Final Report

An Investigation of the Effect of Molecular Changes on Binding Preferences of Amidines

Initiator: Dan Adsmond

Funds Granted: $2950

Beginning and Ending Dates: May 15, 2000 – May 15, 2001

Date Submitted: 10/3/2003

LATE.

An Investigation of the Effect of Molecular Changes on Binding Preferences of Amidines

Final Grant Report

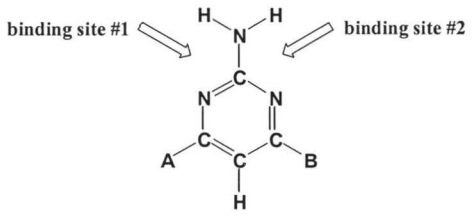
Proposal Abstract

The primary goal of the proposed research is to improve understanding of the effects of neighboring atoms on the selectivity of amidine binding sites. An amidine binding site consists of four atoms: a hydrogen, a nitrogen, a carbon, and another nitrogen chemically bonded together in the order listed. The function of a large variety of biologically and medically important compounds, for example DNA bases, is dependent on the ability of their amidine binding sites to selectively bind to specific binding sites on other molecules. To gain a better understanding of the sensitivity of that selectivity to molecular modification we propose to grow crystals of compounds which contain both the amidine binding site and a chemically different carboxylic acid binding site and to use a combination of nuclear magnetic resonance (NMR), infrared spectroscopy(IR), and x-ray crystallography to identify the specific binding interactions that occur in the crystals. By changing the atoms attached next to the amidine binding site and by using a variety of carboxylic acids we will be able to gain insight into the influence of neighboring atoms on the amidine binding selectivity.

Background

2-aminopyrimidine was selected for this research because it has two identical amidine binding sites, each of which may be modified by changing neighboring atoms. We purchased the three modified aminopyrimidine compounds represented in Scheme 1.

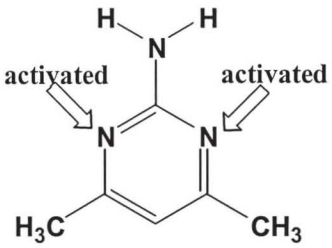
Scheme 1



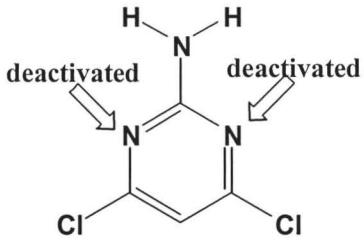
Pyrimidine	A	B
1	CH_3	CH_3
2	Cl	Cl
3	Cl	CH_3

In compound 1, both A and B are methyl groups, activating both binding sites. In compound 2, both A and B are chloro groups deactivating the binding sites. In compound 3, A is a chloro group deactivating site 1 while B is a methyl group activating site 2.

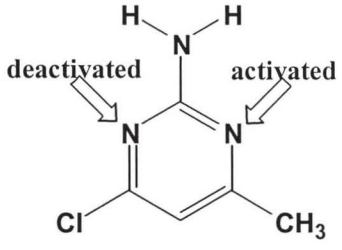
Scheme 2



1



2



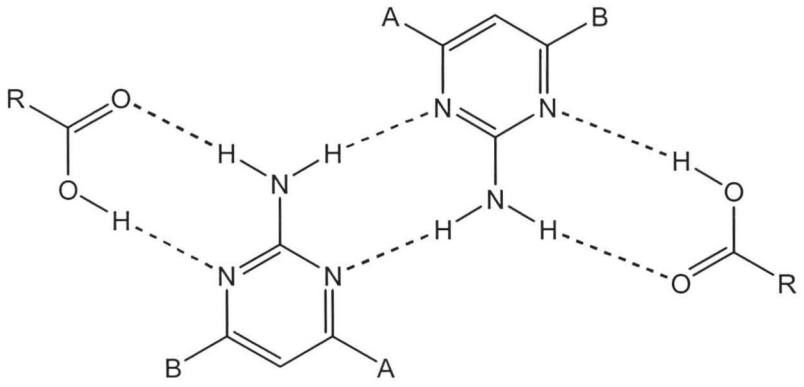
3

The prediction was that the activated binding sites would show a preference for binding to carboxylic acids while the deactivated sites would show a preference for binding to other amidine sites. Compound 1, with both sites activated by methyl groups, would be expected to bind to acid molecules

