevant repeating units, most often with sufficient hydrophilicity. The challenges are therefore different, as it tends to find a synergistic manner to combine both the biorelevant characters of the monomers (and the biopolymer), and the dynamic bonds between them, to enhance the properties of the initial biomolecule. Due to their biocompatibility, acylhydrazone functions are one of the most used
680 dynamic bonds for this kind of strategy. The applications for the three major families of biodynamers (glycopolymers, proteoids or analogs of proteins, and DyNA standing for dynamic DNA) will be described here.^{68,104,130}

The term “glycodynamers” can be applied to three different polymer designs.^{104,131} Firstly, they can be dynamers with modified carbohydrates, dynamically linked to the main chain.
690 These types were studied by Ruff and Lehn¹³² through polymerization with analogs of arabinofuranoside oligosaccharides, containing oxime bonds, but also by Roberts and Thomas¹¹¹, whose polyacylhydrazone was already described above. Secondly, glycodynamers can also be made of carbohydrate-pending repeating units with acylhydrazone dynamic bonds, as studied by Kirker *et al.*¹³³ who cross-linked different types of
695 aminopolysaccharides (hyaluronan and chondroitin) with poly(ethylene glycol) propionaldehyde, forming acylhydrazone bonds. They demonstrated that, once turned into films, these gels were able to accelerate the regeneration of wounded tissues. Ruff and Lehn¹³¹ also worked on these types of glycodynamers, using dialdehydes and dihydrazides, both containing pendent carbohydrates (Figure 34), to obtain rod-like structures once the
700 polymer was synthesized. They concluded that aromatic dialdehydes led to high molar mass polymers, but with a slow reversibility, while aliphatic dialdehydes led to low molar mass polymers with a reversibility property under mild conditions. Both polymers can have applications in different fields, depending on if the preference goes to slow degradation or

high adaptation. Finally, the last type of glycodynamers is made of a polymer scaffold with
705 post-polymerization grafting of acylhydrazone-linked carbohydrate, as already studied before
through the work of Xu *et al.*¹²⁷ about the *E. coli* protein inhibition with galactose derivatives
grafted on a polymer-scaffold.

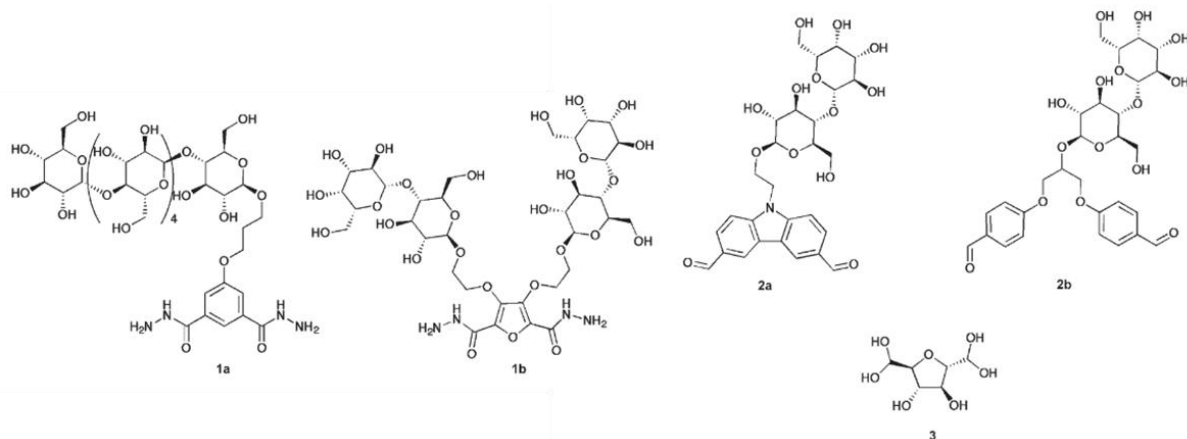
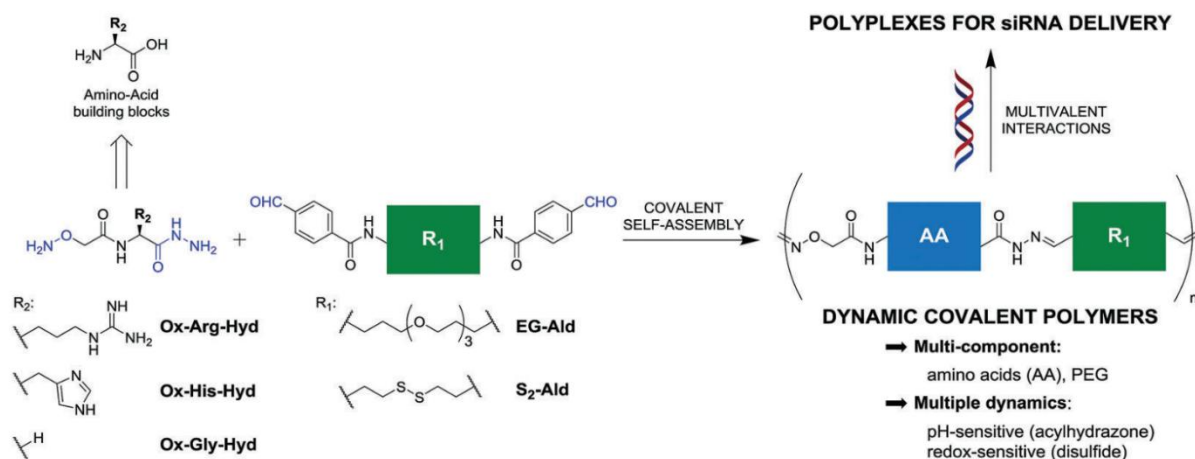


