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Preparation of N-(Haloaryl) succinimides

Okey Noe II

Preparation of *N*-(Haloaryl)succinimides

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Finally, I am indebted to my parents for their encouragement and sacrifices during this study.

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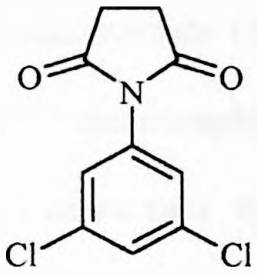
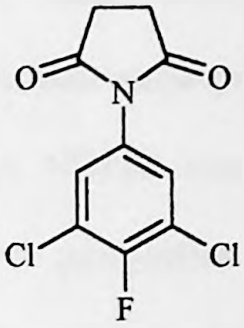


N-(3,5-Dichlorophenyl)succinimide
NDPS
I



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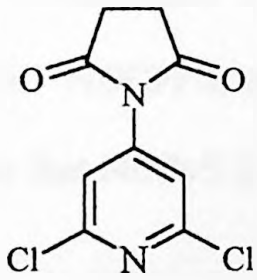

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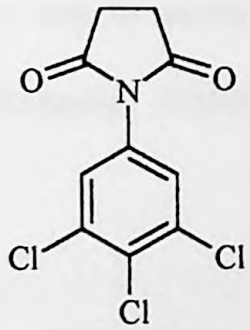

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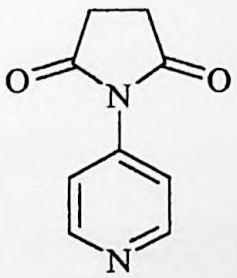

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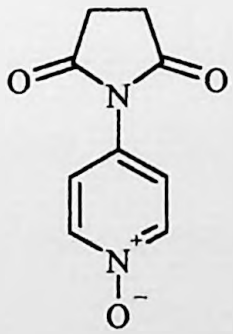
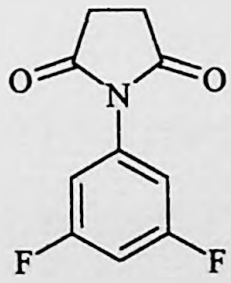


N-(4-Pyridyl-*N*'-oxide)succinimide

XI



N-(3,5-Difluorophenyl)succinimide
NDFPS

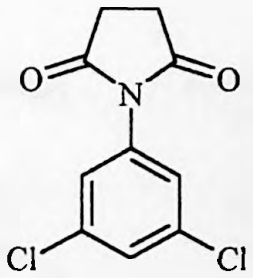
Abstract

N-(3,5-Dichloro-4-fluorophenyl)succinimide (NDCFPS) and *N*-(3,5-dichloro-4-pyridyl)succinimide (NDPyS) were synthesized using procedures employed previously for *N*-(3,5-dichlorophenyl)succinimide (NDPS). However, NDPyS was not formed cleanly: extraction from by-products was necessary and purification by column chromatograpy was required. Several potential alternative routes to NDPyS were investigated without success.

Both compounds were administered to male Fischer 344 rats to assess nephrotoxic potential. NDCFPS, contrary to expectations, was non-nephrotoxic. Preliminary results indicate that NDPyS is also non-nephrotoxic.

I. Introduction

During the 1970's, a number of *N*-(halophenyl)succinimides were synthesized and examined for potential use as agricultural fungicides and antimicrobial agents.[1-5] One of the compounds synthesized, *N*-(3,5-dichlorophenyl)succinimide (NDPS, I), proved to

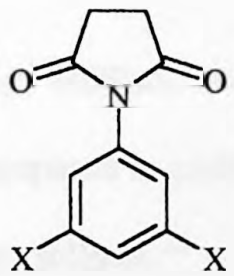


NDPS; I

be a highly effective agricultural fungicide.[1,2] However, the use of NDPS was never widespread due to its demonstrated nephrotoxicity in male Fischer 344 rats following acute and chronic exposure. Acute exposure resulted in acute tubular necrosis,[6-8] while chronic exposure resulted in interstitial nephritis.[9,10] Although NDPS is not known to be carcinogenic, it has been shown to promote the nephrocarcinogenic properties of several chemicals, including citrinin (found in fungus growing in stored rice, barley, corn and dried fish),[11] streptozotocin (an antibacterial and antitumor agent),[12] and 2-(ethylnitrosamino)ethanol.[13]

Since NDPS is a nephrotoxicant, many studies have been done to investigate the structure-nephrotoxicity relationship in *N*-(halophenyl)succinimides.[14-20] These studies show that 3,5-dihalosubstitution of the phenyl ring with respect to the succinimide is essential for nephrotoxicity. Previous studies have shown compounds with substitution at the 3 and/or 4 position of the phenyl ring such as *N*-(3-chlorophenyl)succinimide, *N*-

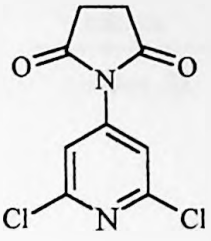
(4-chlorophenyl)succinimide, *N*-(3,4-dichlorophenyl)succinimide to be weakly nephrotoxic.[16,20] Also, compounds with aromatic substituents of different size and shape, such as *N*-(1-naphthyl)succinimide, *N*-(1-anthracenyl)succinimide and *N*-(9-anthracenyl)-succinimide only weakly affected renal function.[21] In addition, a series of *N*-(3,5-disubstitutedphenyl)succinimides were also found to be non-nephrotoxic. The series was:



X = NO_2
CH_3
H
OCH_3
O_2CCH_3

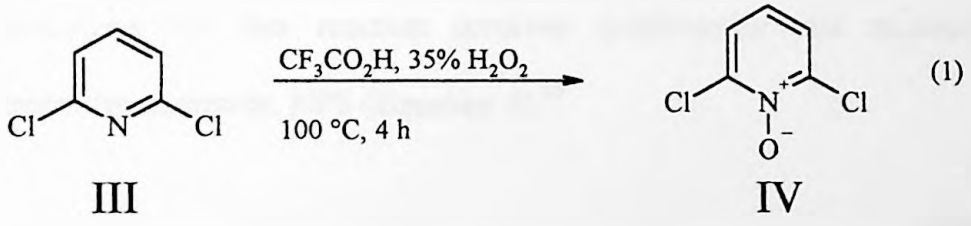
This study determined that 3,5-disubstution on the phenyl ring is not enough for this class of compounds to be nephrotoxicants. In order for marked nephrotoxicity to occur, the substituents at the 3 and 5 positions must be halogens[22] (halogens in order of decreasing nephrotoxicity: Cl>>I>Br>F).[19]

Although the nephrotoxic effects of *N*-(3,5-dihalophenyl)succinimides have been studied, the effects of heteroaromatic succinimides, such as pyridine, thiophene, and pyrimidine succinimides have not been examined. Since none of these compounds have been studied, it is of interest to determine whether these compounds behave like the phenyl derivatives. Since 4-amino-2,6-dichloropyridine is a known compound and a precursor for succinimide formation, the first heterocyclic succinimide that was attempted was *N*-(3,5-dichloro-4-pyridyl)succinimide (NDPyS, II). This compound was also of interest due to its structural similarity to NDPS.

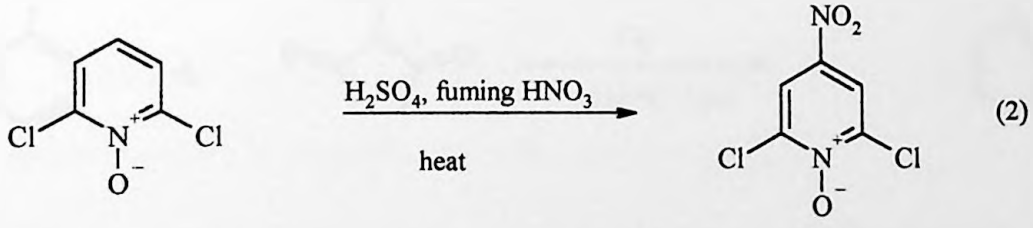


NDPyS, II

The starting material for NDPyS, 4-amino-2,6-dichloropyridine (VI), is a known compound that has been prepared in yields as high as 99%.[23] The synthesis for 4-amino-2,6-dichloropyridine was as follows:[23]



III IV (1)



