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ISCI 1A24 - Chemistry Component

RP4 Chemistry Assignment

Bendamustine: Nitrogen Mustards as Cancer Treatments

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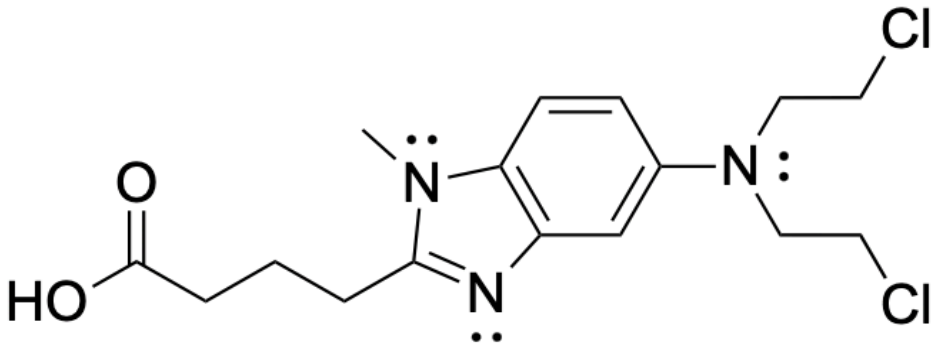

Figure 1: A diagram showing the structure of bendamustine, a nitrogen mustard drug that has been used in the treatment of chronic lymphocytic leukemia (CLL) and indolent B-cell non-Hodgkin lymphoma (NHL) (National Library of Medicine 2025). Bendamustine's chemical structure is composed of a carboxylic acid group, an aromatic ring (benzimidazole) and an amine group that is part of the nitrogen mustard group -$N(CH_2CH_2Cl)_2$.

Shown in Figure 1, bendamustine is a chemotherapy drug that is administered intravenously (as an injection) and is primarily used to treat blood-related cancers such as Chronic Lymphocytic Leukemia (CLL), Non-Hodgkin's Lymphoma (NHL) and myeloma (Cancer Research UK, n.d.). Below is how bendamustine acts as a base when reacted with water (Figure 2). Note that since bendamustine is a nitrogen mustard, it can behave similarly to ammonia.