forming cocrystals in either a 1:1 or a 1:2 pyrimidine to acid ratio as shown in Scheme 3. Compound 2, with both sites deactivated, would not be expected to bind to carboxylic acids at all and would form no cocrystals. Compound 3 with one activated site would be expected to form cocrystals but only in a 1:1 ratio where the deactivated site binds to another pyrimine.

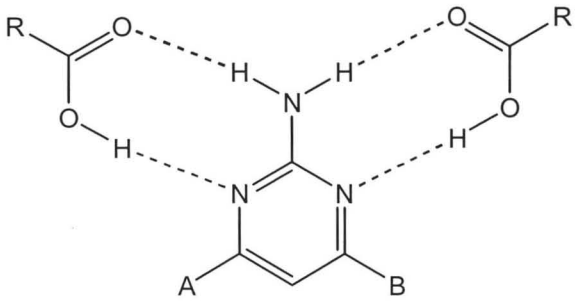
Scheme 3

Binding Expected in a 1:1 Pyrimidine/Acid Cocrystal



acid---------pyrimidine--------pyrimidine--------acid

Binding Expected in a 1:2 Pyrimidine/Acid Cocrystal



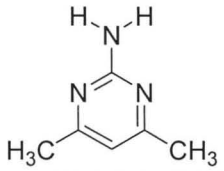
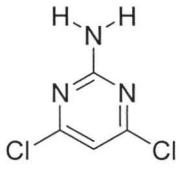
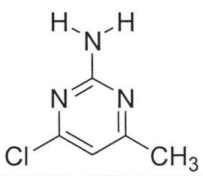
acid--------pyrimidine--------acid

Experimental

During the spring of 2000, chemicals and materials were purchased and a student, Dan Grabill, was hired to carry out the research. Dan completed approximately 160 hours of research during the summer and another 80 hours during the fall and winter semesters of the 200-2001 school year.

We carried out 78 different experiments, growing crystals from solutions containing one of the three different aminopyrimidine compounds and one of 10 different carboxylic acid compounds. The experiments that were set up are summarized in Table I where each of the 3 pyrimidine molecules pictured was crystallized with each of the 10 acids listed in the pyrimidine:acid molar ratios shown. The crystals were filtered from solution and analyzed by NMR to determine if they were pure pyrimidine crystals, pure carboxylic acid crystals, or cocrystals containing both pyrimidine and carboxylic acid molecules. NMR was used to determine the relative amounts of the two components in the cocrystals. All cocrystals were analyzed by FTIR to confirm the presence of an amidine/carboxylic acid binding interaction. It should be noted that due to failure of the NMR instrument to consistently produce data of acceptable quality, most crystals were sent to Mr. Mohammad Zia-Ebrahimi at Eli Lilly and Company for NMR analysis.

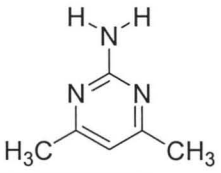
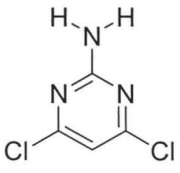
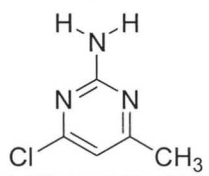
Table I Cocrystallization Experiments Performed

Carboxylic Acid	1 	2 	3 
p-Anisic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
m-Anisic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
m-Toluic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
Trichloroacetic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
Benzoic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
p-Nitrobenzoic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
m-Chlorobenzoic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
p-Chlorobenzoic Acid	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**	**1:1, 1:2, 2:1**
Pentafluorobenzoic Acid	**1:1**	**1:1**	**1:1**
α-Fluorocinnamic Acid	**1:1**	**1:1**	**1:1**