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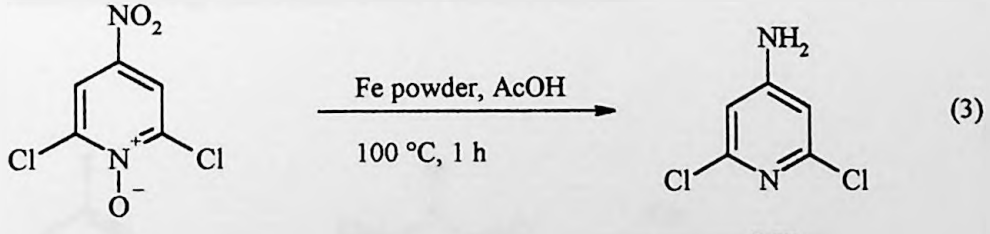
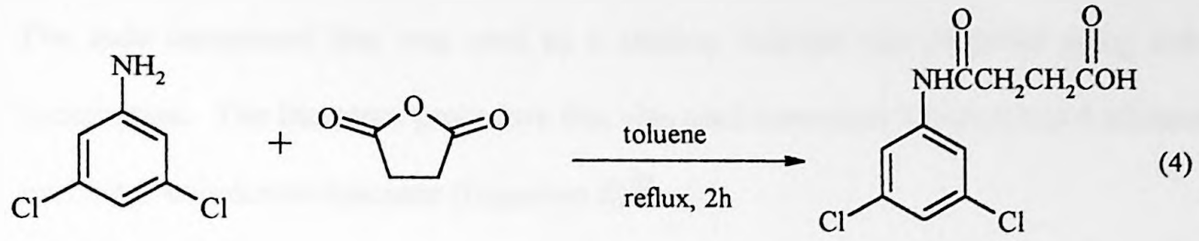


VI (3)

From 4-amino-2,6-dichloropyridine, preparation of NDPyS was attempted using the synthesis by Fujinami for *N*-arylsuccinimides, including NDPS (Equations 4 and 5).[1]



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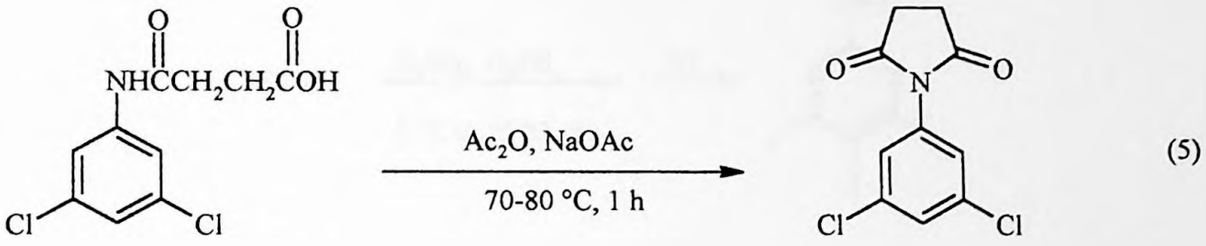
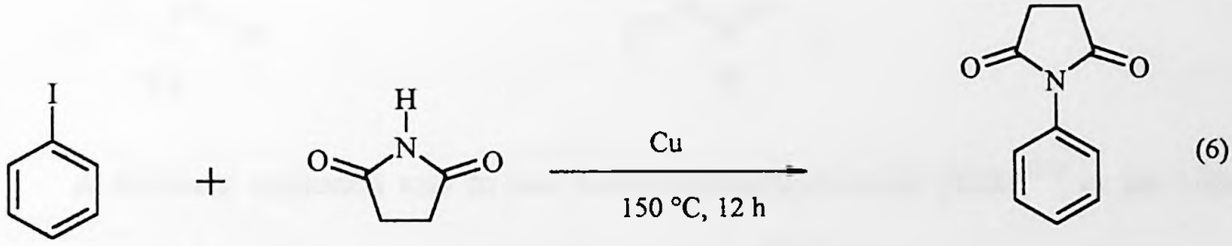


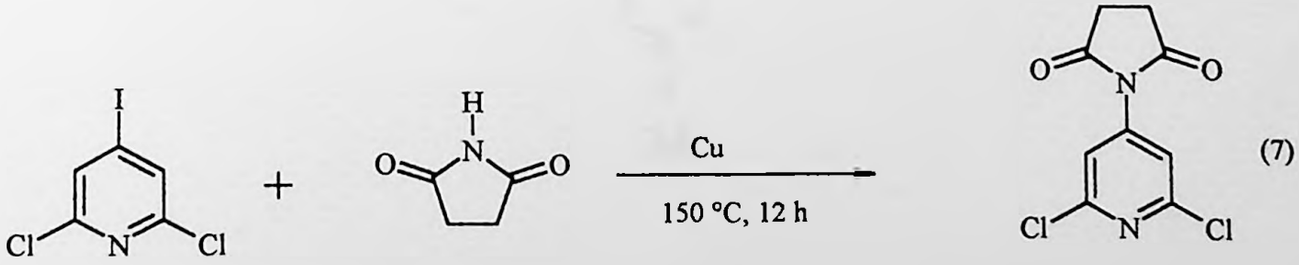
(5)

Another route for succinimide formation involved an Ullman coupling reaction. The precedent for this reaction involved iodobenzene and succinimide to form *N*-phenylsuccinimide, NPS (Equation 6).[24]



(6)

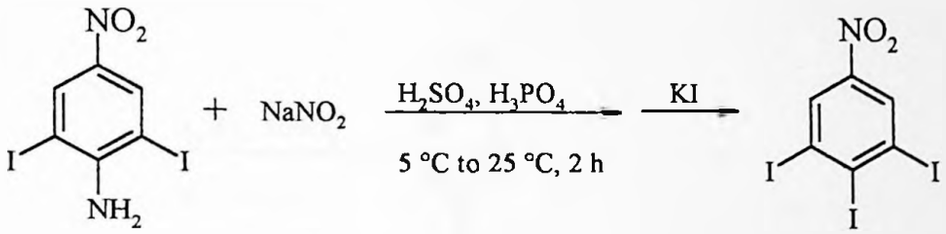
This type of procedure could be a useful approach to NDPyS formation. A possible reaction using this route would be as follows:



VII

(7)

The iodo compound that was used as a starting material was prepared using iodo-de-diazonation. The literature procedure that was used converted 2,6-dichloro-4-nitroaniline into 3,4,5-triiodonitrobenzene (Equation 8).[25]



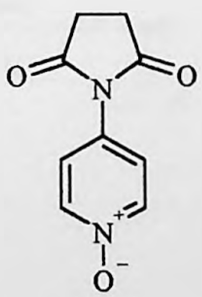
(8)

Since this is a standard procedure for iodo-de-diazonation, it could be used to synthesize 2,6-dichloro-4-iodopyridine (Equation 9).

VI X

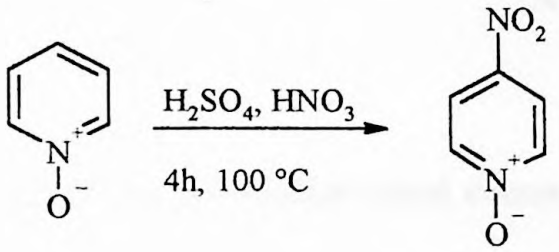
(9)

A different approach was to use 4-chloropyridine-*N*-oxide (XIII)[27-29] in the Ullman reaction to form *N*-(4-pyridyl-*N*'-oxide)succinimide (XI).



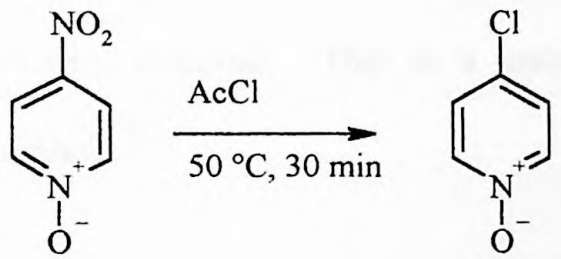
XI

The preparation of 4-chloropyridine-*N*-oxide was as follows:



(10)

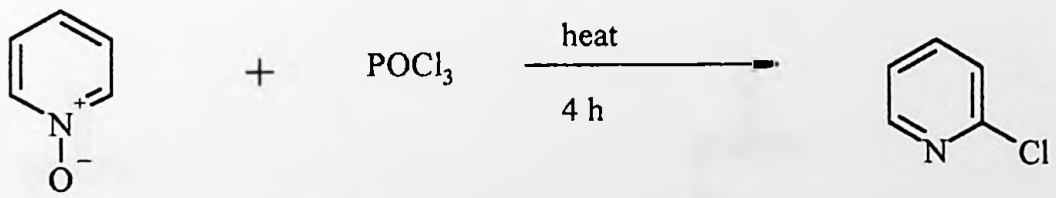
XII



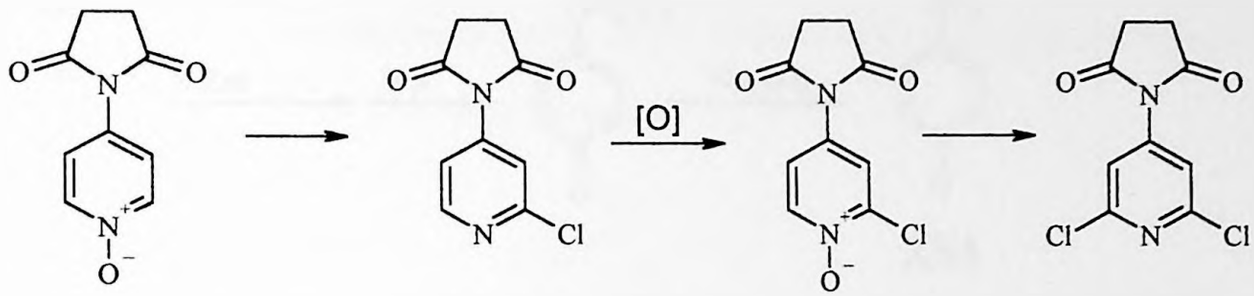
(11)

XIII

After the formation of XI , chlorination-deoxygenation[30-31] can be used to chlorinate the position alpha to the *N*-oxide, as in the representative conversion of pyridine-*N*-oxide to 2-chloropyridine.