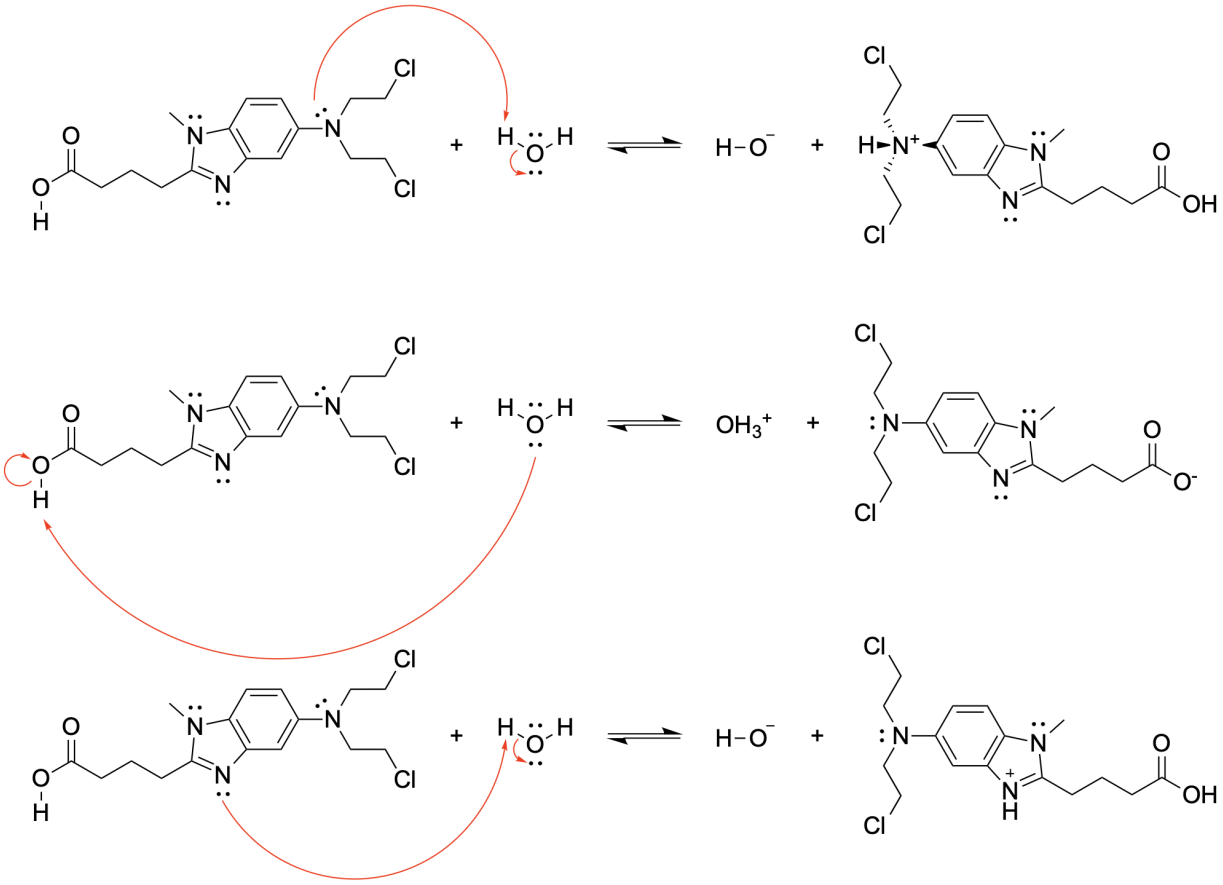

Figure 2: Bendamustine resembles ammonia in that it has a tertiary amine group that bonds to three independent groups. Both molecules act as bases in water in the same way: by having the amine switch out its lone pair for a hydrogen from H_2O. However, bendamustine also has a carboxylic acid group that can react with water to form OH_3^+. One of the Nitrogens on the benzimidazole ring can also likely react with water in highly acidic conditions.

According to ChemDraw, bendamustine's predicted pK_a value is 4.617. Meanwhile, according to DrugBank, bendamustine has two pK_a values, one for acidic and one for basic conditions: 4.38

and 6.65 (DrugBank 2025). We can find out how close these values are by forming the acidic and basic groups in ChemDraw and estimating the pK_a there. What this found is that the carboxylic acid group had a pK_a of 4.759, while the nitrogen mustard group had a pK_a of 8.502. I was unable to find a source for the pK_a of the Nitrogen on the benzimidazole, but I can assume that it is likely more basic than the nitrogen mustard, as nitrogens on rings do not tend to react easily. Using the Henderson Hasselbalch equation, at a pH of 1, the ratio of O^- to OH is $4.17x10^{-4}$, thus meaning that the carboxylic acid group is unlikely to dissociate. The ratio of N to HN^+ is $2.24x10^{-6}$, meaning that there is a significant amount of HN^+ At such a low pH, the concentration of H_3O^+ will be high, making it more likely for the nitrogens to release their electrons and accept hydrogen. I also estimate that the other N group will likely release its lone pair, thus accepting an H^+ group. This forms molecule (1).

At physiological pH, the ratio of O^- to OH is 1047.13 and the ratio of N to HN^+ according to Henderson Hasselbalch is 5.623. I estimate that the other N group will most likely not release its lone pair, meaning that it does not change. This indicates that most OH and some N groups react, forming a roughly 1 to 5 ratio of molecules (2) and (3).

At a high pH of 13, the ratio of O^- to OH is $4.16x10^8$ and the ratio of N to HN^+ is $2.24x10^6$. Therefore, it can be concluded that most carboxylic acid groups will dissociate and none of the nitrogens will release their electrons. This forms molecule (3). See the figure below (Figure 3) for the three versions of bendamustine, at a low pH (<1.0), a high pH (12) and physiological pH (7.4).

It is important at a physiological pH of 7.4 that the N on the left side has a lone pair, as this electron pair is important during its reaction with DNA components. Thus, bendamustine is likely more effective at higher pH values, as this increases the relative concentration of non-ionic nitrogens.