Data

Table II shows the results of these experiments where cocrystal formation indicates selective binding between the two compounds. Although the acids ranged in acidity (pKa) 0.6 to 4.5, the binding behavior appeared to be independent of acidity. The affinity for acid-amidine binding, however, appeared to be strongly dependent upon the nature of the groups flanking the amidine group. Most of the acids selectively bound to the amidine groups of compounds 1 and 3, where at least one of the neighboring groups was an activating methyl group; but none of the acids bound to the amidine groups of compound 2, where the neighboring groups were both deactivating chloro groups. The only acid not forming crystals at all was trichloroacetic acid. This phenomenon may be due to its low melting point.

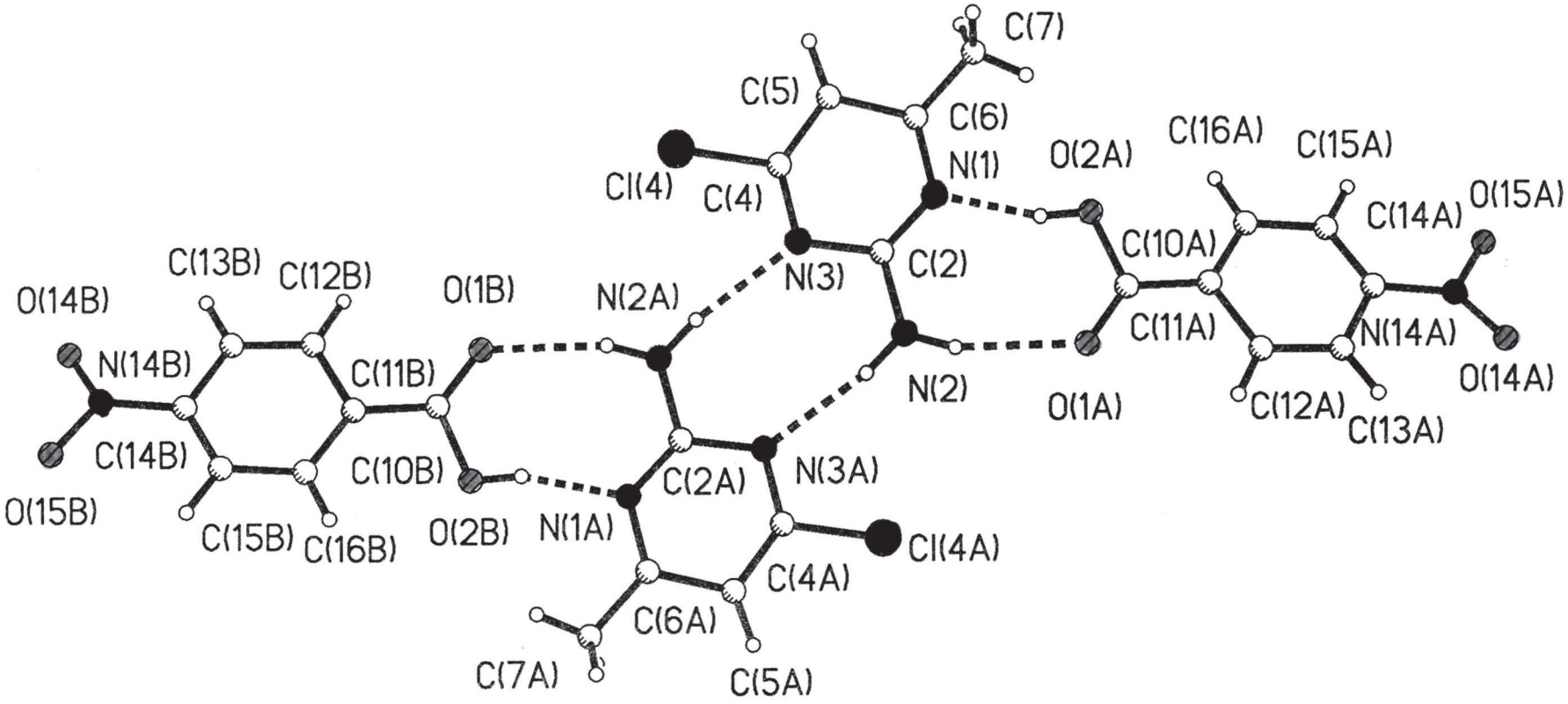
Table II Did Cocrystals Form?