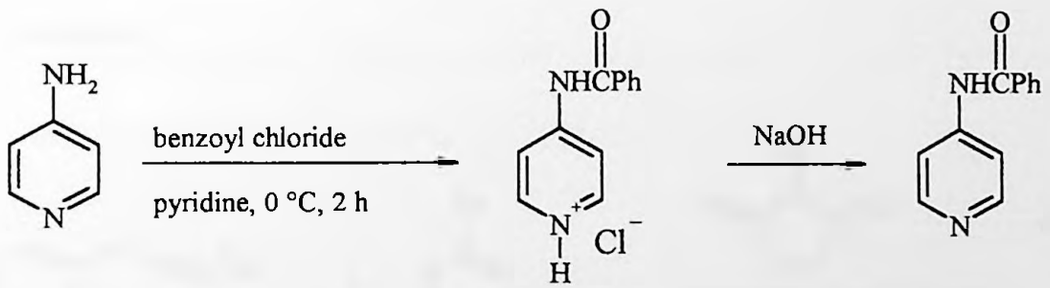
(12)



(13)

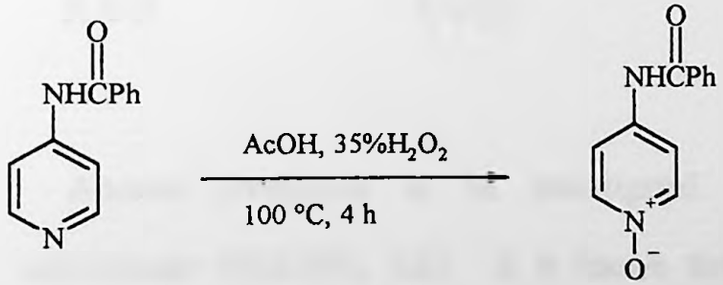
Once the monochlorinated compound was formed, the scheme in Equation 13 (above) could be used in a possible preparation of NDPyS.

Another synthetic route used 4-aminopyridine-*N*-oxide, compound XVI, as the starting material. This is a known compound and was prepared by the following method:[32,33]



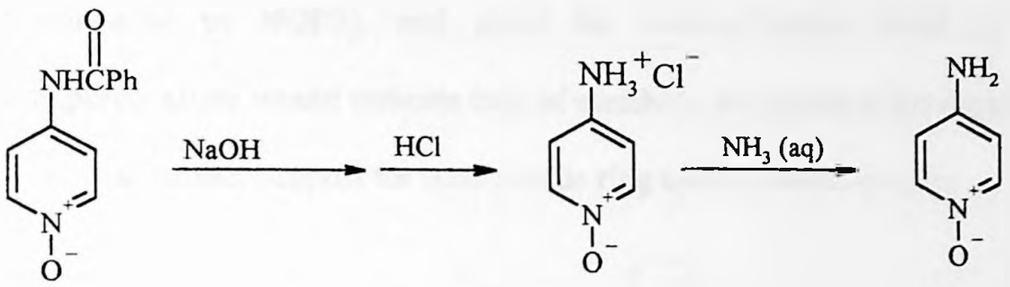
(14)

XIV



(15)

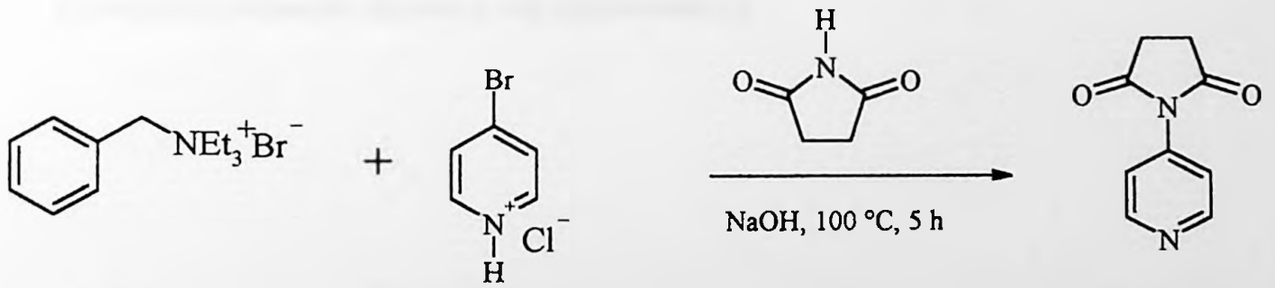
XV



XVI (16)

Once 4-aminopyridine-*N*-oxide was synthesized, it was proposed to proceed using reactions similar to Equation 13.

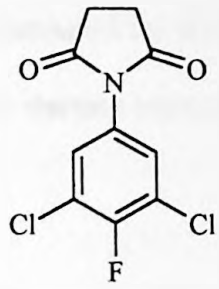
A final route was proposed using two known compounds, benzyltriethylammonium bromide, compound XVII, and 4-bromopyridine-HCl, compound XVIII, to form *N*-(4-pyridyl)succinimide (NPyS), compound XIX. It was prepared by the following reaction:[34]



XVII XVIII XIX (17)

Another compound to be investigated was *N*-(3,5-dichloro-4-fluorophenyl)-succinimide (NDCFPS, XX). It is known that *N*-(3,4,5-trichlorophenyl)succinimide (NTCPS) has nephrotoxic potential similar to NDPS,[18] and it was of interest to compare NTCPS and NDCFPS. The assumption was that NDCFPS would have similar nephrotoxicity. Since fluorine is similar in size to hydrogen (i.e., no steric difference

compared to NDPS), and since the carbon-fluorine bond is relatively strong, nephrotoxicity would indicate lack of metabolic oxidation at the 4-position of NDPS and provide further support for succinimide ring hydroxylation *in vivo*.



NDCFPS, XX

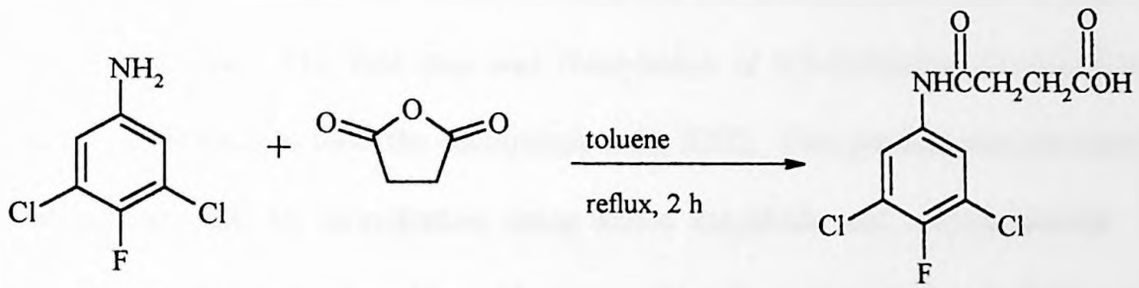
The proposed synthesis for NDCFPS was the same as the known route for NDPS (Equations 4 and 5).[1]

The objectives of this research were to prepare the desired succinimides in satisfactory yields, to compare various potential synthetic methods, and to further understand other correlations between structure and nephrotoxicity.

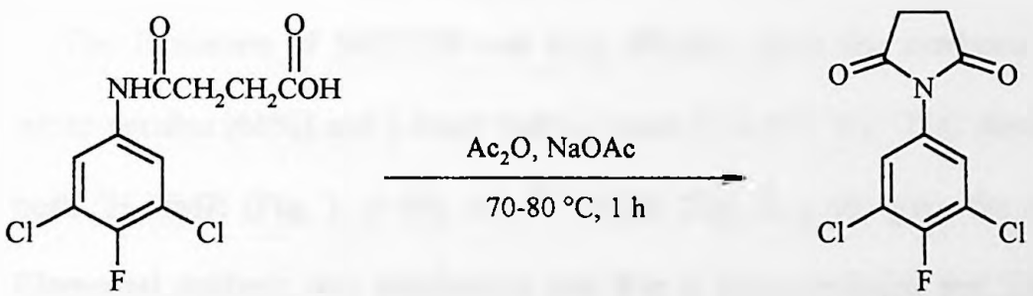
II. Results and Discussion

A. The synthesis of N-(3,5-dichloro-4-fluorophenyl)succinimide

The object of this synthesis was to determine whether *N*-(3,5-dichloro-4-fluorophenyl)succinimide could be prepared by the same procedure as NDPS[1] with 2,6-dichloro-4-fluoroaniline (XXI) as the starting material.