Figure 34 - Carbohydrate-based monomers used by Ruff and Lehn, with dihydrazide (1a, 1b)
710 and dialdehydes (2a, 2b, 3).¹³²

710

Dynamic proteoids, or analogs of proteins, with acylhydrazone reversible bonds has
been studied by several groups. The use of amphiphilic dialdehydes, with pendant hexaglyme,
together with amino acid hydrazides, was explored by Lehn's group.^{8,104,130} These kinds of
715 copolymers were qualified as "doubly" dynamic, because of the imine and acylhydrazone
resulting bonds. In an acetate buffer (pD = 5 in D₂O), they found that the polymerization is
driven by the folding of these polymers, into spherical or cylindrical structures, and that the
reaction rate and the final structure depends on the side-chain moieties on the amino
hydrazides. Similarly, amino acids (Arg, His, Gly) have been turned into aminoxy and
720 hydrazides terminations by Bouillon *et al.*^{134,135}, and reacted with dialdehydes to form oxime
and acylhydrazone bonds. The polymers were synthesized in DMSO but were then used in an
aqueous buffer (pH = 7.0) with physiological saline conditions to effectively complex long
DNA and siRNA, and to deliver siRNA in living cells (Figure 35).



725

Figure 35 - Aminoxy-hydrazides monomers synthesized by Bouillon *et al.*¹³⁴ from amino acids and polymerized with dialdehydes to form a dynamer able to complex and deliver RNA in living cells.

730

Cyclic peptides, functionalized with aldehyde moieties, have been used as a scaffold for the reaction with cationic hydrazides. These clusters were reported by Bartolami *et al.*^{68,136,137} to be able to reversibly complex DNA and release it inside cells.

735

Reuther *et al.*^{138,139} cleverly prepared peptides including some modified amino acids, bearing an aldehyde or hydrazide moiety as a side chain (Figure 36). Depending on the number of modified amino acids in the peptide, it became possible to cross-link the moieties to form loops, with dihydrazides and dialdehydes as cross-linkers, in a PBS/ACN (1/1 v/v) medium. These loops are used to change the quaternary structure of the peptides and can display an enhanced antimicrobial activity.