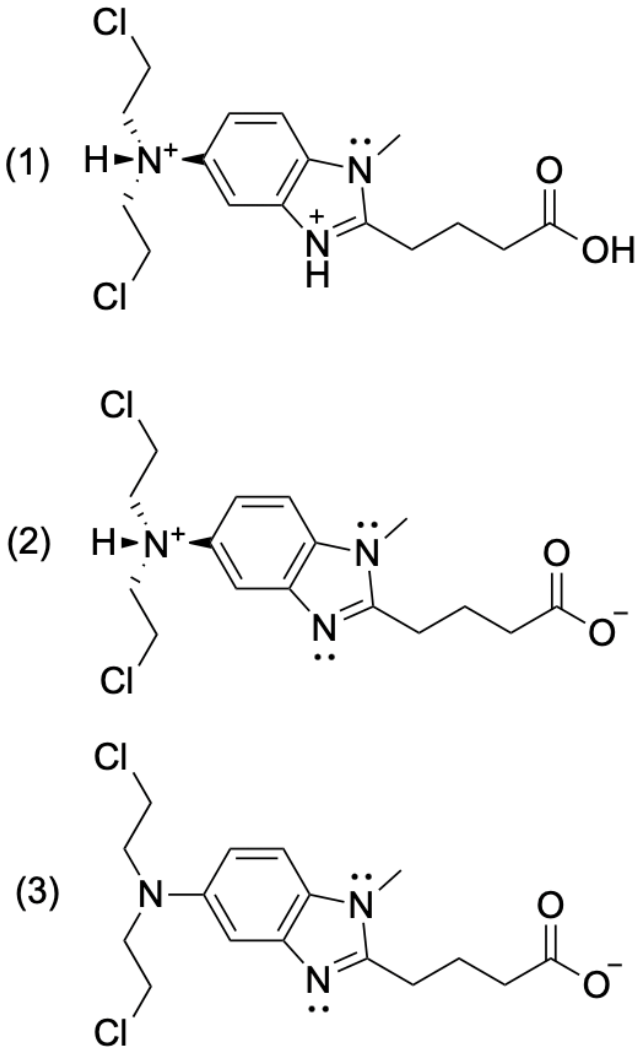

Figure 3: Shown here are the various possible forms that bendamustine can take at various pH values. At low pH, most molecules will likely form (1). When at pH 7.4, there is a 5-1 ratio of (3) to (2), as calculated above. Finally, at high pH values, the majority of bendamustine molecules will be at formation (3).

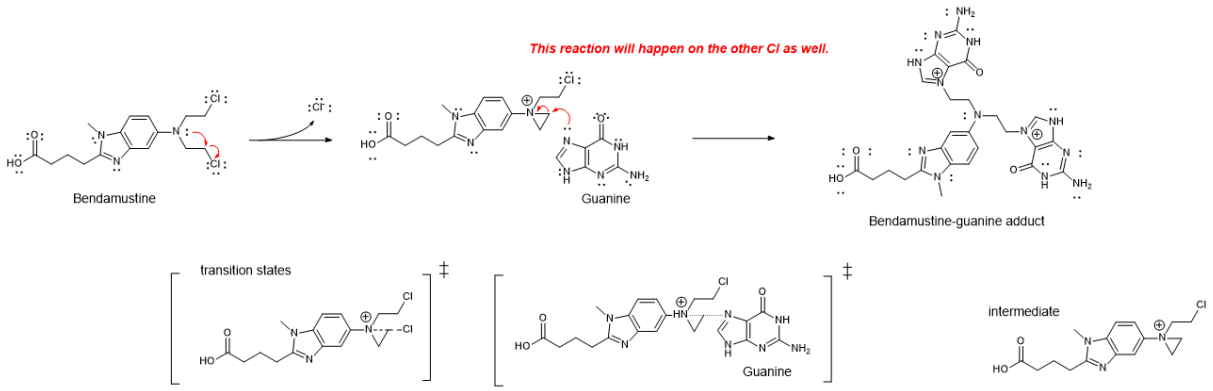

Figure 4: Reaction mechanism for the reaction of bendamustine with guanine.

The nitrogen mustard group on bendamustine and the N7 (nitrogen) on guanine are the sites of reactions for bendamustine's cross-linking of DNA (Polavarapu et al. 2012). In the first step, one chloride will be removed from the nitrogen mustard, meaning that chloride is the leaving group. The carbon that this chloride was bonded to is now a carbocation. This carbocation acts as an electrophile and the lone pair on the nitrogen in the nitrogen mustard group acts as a nucleophile, resulting in an aziridinium ion on the bendamustine. This nucleophilic substitution reaction is an S_N2 reaction because the carbocation acting as the electrophile is a primary carbon; it was attached to only one carbon group and two hydrogens. In the second step, the bond between the same carbon and nitrogen in the previous step breaks and the two electrons from that bond become a lone pair on the nitrogen. At the same time, the newly-formed carbocation bonds to the N7 on a guanine molecule using the lone pair on the N7. In this nucleophilic substitution reaction, the carbocation is the electrophile and the N7 has a lone pair and is the nucleophile.