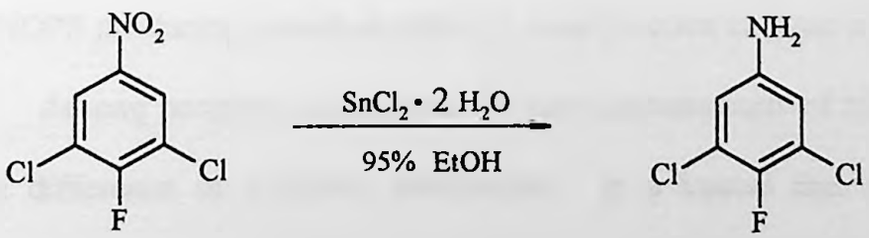


XXI XXII (18)



XX (19)

However, this route required preparation of 3,5-dichloro-4-fluoroaniline. This was accomplished by using 3,5-dichloro-4-fluoronitrobenzene as the starting material. The compound was reduced to the desired amino compound by tin(II) chloride dihydrate in 95% ethanol.[35]



(20)

Bellamy and Ou demonstrated that the use of the stannous chloride in 95% ethanol or ethyl acetate is quite effective and provides milder conditions than procedures utilizing concentrated aqueous HCl.[36] The reduction of 3.5-dichloro-4-fluoronitrobenzene was very efficient using this method (96%). The melting point was sharp and TLC showed one spot, indicating a pure product.

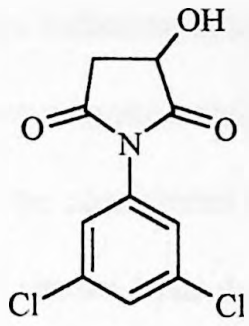
Once 3,5-dichloro-4-fluoroaniline was made, the procedure for NDPS (Equations 4 and 5) was used. The first step was *N*-acylation of 3,5-dichloro-4-fluoroaniline using succinic anhydride to form the succinamic acid, XXII. This product was converted to the succinimide, XX, by dehydration using acetic anhydride and sodium acetate. Acetic anhydride and the carboxylic acid presumably form a mixed anhydride, which is susceptible to nucleophilic attack and allows formation of the five-membered ring.

The formation of NDCFPS was very efficient using this synthesis, producing long white needles (68%) and a sharp melting point (183-185 °C). TLC showed one spot and both ^{1}H NMR (Fig. 1, p 44) and ^{13}C NMR (Fig. 2, p 45) gave the expected spectra. Elemental analysis was satisfactory and thus it was concluded that NDCFPS could be synthesized using the procedure for NDPS.

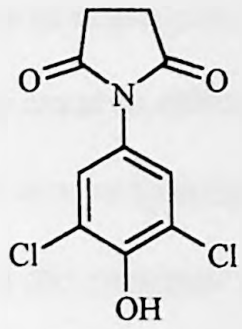
Once NDCFPS was synthesized, it was tested on male Fischer 344 rats to determine nephrotoxicity. The study determined that addition of a fluoro group to the 4-position of the phenyl ring produced a NDPS derivative that was non-nephrotoxic. NDCFPS induced only minor effects on renal function at doses up to 0.8 mmol/kg as opposed to NDPS producing a marked effect on renal function at doses as low as 0.4 mmol/kg.[37]

Among possible explanations for non-nephrotoxicity of NDCFPS relative to NDPS is a difference in oxidative metabolism. It is known that NDPS is metabolized to a

nephrotoxicant via oxidation on the succinimide ring (IA)[38-40] and that it also undergoes oxidation at the 4-position of the phenyl ring (IB).[41]



IA



IB

If the latter oxidation played a role in nephrotoxicity, the addition of a molecule or atom at the 4-position as in NDCFPS or *N*-(3,4,5-trichlorophenyl)succinimide (NTCPS) may slow oxidation and diminish nephrotoxicity. Such an effect was not expected since Harvison has shown that *N*-(3,5-dichloro-4-hydroxylphenyl)succinimide (IB), a potential metabolite from oxidation at the 4-position, is a non-nephrotoxicant *in vivo* at doses of 0.4 mmol/kg.[42] However, the fact that NTCPS is a nephrotoxicant similar in potency to NDPS indicates that diminishing oxidation at the 4-position of the phenyl ring cannot by itself explain the non-nephrotoxic characteristics of NDCFPS.

Another explanation could be that NDCFPS is removed from the body faster than NDPS. In previous studies, *N*-(3,5-difluorophenyl)succinimide (NDFPS) was found to be non-nephrotoxic in doses of up to 1.0 mmol/kg.[19] It was concluded that NDFPS metabolites were excreted much faster than NDPS metabolites from Fischer 344 rats,[43] and a larger amount of NDFPS was hydrolyzed and excreted relative to NDPS. Thus, it appeared the presence of fluorine might promote a faster rate of hydrolysis.

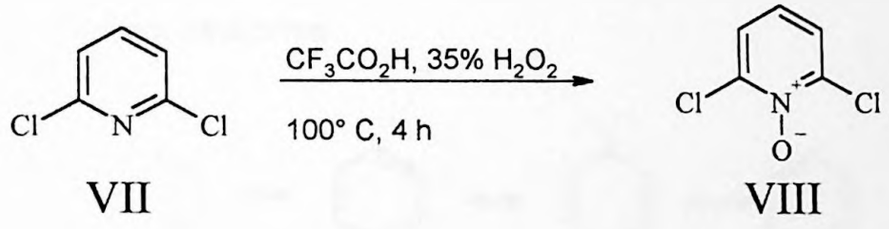
Relative rates of hydrolysis for NDPS and NDCFPS were examined under physiological conditions (pH = 7.4, 37 °C) by monitoring changes in the UV spectra at 290 nm. These studies indicated essentially identical rates of hydrolysis.[67] However, the possibility remains that enzyme-catalyzed hydrolyses may occur at different rates, and no definitive studies can be considered until the enzyme for *in vivo* hydrolysis is identified. Previous studies have shown hydrolysis products, such as the precursor succinamic acid, are less nephrotoxic than either the succinimide or the oxidiation metabolite.[17,40] Therefore, it is reasonable that an increased rate of hydrolysis of NDCFPS could partially explain the reduced nephrotoxicity of this compound.

However, this cannot fully account for the marked difference in nephrotoxicity. In the study of NDFPS, the percentage of NDFPS that was converted to the oxidized metabolite was comparable to NDPS.[44] If this was the sole factor involved in nephrotoxicity, a higher percentage of NDFPS should be converted to its oxidized metabolite than for NDPS. Even though the percentages of the oxidized metabolites are comparable, NDFPS metabolites are much weaker nephrotoxicants (~1.0 mmol/kg dose) than the NDPS metabolites (0.1 mmol/kg dose).[39,44] Therefore a combination of succinimide ring hydrolysis and an increase in excretion rate for metabolites capable of being nephrotoxicants could possibly account for the reduced nephrotoxic potential of fluorophenyl succinimides.

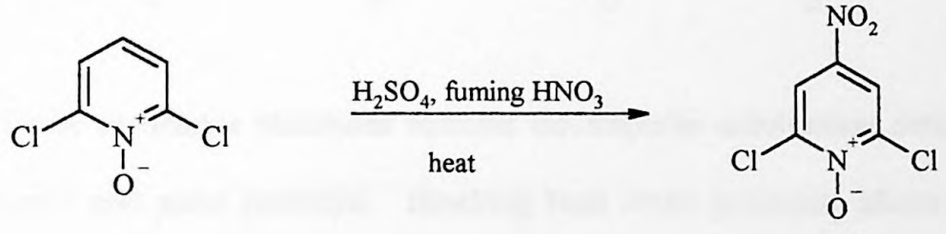
B. The synthesis of N-(3,5-dichloro-4-pyridyl)succinimide

1. Using 4-amino-2,6-dichloropyridine in the NDPS synthesis.

The preparation of the precursor for NDPyS was originally reported by Rousseau and Robins (Equations 1-3).[23]



VII VIII (1)