Peptide	Sequence
DiX ₁	N-RTRXFTXGRYF-C
DiX ₂	N-RTXRFTGXRY-C
DiX ₃	N-RXTRFTGRXY-C
DiX ₄	N-XRTRFTGRYXG-C
TetX ₁	N-RTXVXTXRXY-C
TetX ₂	N-XGXTDHRLDXGXE-C
TetX ₃	N-XIHEXQNRXSARXVTW-C
HexX	N-XRLTXTFXSFTXGTRXSGTXLRTXTRE-C

740

Figure 36 - Peptide sequence showing the modified amino acids bearing a hydrazide or aldehyde moiety (with the X). These pending moieties can then be cross-linked to create loops.¹³⁹

745

Analogously to proteoids, polymers of amino acids analogs, the dynamic DNA (or DyNA) are polymers of nucleic acid analogs, with reversible covalent bonds such as acylhydrazone, while keeping a maximum of DNA properties. Lehn's group^{88,104,140} prepared a polymer-scaffold with pendant nucleobases, in physiological pH medium, and showed their capacity to undergo monomer exchange and adaptability to external biological templates (Figure 37). These DyNA can also be synthesized using regular DNA as a template to obtain its complementary, similarly to the RNA.

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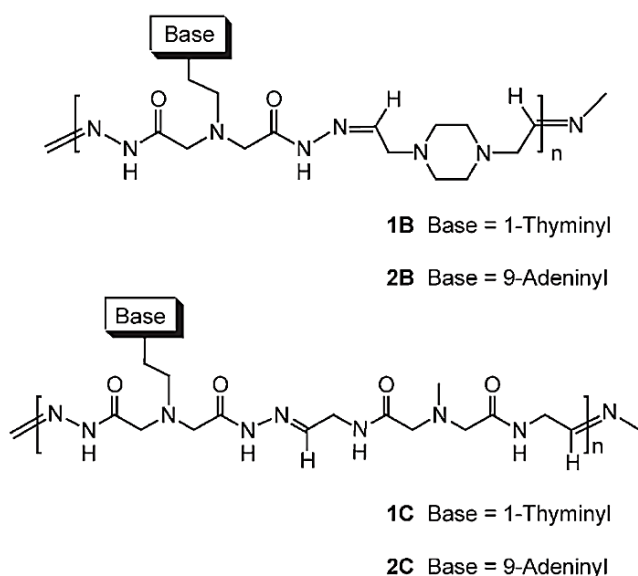


Figure 37 - Two different DyNAs prepared by Sreenivasachary *et al.*, bearing pending nucleobases.⁸⁸

755

Conclusion and Discussion

To conclude this review about water-soluble acylhydrazone (macro)molecules, the literature shows that the water-solubility of such molecules, depending on their molecular structure, is not trivial. Concerning single bond acylhydrazone molecules, the solubility can be predicted with physicochemical parameters such as logP. A common strategy is to synthesize the molecules in polar organic solvents (such as ethanol, DMSO, or acetonitrile), and to dilute them in water for the aimed use. The drawbacks of this approach are the inevitable presence of organic solvent in the final medium, and a relatively low final concentration, which can be obstacles for some valorizations. Parallely, a direct synthesis in water can sometimes imply the requirement of organic co-solvents, as they can be used to ensure good solubilization of both the reactants and the products. However, if the goal is to have an organic-solvent-free solution, the structure of the reactants must be carefully designed, as the solubility of the final products mostly depends on them. As examples, carbohydrate-based molecules, or ionic ones, were found to be water-soluble without any organic solvent.

What is true for single acylhydrazone bond molecules becomes even more important for multiple acylhydrazone bond ones, or polyacylhydrazone, because the intra - and inter - hydrogen bonds become an obstacle when the number of acylhydrazone functions increases. For these polymeric acylhydrazones, with functions in the main chain or the side chains, there are various applications in water medium. From the fundamental fields of rod-like structures to more applied domains such as enzyme inhibition or drug delivery, the dynamic aspect of these bonds provides great potency. In order to succeed in these applications, the polyacylhydrazones, whether synthesized in water or in organic solvents, need to be water-soluble enough despite the high number of hydrogen bonds.

Various strategies have been developed in the literature to ensure a satisfactory water-solubility such as: i) a solubilization in an organic solvent then a dilution in water, ii) a direct solubilization in a water-organic co-solvent, iii) a use of ionic monomers or pending moieties, iv) or the biopolymer development with the use of highly water-soluble monomers.

Despite being a major limitation for the development of polyacylhydrazones, the water-solubility is seldomly deeply analyzed in most of the papers cited in this review. In order to match the rapidly growing application field of these polymers, such studies would be welcome to help produce better-designed water-soluble polyacylhydrazones.

Acknowledgments

This work was financially supported by a grant from the French Ministry of Higher Education and Research.

795

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