Amino Acids and Peptides I

An Introduction to Protein

In the 1950’s Francis Crick formulated the “central dogma of molecular biology” in which information flows from DNA to RNA and finally to protein. Although certain exceptions to this model have emerged over the past 50 years, such as the discovery of RNA as the source of genetic information in certain viruses and the role of catalytic RNA in translation, this formulation has proved to be nearly ubiquitous.

With this in mind it’s only appropriate to think of protein as the functional macromolecule of life; with DNA providing information storage and RNA acting as both decoder and messenger. However, as a consequence of this reality, proteins must be capable of performing an incredible number of both highly varied and highly specific tasks. For example, one protein must be capable of importing glucose across an impermeable cellular membrane while another protein must be capable of recognizing and catalyzing the phosphorylation of target macromolecules upon stimulation. Therefore, nature cleverly designed a single class of biomacromolecules capable of performing disparate functions. Among the varied responsibilities of proteins are molecular and ion transport, signaling, structural integrity, and reaction catalysis. Remarkably, nature has used many of the principles now common to you, as students of organic chemistry, to accomplish the extraordinary demands made of proteins.

Amino Acids

Proteins are composed of monomeric units known as amino acids. The linear sequence of amino acids in a protein contains all the necessary information for highly specific 3-dimensional folding. Therefore, knowing something about the properties of amino acids should help us to better understand the properties of polypeptides and proteins. Furthermore, amino acids and their derivatives are individually important as energy metabolites, essential nutrients, and organic reagents.

Analysis of a vast number of proteins has revealed that all proteins are composed of the same 20 “standard” amino acids. These amino acids are termed α-amino acids because, with the exception of proline, their generic structure includes a primary amino group and a carboxylic acid bonded to the same carbon, known as the α-carbon (Cα) (Figure 1). Also bonded to the Cα of each amino acid is one of 20 different standard side chains, each of which possesses unique chemical properties. In the physiological pH range the amino group (pKα≈9.5) is protonated and the carboxylic acid (pKα≈2) is deprotonated forming a zwitterion (Figure 2). As amino acids are capable of acting as either acids or bases they are said to be amphoteric and are referred to as ampholytes.
One of the most fundamental properties of amino acids is their ability to polymerize. Polymerization of amino acids can be generically conceptualized as the elimination of water (Figure 3) in order to form a CO-NH covalent linkage known as the peptide bond (known generically in organic chemistry as an amide bond). Polymers consisting of two, three, several, and many amino acid residues are known as dipeptides, tripeptides, oligopeptides (4-10 amino acids), and polypeptides (many amino acids), respectively.

Clearly, differences between amino acids are defined by the differences between their respective side chains (remember that the other 3 groups bonded to the C<sub>α</sub> always remain the same). Biochemists have simplified these differences by assigning each amino acid to one of three groups. These assignments are based directly on the chemical properties of the side chains and are easily comprehended using your knowledge of organic chemistry. The three categories are: (1) amino acids with nonpolar R groups, (2) amino acids with uncharged polar R groups, and (3) amino acids with charged polar R groups. There are nine amino acids classified as having nonpolar R groups, six amino acids classified as having polar uncharged R groups, and five amino acids classified as having polar charged side groups.

When assigning amino acids to a given group, it’s important to remember that the charge of a given functionality is based on its pK<sub>a</sub> and the pH of the solution. For amino acids, the relevant pH is physiological pH (pH~7.4). Therefore, using the Henderson-Hasselbalch equation (Equation 1) it’s possible to determine whether or not side chain functionalities are predominately charged or uncharged at pH 7.4.

\[
pH = pK_a + \log ([A^-]/[HA])
\]

and \( K_a \times K_b = K_w \)  
(where \( K_w = 1.0 \times 10^{-14} \))

Equation 1

An alphabetical list of all 20 standard α-amino acids is provided below (Figure 4). The amino acids are listed with their name, their three letter abbreviation, and their one letter abbreviation. Biochemists often use the abbreviations as a way to save time and space when dealing with peptides.
Figure 4
Please use what you know about acid base chemistry and the information provided in the packet to place each amino acid into its appropriate group in the chart below. It’s important to note that the $pK_a$ listed on the table provides the $pK_a$ of the most acidic proton at pH 7.4 and that the $pK_b$ indicates that the uncharged functionality will act as a base at pH 7.4.