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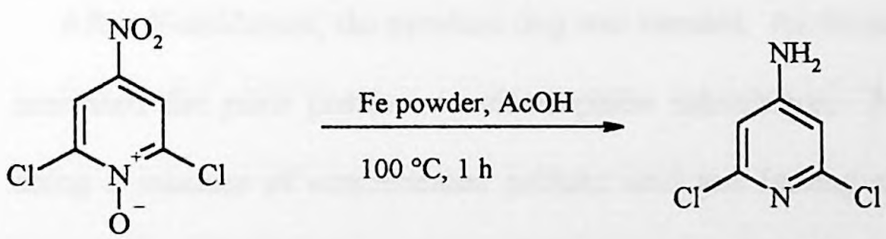
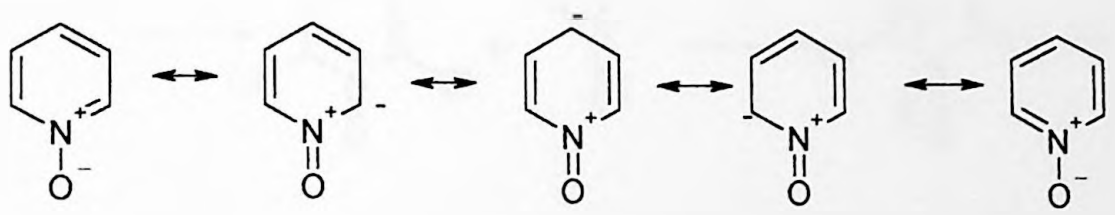


X (3)

The first step in preparing 4-amino-2,6-dichloropyridine was *N*-oxidation of 2,6-dichloropyridine. This was accomplished by using hydrogen peroxide and trifluoroacetic acid. Unlike other reagents used for *N*-oxidation of the pyridine ring (perbenzoic acid and perphthalic acid), this combination was effective in spite of steric or inductive effects

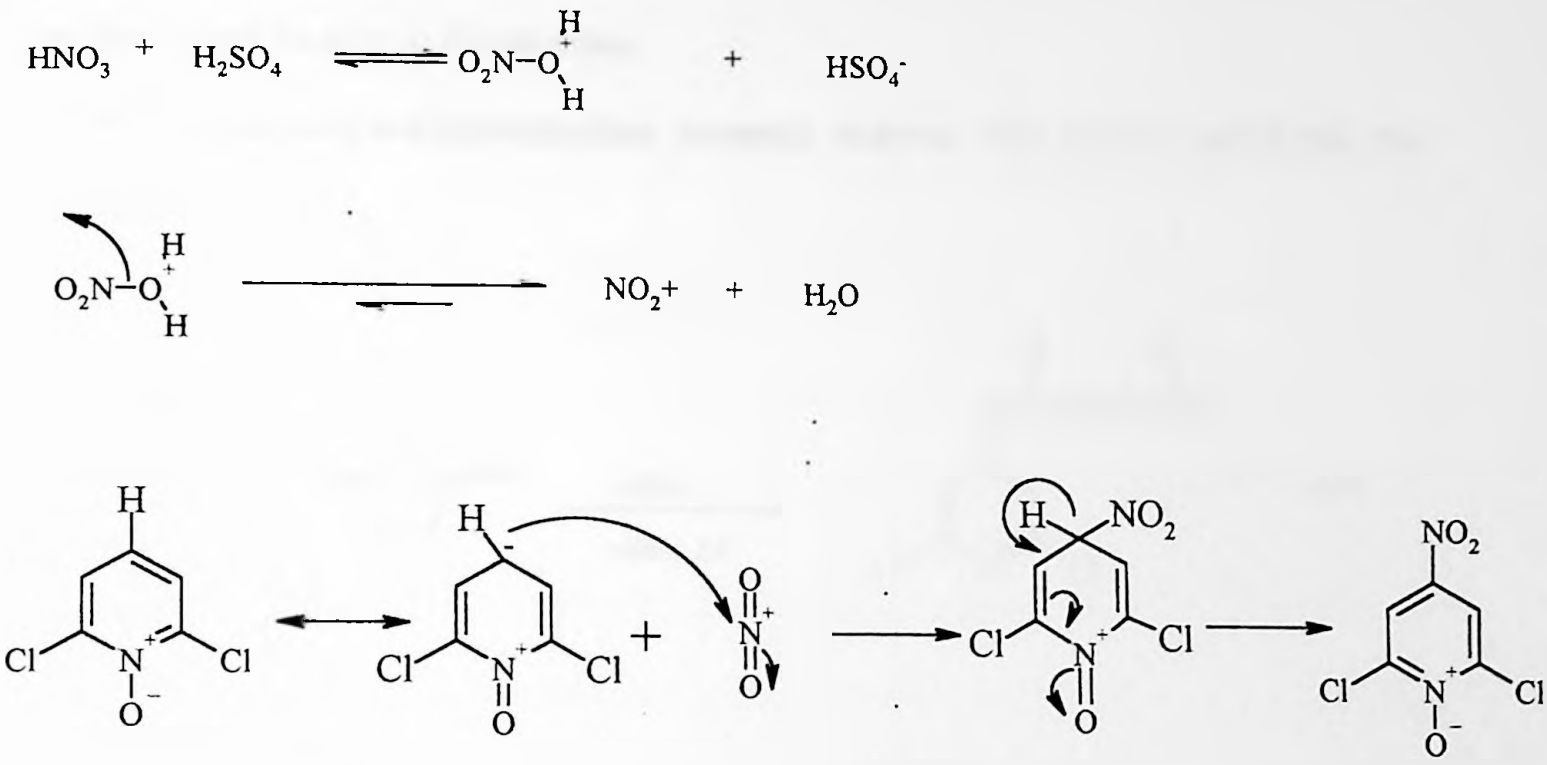
of alpha substituents.[45] Although the pyridine ring is an aromatic species, the nitrogen atom has a lone pair of electrons available for bonding without disturbing aromaticity.

Formation of the *N*-oxide caused the compound to become activated toward electrophilic substitution. Without *N*-oxidation, pyridine is relatively inert toward electrophilic substitution. Substitution at the *meta* position is achieved only under harsh conditions and the yields are poor.[46] *N*-Oxide activation can be accounted for by looking at resonance structures:



These resonance structures indicate electrophilic substitution should be directed to the *ortho* and *para* positions. Blocking both *ortho* positions, allows reaction at the *para* position only.

After *N*-oxidation, the pyridine ring was nitrated. As discussed above, the *N*-oxidation activated the *para* position to electrophilic substitution. Nitration was accomplished using a mixture of concentrated sulfuric acid and fuming nitric acid. The mixture of nitric acid and sulfuric acid formed the nitronium ion, which is sufficiently electrophilic to add to the aromatic ring. The formation of the nitronium ion and electrophilic addition is shown below:

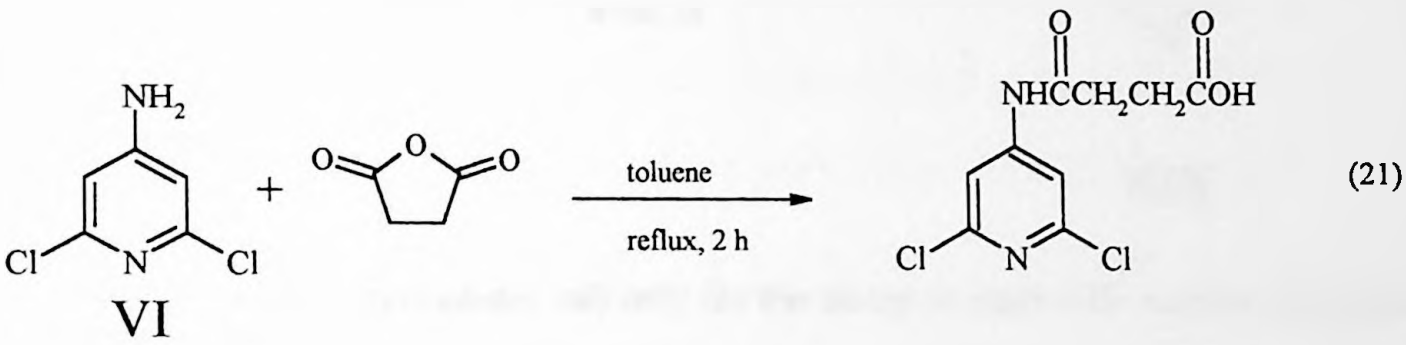


The yields for these reactions are usually around 80% and the reaction carried out gave similar results (76%).

Once 4-nitro-2,6-dichloropyridine-*N*-oxide was prepared, the compound was reduced to the desired 4-amino-2,6-dichloropyridine by a dissolving metal procedure that used glacial acetic acid and iron powder.[47] This procedure reduces aromatic nitro compounds very well. The procedure also reduced the *N*-oxide without reducing the aromatic ring. The proper reducing agent in this reaction was important since stronger reducing agents such as lithium aluminum hydride and sodium borohydride give various products without reduction of the nitro compound.[48] Another procedure that reduced this compound used Raney nickel under pressure;[23] however the procedure used was efficient in the reduction and more convenient since the reaction was not performed under pressure. The

experiment afforded ivory crystals recrystallized from water. However, the crude product could be used in the next step.