This nucleophilic substitution reaction is also S_N2 because the carbocation in this step is once again primary since it is attached to only one carbon group, along with one nitrogen and one hydrogen. Primary carbocations have less electron density and less steric hindrance around the carbon compared to secondary and tertiary carbocations, which is why they follow an S_N2 reaction pathway as opposed to an S_N1 pathway (Figure 5). Once this reaction is repeated on the other side of the nitrogen mustard group on the bendamustine molecule with another guanine, the DNA molecule or molecules that these two guanines are found within will be cross-linked.

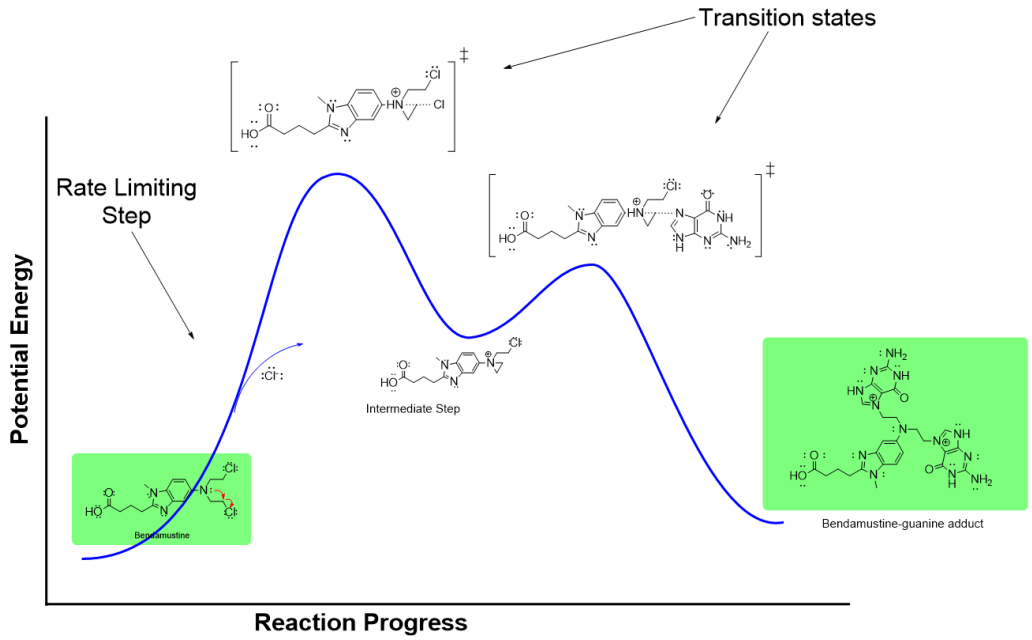

Figure 5: The reaction profile of the alkylating agent bendamustine, acting on guanine, a nitrogenous base, created using ChemDraw. This is a two-step, S_N2 reaction, with the first step (creating the first intermediate) being the rate-determining step. As the reaction progresses, two transition states and one intermediate are created, and the end product is a

bendamustine-guanine adduct. Since the potential energy ends at a higher value than where it started, one can conclude that this reaction is endothermic.

Alkylating agents are a class of anticancer drugs that work by substituting alkyl groups for hydrogen atoms on DNA, resulting in the formation of cross-links within the DNA chain (National Library of Medicine 2015). Cross-linking is when two parts of DNA become chemically stuck together by the alkylating agent, preventing the DNA from properly unzipping (which is essential for replication and transcription), preventing it from replicating and triggering cell death (apoptosis). In short, the interference of alkylating agents stops cells from dividing, being particularly effective for rapidly dividing cells such as cancer cells that don't have time for DNA repair. This makes them powerful tools in cancer treatment (Canadian Cancer Society 2024). Nitrogen mustards are classified as alkylating agents as their major mechanism is creating DNA cross-links that result in cytotoxicity by forming reactive intermediates that add alkyl groups to DNA bases disrupting their structure and function. Nitrogen mustards exert their mechanism through a chloroethylamine group (Imyanitov and Iyevleva 2021).

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