<table>
<thead>
<tr>
<th>Nonpolar Amino Acids</th>
<th>Uncharged Polar Amino Acids</th>
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| Charged Polar Amino Acids |
All amino acids isolated from nature are optically active and biochemists commonly use several different systems to assign their stereochemical configuration. The Fisher Convention relates the configuration of groups about an asymmetric center to that of glyceraldehyde. Glyceraldehyde has one asymmetric center and by convention, introduced by Emil Fischer in 1891, its dextro- and levorotatory stereoisomers are denoted D-glyceraldehyde and L-glyceraldehyde, respectively. Knowing that he had a 50% chance of being correct, Fisher guessed at what the configurations of these stereoisomers were (Figure 5). Fisher also developed a shorthand method for drawing molecules, known as Fisher Projections, in which horizontal bonds extend out of the plane of the page and vertical bonds are directed into the plane of the page (Figure 5).

The configuration of other groups about a chiral center can then be related to that of glyceraldehyde by chemically converting these groups, through reactions of known stereochemistry, to those of glyceraldehyde. For α-amino acids the amino, carboxyl, R, and H groups are related to the hydroxyl, aldehyde, CH$_2$OH, and H groups of glyceraldehyde, respectively (Figure 6). In this way, L-glyceraldehyde and L-α-amino acids are said to have the same relative configuration. All naturally occurring α-amino acids have the L stereochemical configuration.

However, another system of stereocchemical classification is often more functional than the Fisher Convention. The Cahn-Ingold-Prelog or (RS) System is familiar to you from organic chemistry and assigns an absolute configuration to asymmetric centers by prioritizing their substituent groups. Using absolute configurations to assess stereochemistry is useful as it avoids certain ambiguities that arise when using the Fisher Convention; namely involving multistereocenter molecules.
Still Confused? Now it’s time to flex your indexing muscles. Start with Ege or go right to the source; there are a number of high quality biochemistry texts available at the Science Library and at the SLC (inquire at the front desk).

Problem Set 1

1. Draw the step-wise mechanism for the formation of a Val-Trp peptide bond (mildly acidic conditions).


3. What is the absolute configuration of the L-α-amino acids? Are there any exceptions to this rule? Are there any amino acids that contain more than one stereocenter and if so, which?

4. What kinds of non-covalent interactions are the side chains capable of forming (assuming that the polypeptides are in an aqueous solution)? Select appropriate amino acids and use diagrams to exemplify these interactions.

5. Draw the following oligopeptides in their predominate ionic forms at pH 3 (the pH of a food filled stomach) and at pH 7.4: Y-I-C and K-F-E.
Think 3-Dimensionally

When thinking about biological macromolecules it’s always important to remember that these molecules are nothing more than extended organic molecules. That is to say, the properties of biomolecules are defined by the same kinds of variables you have been learning about in organic chemistry; such as the presence of reactive functional groups, the reaction conditions, and stereochemical concerns.

Of particular importance when considering the properties of polypeptides (and most all biological macromolecules) is stereochemistry. In fact, every naturally occurring linear sequence of amino acids will repeatedly fold into a unique 3-dimensional structure under the correct conditions (pH, ionic strength of the solution, etc.). In some cases, changes in 3-dimensional structure shift reactive residues (particular amino acids) by only nanometers (1nm = 1x10^-9 m) will completely abolish a protein’s biological activity (this is especially true for enzymes).

The repeating sequence of atoms along the core of a polypeptide chain is referred to as the polypeptide backbone (Figure 9) and the 3-dimensional structure of a polypeptide is dependent on how these bonds are each respectively rotated. There are four terms used to describe the structure of polypeptides: (1) the primary structure (1°) of a polypeptide is defined as the linear sequence of amino acids, (2) the secondary structure (2°) is defined as the local spatial arrangement of the polypeptide backbone without regard to the side chains, (3) the tertiary structure (3°) is defined as the global 3-dimensional structure, (4) and the quaternary structure (4°) refers to the spatial arrangement of more than one interacting polypeptide.