With 4-amino-2,6-dichloropyridine prepared, reaction with succinic anhydride was attempted.



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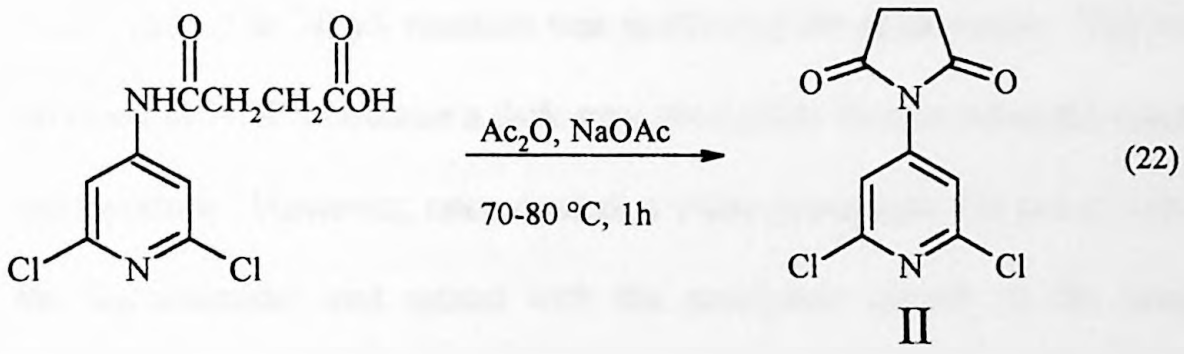

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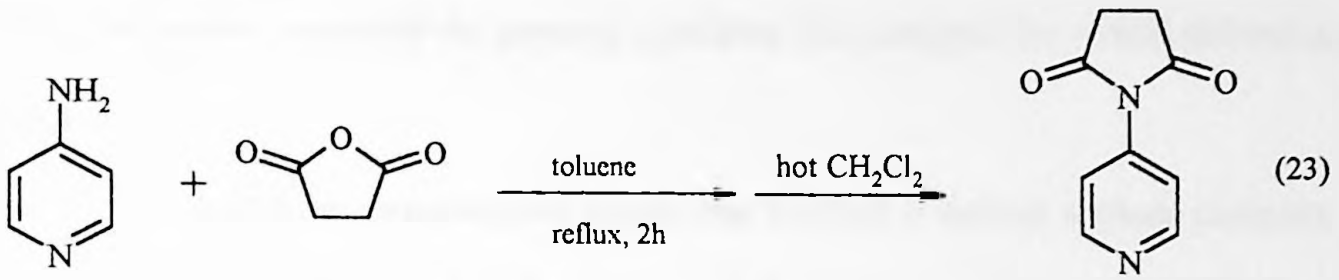
II

However, upon heating the solution, very little precipitate formed, unlike the NDCFPS procedure. After the solution was taken off the heat, a fluffy off white precipitate began to form. After placing in an ice bath, an enormous amount of product formed. The product was used in the next step. However, the dehydration did not work well as the precipitate was dark gray and the melting point range was large.

A workup presented in a U.S. patent by Werner[49] and a paper by Browne and Polya[50] described the preparation of *N*-(4-pyridyl)succinimide (NPyS) in a manner similar to the preparation of NDPS.



(23)

XIX

However, these procedures call only for the amine to react with succinic anhydride to form the succinimide; no succinamic acid intermediate was isolated as in the Fujinami technique. The NPyS reaction was performed for observation. The reaction seemed to proceed as NDPS because a dark gray precipitate formed when the reaction was at reflux temperature. However, once cooled, a white precipitate fell out of solution (presumably the succinimide) and mixed with the precipitate already in the reaction vessel. To recover the succinimide, the solids were extracted with hot methylene chloride and the methylene chloride was evaporated to afford a white solid. A melting point and ^{1}H NMR spectrum were obtained to characterize the succinimide.

Since this procedure worked for NPyS, it was assumed that 4-amino-2,6-dichloropyridine would work as well. As mentioned above, very little precipitate formed during the reflux period, but when the solution was cooled, an enormous amount of product formed. The solid was washed with methylene chloride and the solvent was evaporated to leave a white solid. Several analytical techniques were performed. The melting point was precise, both ^{1}H and ^{13}C NMR (Fig. 3 and 4, p 46 and 47 respectively) gave the expected results and mass spectrometry gave a correct mass to charge ratio.

However, TLC and elemental analysis did not give satisfactory results. Several attempts to purify the product by recrystallization were ineffective. It was necessary to use column chromatography to purify the product , yielding TLC material for which showed a single spot.

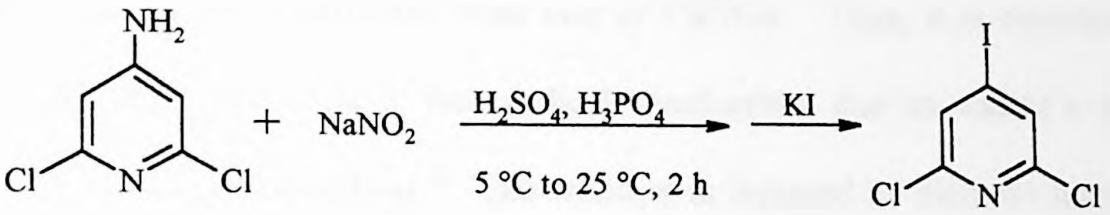
It is evident from experimental results that NDPyS is formed without isolation of the precursor succinamic acid and subsequent dehydration with acetic anhydride. The solubility of the succinamic acid in either toluene or xylene presumably allows dehydration under reflux conditions. This is reasonable since 5-membered rings form at relatively fast rates. Cyclization is prevented when the succinamic acid precipitates, as is the case for the precursors to NDPS and other succinimides lacking heteroatoms in the aryl ring.

To determine if prolonged heating of the solution would increase yield, the experiment was repeated several times at different reflux periods. Upon longer reflux times, the yield diminished. At 24 h, the solution was bright orange (not yellow as expected) and no precipitate could be recovered. No reflux period was performed under 2 h, so it was not determined if a shorter reflux period could produce a better yield.

After its characterization, NDPyS was administered to male Fischer 344 rats to determine its neprotoxicant effects. Although testing is in the early stages, it appears that NDPyS is non-nephrotoxic when compared to NDPS.[68] Definite conclusions await further experimental trials.

2. Preparation of NDPyS using 4-iodo-2,6-dichloropyridine.

Another synthetic route was attempted in preparing NDPyS by using 4-iodo-2,6-dichloropyridine and performing an Ullmann coupling reaction.[25]



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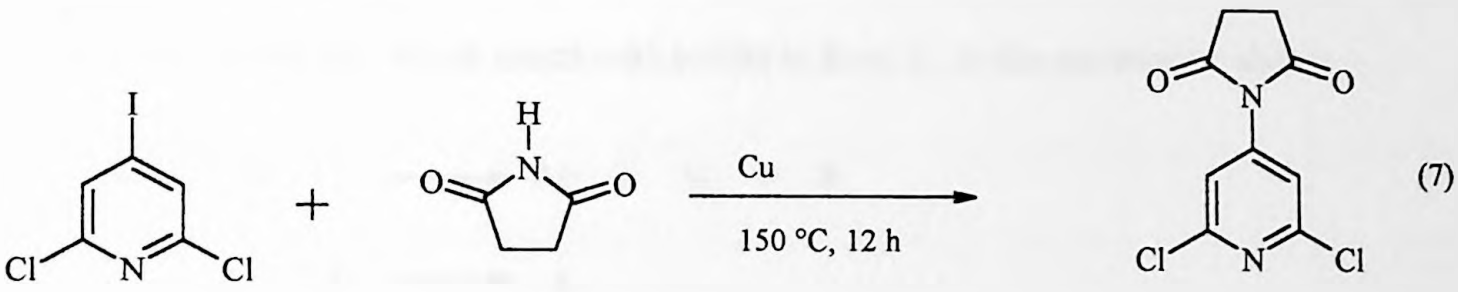


(7)

The iodo compound was prepared from 4-amino-2,6-dichloropyridine, a compound that has been discussed in some detail. The iodine atom was introduced to the aromatic ring by an iodo-de-diazonation reaction. The diazonium salt was prepared by adding 4-amino-2,6-dichloropyridine in acidic solution (H_2SO_4 in this procedure) to an acidic solution containing sodium nitrite in slight excess. Sodium nitrite in acidic solution formed nitrous acid, HNO_2, which causes the diazonation to proceed.