Carboxylic Acid	1 	2 	3 
p-anisic Acid	**Yes**	**No**	**Yes**
m-Anisic Acid	**Yes**	**No**	**Yes**
m-Toluic Acid	**Yes**	**No**	**Yes**
Trichloroacetic Acid	**No**	**No**	**No**
Benzoic Acid	**Yes**	**No**	**Yes**
p-Nitrobenzoic Acid	**Yes**	**No**	**Yes**
m-Chlorobenzoic Acid	**Yes**	**No**	**Yes**
p-Chlorobenzoic Acid	**Yes**	**No**	**Yes**
Pentafluorobenzoic Acid	**Yes**	**No**	**Yes**
α-Fluorocinnamic Acid	**Yes**	**No**	**Yes**

A 1:1 pyrimidine:acid ratio was observed in all cocrystals despite the pyrimidine:acid ratio of the original solution. The 1:1 ratio was expected for compound 3 where one of the pyrimidine binding sites was deactivated an unable to bind to a second acid molecule. It was, however, surprising to see that no compounds formed 1:2 cocrystals with compound 1 which has two activated binding sites and is able to bind to two acid molecules. Further research will be required in order to understand the factors influencing the 1:1 preference.

To demonstrated that the acid molecules only bind to the activated side of compound 3, and not its deactivated site, samples of a 1:1 cocrystal of compound 3 and p-nitrobenzoic acid were mailed to Dr. Mark Whitener for single crystal structure determination. At the conclusion of the x-ray data gathering period, Adsmond flew to Montclair State University for two days of data analysis. Figure 1 shows the crystal structure as solved by Whitener. As expected, the acid molecules are seen binding only to the activated binding site next to the methyl group of the pyrimidine molecule.

Figure 1 Crystal Structure of the 2-Amino-4-chloro-6-methylpyrimidine / p-Nitrobenzoic Acid 1:1 Cocrystal (blue = nitrogen, red = oxygen, green = chlorine, large white = carbon, small white = hydrogen)



In conclusion, this research has shown that the hydrogen-bond selectivity of the amidine group is sensitive to changes in neighboring substituents and that the accepting ability of the amidine may be decreased by an electron-withdrawing chloro group to the point where carboxylic acids will no longer bind to it. The final results and conclusions were presented by Grabill and Adsmond at the American Chemical Society Midwest/Great Lakes Regional Meeting in Grand Rapids, MI, June 11-13, 2001 in the form of a paper entitled "An Investigation of the Effects of Molecular Changes on the Binding Preferences of Amidines"

Impact of Project

As stated in the original proposal, the intention was that this research would be the beginning of an ongoing program of research in the area of molecular recognition here at Ferris. The intention was to seek external funding and to expand the study to involve more students and to include more complicated amidine-containing molecules such as sulfa drugs.

I am pleased to report that, partly as a result of this research grant, Ferris has received 3 years of funding from the National Science Foundation for a grant entitled "FT-NMR Upgrade for a Collaborative Research-Based Organic Chemistry 2 Laboratory Course: Development and Implementation." This grant allowed us to purchase a $55,000 NMR spectrometer upgrade that collects data of excellent quality in one tenth the time previously required. All maintenance problems with the instrument have been virtually eliminated. The grant also provides support for students to carry out 15 weeks of research in the laboratory portion of the Organic Chemistry 2 course here at Ferris. So far approximately 40 students have been involved in the research lab and about half of them have carried out research involving the binding between amidine-containing sulfa drugs and carboxylic acids.