In order to understand the 3-dimensional arrangements of polypeptides, we must first take a look at a much smaller portion of the chain, the peptide group (the atoms of the peptide bond). Due to resonance (Figure 7), the peptide bond has a rigid planar structure and the nitrogen atom is largely sp² hybridized. Remember that any distortion from planarity results in an increase in energy due to a loss of the stabilizing effects of the resonance. With this in mind, there are two possible arrangements of the peptide group in which the peptide bond maintains planarity. In a cis-peptide bond, two adjacent Cα groups are on the same side of the peptide bond, or are cis to one another. In a trans-peptide bond, these two Cα groups are on opposite sides of the peptide bond, or are trans to one another (Figure 8). Due largely to steric interference between the substituents of the adjacent Cα atoms peptide groups primarily assume the trans conformation. In fact, the cis conformation is, on average, approximately ~8 kJ/mol less stable than the trans conformation.
The rigid plane formed by the trans peptide bond is known as an **amide plane** (Figure 9). The consequence of this rigidity is that rotation of the polypeptide backbone is only possible about the C$_\alpha$. This being the case, the conformation of the backbone can be described by **torsion angles** (or dihedral angles). These two angles are defined by the degree of rotation about the C$_\alpha$-N bond (denoted $\Phi$) and the C$_\alpha$-C bond (denoted $\Psi$), respectively (Figure 10).

The angles $\Phi$ and $\Psi$ are defined as 180° when the polypeptide backbone is in its planar fully extended (all-trans) conformation and increase for a clockwise rotation as viewed from the C$_\alpha$. As in any molecule, there are certain steric constraints on allowed conformations that are based on electron repulsion between substituents on adjacent and nearby backbone centers. Interactions of this kind can easily be examined using the familiar Newman projection. In peptides, dihedral angles of 180° correspond to a staggered conformation and dihedral angles of 0° correspond to an eclipsed conformation. When substituents are large, as is often the case for polypeptides (consider the space filled by the side chains and the van der Waals radii of oxygen, etc.), some high energy conformations may be sterically forbidden.

In order to better understand 3-dimensional polypeptide conformations, allowed dihedral angles have been calculated using a tripeptide of each respective amino acid at the central C$_\alpha$ for all values of $\Phi$ and $\Psi$. Those dihedral angles that result in any nonbonding interatomic interactions at distances that cause an overlap of the van der Waals radii are considered forbidden. A common way of summarizing the allowed and forbidden regions is on a **Ramachandran diagram** (Figure 11). As might be expected, for most polypeptides only three small regions of the
Ramachandran diagram are conformationally accessible. The Ramachandran diagram provided below is of tri-L-alanine, in which the Cβ (the first carbon of the side chain) is unbranched. Other amino acids that are unbranched at Cβ have Ramachandran diagrams that are very similar to that of L-alanine while bulkier amino acids and those that are branched at Cβ have allowed regions that are somewhat smaller than those for L-Ala. Therefore, it's clear that the allowed conformations for polypeptides are highly restricted. However, as we shall see, there are still a number of highly stable regular local structures whose dihedral angles fall into universally allowed regions.

![Ramachandran diagram]

**Figure 11**

**Secondary Structure (2°)**

As mentioned previously, the 2° structure of a polypeptide is defined as the local spatial arrangement of the polypeptide backbone. Certain regions of 2° structure have well defined regular configurations that correspond to allowed conformations. However, in order for any 2° structural element to have more than a transient existence it must be “glued” together in some way. That is to say, there must be some energetically favorable stabilizing force holding the local arrangement in a particular conformation. Among the familiar 2° structural elements meeting these criteria (allowed angles and “glue”), the α-helix is especially stable and is therefore frequently present in polypeptide 3-dimensional structure.
Helices are striking and impressive structures. They form in polypeptides when the backbone chain is twisted slightly about each of its $C_\alpha$ atoms. An important feature of helices is that they are chiral; helices may be either right handed or left handed. The conformation of the helix can be defined by the dihedral angles of the helix residues or by the number, $n$, of residues per helical turn and its pitch, $p$ (the distance the helix rises along its axis per helical turn) (Figure 12). In proteins, $n$ needn’t be an integral number of residues, and in fact rarely is.

The $\alpha$-helix is the only helical polypeptide conformation that simultaneously has allowed dihedral angles and a stabilizing network of hydrogen bonds. The bonding pattern of the $\alpha$-helix acts as the “glue” and is what lends this $2^\circ$ structural element its relative rigidity and strength. These helices have an average span of $\sim$12 residues (approximately 3 turns), but $\alpha$-helices have been found to contain as many 53 residues.