Once the diazonium ion was prepared, the solution (the salts are usually not isolated as a solid) was used to prepare the iodo compound. A slight excess of potassium iodide in water was added to the diazonium salt solution. The addition of potassium iodide caused the reaction to turn immediately purple (production of iodine) with formation of a

precipitate. The mechanism of this reaction is not fully understood. Although this reaction does not use a copper catalyst as in the Sandmeyer reaction for chloro- and bromo-de-diazonation, several mechanisms have been proposed that are similar to that for the Sandmeyer reaction. Waters determined that the redox potential of the I_2/I^- couple was not much different from that of Cu^{II}/Cu^{I}. Thus, it is reasonable that this reaction could proceed via a free radical mechanism due to iodide's ability to reduce the diazonium free radical.[52] The reaction is initiated by electron transfer from I^- to ArN_2^+. Later research showed that I_3^- was the actual attacking species, not I^-.[53] The iodide ion is oxidized to iodine, which reacts with iodide to form I_3^-, as the mechanism shows.

$$ArN_2^+ + I^- \longrightarrow Ar\bullet + N_2 + I\bullet$$

$$2\ I\bullet \longrightarrow I_2$$

$$I_2 + I^- \longrightarrow I_3^-$$

$$ArN_2^+ + I_3^- \longrightarrow ArI + N_2 + I_2$$

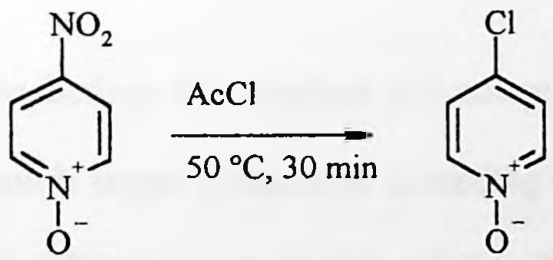
Once 4-iodo-2,6-dichloropyridine was made, it was used in an Ullmann reaction, typically a coupling reaction involving two aryl halides and a copper catalyst.[54] In this case, an active hydrogen compound was arylated by an aryl halide. The iodo compound was added to succinimide and a stoichiometric excess of copper (prepared by the method in the experimental section).

The compound that was prepared was long needles (74%). The melting point was sharp and TLC gave one spot. However, ^1H and ^{13}C NMR did not give the expected results. The number of peaks in ^1H NMR was correct (2), but the integration was wrong. Carbon NMR gave only 4 carbon peaks, less than expected. Also, mass spectrometry

gave a mass to charge ratio that was not consistent with that expected. Through trial and error, no structure could be determined that matched both the ^{13}C NMR and mass spectrometry. However, the large weight of the compound may indicate that the compound polymerized, or coupled into a large molecule.

3. NDPyS formation using 4-chloropyridine-*N*-oxide

This procedure was similar to the previous procedure that used the Ullmann reaction in an attempt to prepare NDPyS. This procedure used 4-chloropyridine-*N*-oxide as the starting material. This compound has been prepared by several different methods. A procedure by den Hertog and Combe[55] reacted 4-nitropyridine-*N*-oxide (discussed previously) with either 25% HCl for 4 h at 160 °C or conc. HCl for 24 h at reflux. However, the most efficient way to prepare this compound is a procedure by Ochiai[26] that used acetyl chloride to cause nucleophilic displacement of the nitro group by chloride.

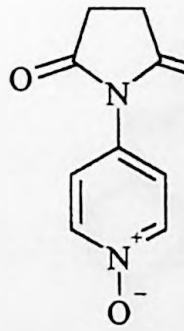


XII XIII (11)

In both of these procedures, the replacement of the nitro group occurs without loss of the *N*-oxide function. Several different melting points have been reported for this compound.[26,55] This discrepancy is probably due to polymorphism (the property of crystallizing in two or more forms with distinct structure) because a related compound, 4-

ethoxypyridine-*N*-oxide, has been shown to exist in three polymorphic forms.[56] During this research, the compound was synthesized using Ochiai's procedure and it appeared that the compound crystallized in two different structures due to the difference in melting points (167 °C and 152 °C, both with decomposition). Both of the compounds gave a single spot on TLC and the same ^{1}H NMR (Fig. 5, p 48)(the NMR was similar to the one found for 4-chloropyridine-*N*-oxide in an Aldrich NMR book).[57]

Once 4-chloropyridine-*N*-oxide was prepared, it was used in an Ullmann reaction, as described previously, to attempt to synthesize *N*-(4-pyridyl-*N*'-oxide)succinimide (XI).

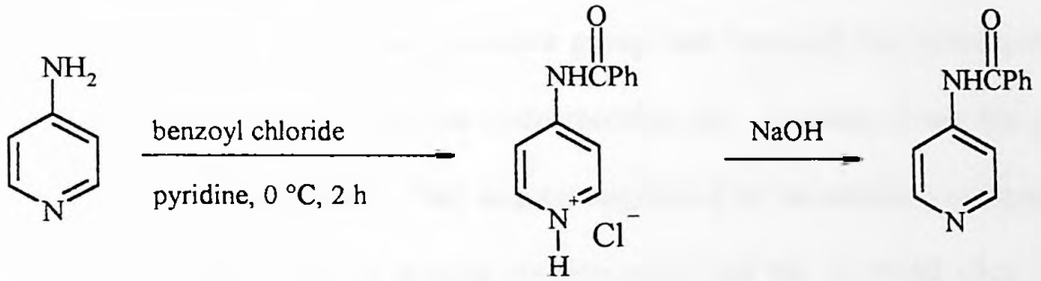


XI

As before, the reaction did not proceed as anticipated. The coupling reaction gave a much larger compound according to mass spectrometry. Also, TLC gave three spots, signifying an impure product. Without the formation of XI, the remainder of the synthesis outlined in the introduction could not be performed.

4. NDPyS formation using 4-aminopyridine-*N*-oxide

Since coupling reactions proved ineffective in the formation of *N*-(pyridyl)succinimides, 4-aminopyridine-*N*-oxide was prepared to use in the NDPS synthesis. The starting material was prepared by a procedure of Pentimalli.[33]



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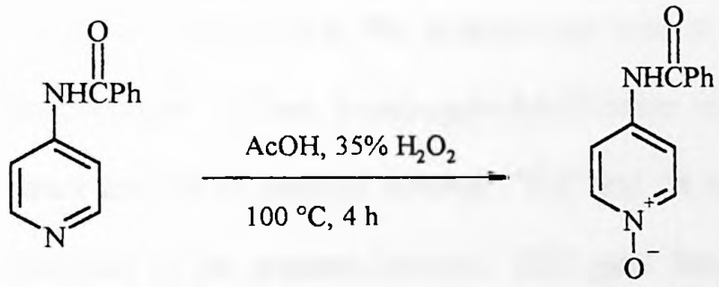
XIV



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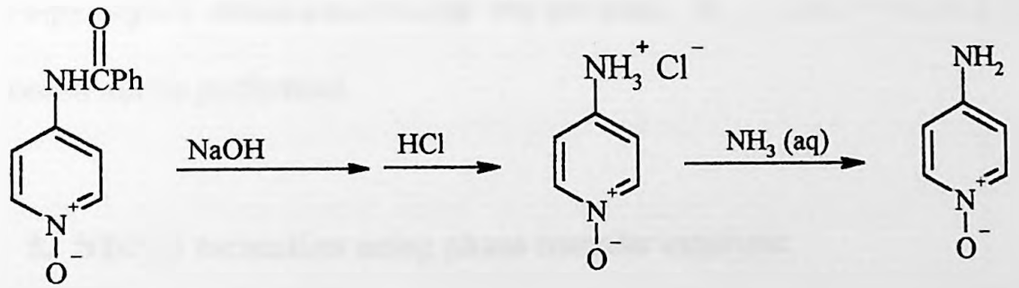
XV



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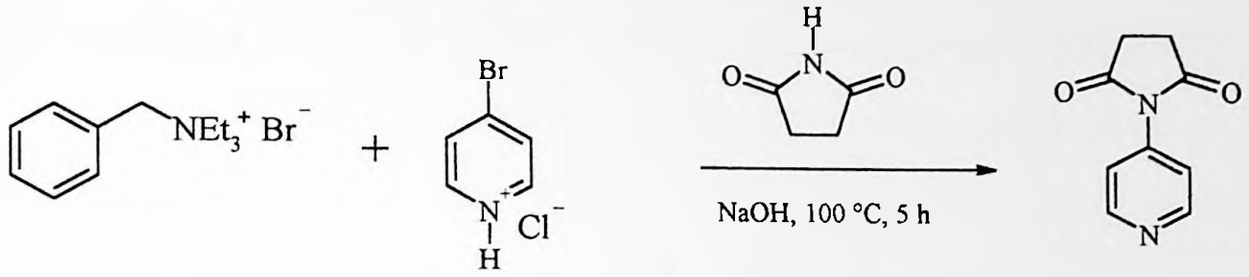
XVI

The procedure used 4-aminopyridine as the starting material and a benzoylation was performed to protect the amino group. The benzoylation was accomplished using benzoyl chloride. The protecting group allowed *N*-oxidation of the pyridine ring without affecting the amino group, which could be oxidized easily during *N*-oxidation. With the protective group in place, *N*-oxidation using glacial acetic acid and hydrogen peroxide was performed. Then the protective group was removed via hydrolysis. The product reported by Pentimalli was the hydrochloride salt. In order to use the product, the salt needed to be neutralized. This was accomplished by the addition of aqueous ammonia.[58] The final product had a precise melting point and the ^{1}H NMR (Fig. 6, p 49) was as expected.