The right handed $\alpha$-helix has dihedral angles of $\Phi = -57^\circ$ and $\Psi = -47^\circ$. The number of residues per turn is $n = 3.6$ and the pitch is 0.54 nm. The $\alpha$-helix is arranged in such a way that the carbonyl group of the $n$th residue points along the axis of the helix towards the N-H group of the (n+4)th residue. This arrangement allows for a strong hydrogen bonding interaction to form at nearly an optimal hydrogen bonding distance. In addition, the $\alpha$-helix core is very tightly packed and therefore maximizes van der Waals interactions across the helix. What is more, the side groups are directed outwards and away from the helix, thereby preventing steric interference. All of these stabilizing features make the right handed $\alpha$-helix a particularly energetically favorable local arrangement.

![Figure 12](https://example.com/f12.png)
Problem Set 2

1. How many bonds does each amino acid contribute to the polypeptide backbone?

2. There is one very notable exception to the trans vs. cis peptide bond rule. Can you think of an amino acid that might not be as sterically hindered when following a cis peptide bond?

3. Please draw the Newman projections for the peptide fragment depicted in Figure 4 with dihedral angles equal to $\Phi=0^\circ$, $\Phi=180^\circ$, $\Psi=0^\circ$, $\Psi=180^\circ$ (this means four separate Newman projections).

4. Which amino acid would you expect to have the fewest conformational restrictions and why?

5. If you were given all of the $\Phi$ and $\Psi$ angles for a polypeptide what levels of structure would you be able to describe ($2^\circ$, $3^\circ$, $4^\circ$)? Briefly explain your answer.

6. Imagine that a 14 residue $\alpha$-helical region of a 105 residue polypeptide has been identified. When the $\alpha$-helix is examined, it is noted that one face of the cylinder is dominated by polar charged amino acids while the other is dominated by non-polar amino acids. What conclusion might you be able to draw about the location of this side chain in the correctly folded protein (remember that this protein is in aqueous solution). It might be helpful to look back at some of the terminology and ideas about solubility you have learned.
Amino Acids and Peptides III

Relevance to Chemistry

The study and utilization of amino acids is commonplace in the modern laboratory; the chemistry of these molecules deserves special attention. Research done in many fields (biology, chemistry, chemical biology and biochemistry, for example) relies upon amino acid chemistry. From a synthetic standpoint, because peptides are polymers, the synthesis of a given polypeptide can involve tens or hundreds of synthetic steps. Much of modern synthetic peptide technology takes advantage of the fact that the same type of amide bond-forming reaction is performed iteratively; a chemist (or even an automaton!) can perform these straight-forward reactions rather rapidly. A drawback to performing so many linear synthetic steps, though, is the fact that not every reaction gives a perfect 100% yield; if a chemist could effect, say, a 90% yield with each amide bond formation, s/he would still be losing 10% per amide bond-coupling, and would soon run short on material. To combat this, numerous reliable and high-yielding synthetic techniques (including specific protecting-group reactions and solid-phase synthetic methodologies) have been developed for use in peptide synthesis.

From a characterization viewpoint, it is often the case that a chemist/biologist desires to learn the amino acid sequence of an interesting polypeptide. In many instances, reliable and efficient means are necessary for the elucidation of the amino acid constituency of a natural protein. Modern chemistry has also provided the practicing chemist with facile degrative pathways that can be used to this end in the laboratory.

Protecting Groups in Peptide Synthesis

At this point in your organic chemistry classes, you should be familiar with a few different kinds of ‘protecting groups,’ namely acetics, ketals, and a few alkyl silanes. When chemists talk about protecting groups, they usually mean some kind of auxiliary functional group that will mask a particular moiety (say, a ketone “protected” as a ketal) in order to render it inert to reaction conditions that might effect an undesirable transformation. The kinds of protecting groups most common in amino acid chemistry are called carbamates (figure 13). The two carbamates most commonly used are called tert-butoxycarbonyl (t-Boc, or just Boc) and 9-Fluorenylmethoxycarbonyl (Fmoc).
Figure 13. Carbamates, Specifically Boc and Fmoc Protecting Groups.

With respect to peptide synthesis, these protecting groups serve the function (in general) of masking the nucleophilicity of a selected amine functionality, and leaving the carboxy terminus of the growing peptide chain free to be coupled to the next desired amino acid. Figure 14 shows an example synthesis of the dipeptide Ala-Phe.

Figure 14. Example Synthesis of Boc-Ala-Phe(OEt).

One can see that the use of the Boc protecting group allows only the phenylalanine-amine to act as a nucleophile. Without this restriction, one can imagine all sorts of amino acid coupling reactions taking place: Phe dimerization, Ala dimerization, and any number of polymerization reactions. Note that the phenylalanine residue is likewise ‘protected’ as the ethyl ester; this prevents Phe molecules from reacting with...
each other. After the Boc-Ala-Phe(OEt) compound is formed, though, there is not a reasonable way to remove one protecting group over the other; this is because both protecting groups (the Boc and the ethyl ester) are easily cleaved under acidic conditions. In order to remove protecting groups selectively, a chemist needs to plan carefully and use functional groups that are sensitive/tolerant to different reaction conditions. This being said, the Fmoc group is sensitive to basic conditions, and is stable to the kinds of acidic conditions that might cleave a Boc group even though both are carbamates. Figure 15 demonstrates the mechanism of cleavage of a Boc group under acidic conditions.

![Figure 15. Cleavage of a Boc Group Under Acidic Conditions.](image)

As for the mechanism of cleavage for an Fmoc group under basic conditions, that will be left up to the student in this week’s problem set.

**Amino Acid Synthesis in the “Solid Phase”**

The technique referred to as "solid phase synthesis" is a bit of a misnomer. The substrate of the reaction is not necessarily in the solid phase; the substrate is temporarily attached to some sort of solid supporting material by a readily-cleaved covalent bond, so in essence this is a solid phase molecule, just not in the sense you might be used to thinking about it. The solid support used in solid phase synthesis is usually a polymer such as polystyrene or polyethylene glycol acrylamide (PEGA). The first amino acid residue is immobilized on the solid support, leaving only one of the termini (either N or C) free to performing coupling reactions with the next (suitably protected) amino acid. The second amino acid's protecting group is the cleaved, a third protected residue is coupled to the second, and so on and so forth. If each of these reactions had to be carried out in solution, this would be a wasteful enterprise, both in terms of material (i.e. reagents and solvents) and labor. With solid phase synthesis, reagents are simply mixed with the solid beads (the 'starting material'), then washed to remove excess contaminant, leaving the newly elongated peptide still bound to the solid bead. The advantage of solid phase synthesis stems from the fact that these deprotect-couple-deprotect-couple etc. reactions are highly repetitive, and
can thus be performed easily. Figure 16 shows the general strategy for solid phase synthesis.

Figure 16. Solid Phase Synthetic Strategy.

One can imagine this process occurring many times to ultimately synthesize a large polypeptide (in this case, the final polypeptide product is then cleaved from the resin using liquid HF). The repetitious nature of solid phase synthesis allows the process to be automated, that is, performed by a machine referred to as a peptide synthesizer.
Problem Set 3

1. Please propose a mechanism for the removal of an Fmoc group under basic conditions. A few pieces of potentially useful information are learned if you and your lab partner perform the following reaction:

\[
\begin{array}{c}
\begin{array}{c}
\text{MeONa} \\
\text{MeOH}
\end{array}
\end{array}
\xrightarrow{\text{\text{O}}} 
\begin{array}{c}
\begin{array}{c}
\text{H}_2\text{N} \\
\text{OH}
\end{array}
\end{array}
\]

If the base used is sodium methoxide, the products of the reaction are methanol, the free amino acid, carbon dioxide, and an unidentified substance. The unknown compound has a molecular formula of \(C_{14}H_{10}\) and shows a strong absorption in the UV-Vis region of the electromagnetic spectrum.

2. The use of strong bases in amino acid synthesis can lead to undesired products. In one such instance, an enolate is formed. Please show how an enolate of Tyr-Arg-Pro tripeptide could be formed. Based on what you drew, what would you guess the \(pK_a\) of the corresponding keto tautomer to be? What kinds of bases are strong enough to form the enolate you drew?

3. Having realized what occurred, you quickly decide to quench the enolate (we'll call it molecule 2) from question #2 in an attempt to recover valuable starting material (which we'll call molecule 1). The solid you recover (sample 3) seems to have physical properties that agree with 1. When you take an \(^1\text{H}-\text{NMR}\) of 3, all of the resonances and splitting patterns are identical to 1, except a few of the resonances show a peculiar difference in terms of their ppm chemical shift. What would you guess has occurred? Please explain.
Think Asymmetrically

Synthetic chemists have found a number of uses for naturally occurring amino acids. L-amino acids are chiral, they contain a lot of functionality relative to their size (there are 20 of them, to boot), and are usually very reasonably priced to order from chemical warehouses. As such, they appeal to organic chemists for their utility and chiral accessibility. In fact, amino acids make up a large portion of what is generally referred to as the “chiral pool” of starting materials. What is usually meant by “chiral pool” is naturally occurring, abundant chiral starting materials such as D-sugars and L-amino acids. In this final installment of the Amino Acids Packet, we hope to show you some of the applicability of amino acids to asymmetric organic synthesis in a few different motifs.

Amino Acids in Steroid Chemistry


This is an example of amino acids as chiral auxiliaries. A chiral auxiliary is an appendage attached to a substrate in order to introduce chirality to an otherwise racemic procedure. Once the desired transformation has been effected, the auxiliary is cleaved under facile conditions to yield the desired product. Figure 17 shows the overall reaction scheme and stereochemical outcome when L-proline is used as a chiral iminium auxiliary.

![Figure 17. Asymmetric Aldol Condensation via an L-Proline Auxiliary.](image)

The reaction from Figure 17 is significant because it sets the first stereocenter in the synthesis of a steroidal skeleton. Since the starting material was achiral (meso), the chirality must have originated from the L-proline. Furthermore, once chirality has been introduced to the molecule, further stereochemical outcome can be affected by the existing stereocenter(s). The two rings in Figure 17 make up the C and D rings of the steroidal skeleton, as described in Figure 18.
Based on this, one can see that two of the four steroidal rings have been formed, including one of the stereocenters.

**Amino Acids as Precursors to Oxizolidinone Auxiliaries**


Another class of important chiral auxiliaries is one called oxizolidinones. The first types of these auxiliaries owed their chirality to the fact that they were synthesized from L-amino acids. Figure 19 shows the scheme used to synthesize an oxazolidinone auxiliary.
This class of auxiliary was developed to perform asymmetric aldol reactions, and have since been improved in applicability and scope. The manner in which the auxiliary is attached and used with a substrate is shown in Figure 20.

![Figure 20. Demonstration of an Asymmetric Aldol Reaction using a Chiral Oxazolidinone Auxiliary.](image)

It is worth noting that two stereocenters are set, using familiar reactions from Chemistry 215.

**Amino Acids as Key Synthetic “Building Blocks”**


Many natural products contain amino acids and their derivatives. The following scheme shown in Figure 21 shows the retrosynthetic strategy of a total synthesis of an alkaloid product (-)-Normalindine, which was isolated from the plant species *Strychnos johnsonii* and *Ophiorrhiza filistipula*.

![Figure 21. Retrosynthetic Strategy for (-) – Normalindine.](image)
In this case, the stereochemical information from the amino acid was used to directly affect the stereochemistry of the product by incorporating the amino acid itself into the product. Of course, lots of other steps are involved in the forward-synthetic direction, but the point is that amino acids were used as a source of chirality in yet another way by synthetic organic chemists.

There are many, many more examples of amino acid use in organic chemistry in the chemical literature; what was shown in this packet was intended to be a sampling to get your imagination working. Hopefully, you the students of Chem 215 H have gained an understanding and appreciation for the diversity of applicability of amino acids in both chemistry and biology.

**Problem Set 4**

1. The sources of the chirality in all three examples shown were L-amino acids. For the oxazolidinone case, the L-Valine was modified, though. In the oxazolidinone example, why do you think the stereoisomer shown in the text was the major observed product? Why this stereoisomer over the others? Think carefully with regard to what we know about aldol chemistry, especially geometries/orbital hybridization. Try building a model for inspiration. (Hint: it has something to do with the fact that the auxiliary forces the enolate into the conformation shown below, i.e. a lot of the free bond rotation is lost).

2. Why do you think the enolate below is so rigidly held in the shown conformation (2nd Hint: think about the resonance forms, especially those due to the Nitrogen atom adjacent to both carbonyls. Think also about typical cation that might be present after formation of an enolate. What might those cations be doing?)?

![Chemical Structure](image-url)