Unfortunately, when this product was used in the NDPS synthesis, the results were unacceptable. When 4-aminopyridine-*N*-oxide reacted with succinic anhydride, only a small amount of product formed. TLC and ^{1}H NMR gave inconclusive results on the structure of the product formed. TLC gave three spots, signifying impurities and ^{1}H NMR gave unexpected peaks, signifying that the desired product was not made. Since *N*-(4-pyridyl-*N*'-oxide)succinimide was not made, the procedure outlined in the introduction could not be performed.

5. NDPyS formation using phase transfer catalysis.

A final synthetic procedure that was studied used 4-bromopyridine-HCl and benzyltriethylammonium bromide in a phase transfer catalysis reaction to form NPyS.[34]



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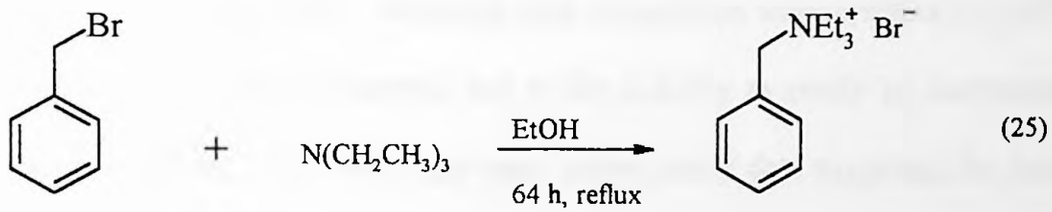
XVII XVIII XIX

The procedure for preparing benzyltriethylammonium bromide was a simple nucleophilic substitution between benzyl bromide and triethylamine.



(25)

XVII

However, 4-bromopyridine-HCl was not as easily prepared. Since a procedure for 4-bromopyridine-*N*-oxide was available, this was the compound that was attempted, since the *N*-oxide could be easily removed. The formation of the bromo compound mimics the formation of 4-chloropyridine-*N*-oxide.[59] However, due to the physical properties of both 4-bromopyridine-*N*-oxide and 4-bromopyridine, these compounds were not successfully synthesized. Both compounds readily decompose upon standing and 4-bromopyridine is fairly unstable at room temperature (mp 27.5-30 °C). Also 4-bromopyridine polymerizes at 135 °C, which creates additional problems.[60] When 4-nitropyridine-*N*-oxide was added to acetyl bromide, the solid that resulted began to

decompose almost immediately. The solid could not be analyzed and therefore this route was disregarded.

C. Summary

Both NDCFPS and NDPyS were prepared in good yields by the procedure used previously for NDPS. NDCFPS formation mimicked NDPS formation, however, preparation of NDPyS was somewhat different due to the solubility of the intermediate succinamic acid. Since the acid never precipitated from solution (like in NDCFPS), the reflux temperature of the solvent allowed dehydration and formation of the ring without using a mixed anhydride. Although both compounds were synthesized, NDPyS gave an unacceptable elemental analysis due to the inability to purify by recrystallization, even though ^{1}H NMR, ^{13}C NMR, and mass spectrometry data suggested the formation of the product. Column chromatography was then used for further purification, yielding product for which TLC gave a single spot.

Coupling reactions that were studied did not give good results. Using mass spectrometry as a guide, the products formed by either the Ullmann reaction were much larger in size than anticipated. The desired products may have formed but apparently reacted further, giving large molecular weight compounds. Although *N*-(4-pyridyl-*N*'-oxide)succinimide was never successfully synthesized, this compound may be a building block for another route in NDPyS synthesis. If this compound could be made, chlorination-deoxygenation (mentioned in the introduction but never performed) could give an efficient way to build NDPyS from *N*-(4-pyridyl-*N*'-oxide)succinimide.

Both compounds were studied to determine their nephrotoxic potential. NDCFPS was studied extensively and it was determined that it was a non-nephrotoxicant compared to NDPS.[37] However, the exact reason for its non-nephrotoxicity was not determined, although it may be a combination of faster hydrolysis of the succinimide ring and the faster clearance of the compound from the rats. NDPyS has not been studied enough to draw any conclusion, but initial studies have shown NDPyS to have similar nephrotoxic characteristics as NDCFPS.[68]

III. Experimental Section

A. Analytical Techniques

All melting points were determined using a calibrated Mel-Temp device.

^{1}H NMR spectra were obtained using a Varian XL-200 spectrometer operating at 200 MHz with various solvents and tetramethylsilane as the internal standard.

Mass spectrometry was performed using a Finnigan MAT LCQ LC/MS system.

Thin-Layer Chromatography (TLC) was performed using silica gel 254F plates and UV detection.

Column Chromatography was performed using a column packed with a slurry of Silica Gel 60 and chloroform. A thin layer of cotton was placed on the bottom of the column and a cut piece of filter paper, topped with sea sand, was placed on top of the mixture. Ethyl acetate was utilized as the eluting solvent while progress down the column was monitored using TLC with ethyl acetate as the eluting solvent.

Elemental analysis was performed by Atlantic Microlab, Inc. of Atlanta, GA.

B. Materials

3,5-Dichloro-4-fluoronitrobenzene (Hoechst); all other reagents were purchased from Aldrich Chemical, Fisher Scientific, Acros, or MCK and were the highest grade avaliable.

C. 3,5-Dichloro-4-fluoroaniline (XXI).[35]

3,5-Dichloro-4-fluoronitrobenzene, (8.247 g, 39.31 mmol), tin(II) chloride (45.097 g, 199.8 mmol), and 95% ethanol (80 mL) were combined in a 250-mL two-necked round-bottom flask fitted with a water condenser, a nitrogen inlet tube, and a magnetic stirring

bar. A mercury-filled bubbler was attached to the water condenser to maintain a nitrogen atmosphere. The reaction vessel was purged with nitrogen, and the reactants were heated to 70 °C. The solids dissolved giving a pale yellow solution. After 30 min, the heating was stopped and the reaction was allowed to cool to room temperature. The solution was poured onto ice and a silky white precipitate formed. The mixture was neutralized using 5% sodium bicarbonate solution and then the product was extracted with ethyl acetate (3 x 125 mL). The organic layer was separated from the aqueous layer, washed with brine, and dried using anhydrous sodium sulfate. The solvent was removed using a rotary evaporator affording off-white needles (6.77 g, 96%) with a melting point of 94-96 °C (lit. 104 °C).[61]

D. *N-(3,5-Dichloro-4-fluorophenyl)succinamic acid (XXII).*[1]

3,5-Dichloro-4-fluoroaniline (6.77 g, 37.65 mmol), succinic anhydride (5.211 g, 51.75 mmol), and toluene (75 mL) were added to a 250-mL round-bottom flask containing a magnetic stirring bar and fitted with a water condenser. The reactants were heated at reflux for 2 h. Almost immediately upon heating, the light yellow solution afforded a fluffy white precipitate that was too thick for the stirring bar to work. After two hours, the white precipitate was collected using vacuum filtration (10.39 g, 98%), mp 187.5-189 °C.

E. N-(3,5-Dichloro-4-fluorophenyl)succinimide (XX).[1]

N-(3,5-Dichloro-4-fluorophenyl)succinamic acid (5.1488g, 18.4 mmol), sodium acetate (0.5199 g), and acetic anhydride (26.5 mL, 281 mmol) were added to a 100-mL

round-bottom flask fitted with a water condenser and a magnetic stirring bar. The reactants were heated at 70-80 °C. The solids dissolved in the liquid upon heating and the mixture became light yellow. After 1 h, the solution was allowed to cool, then poured into approximately 75 mL of cold distilled water. This treatment caused a chalky white precipitate to form. The solid was collected using vacuum filtration and then recrystallized using 95% ethanol, producing long white needles (3.274 g, 68%), mp 183-185 °C (lit. 173-174 °C)[62] ; TLC (100% EtOAc and 80/10/10 $CHCl_3$, toluene, EtOAc) showed one spot; ^{1}H NMR (acetone-d_6): δ 2.9 (s, 2H), 7.3-7.5 (d, 1H); ^{13}C NMR: δ 29 (methyl C), 123, 128, 152,157,176 (aromatic C).

Anal. Calcd. for $C_{10}H_6Cl_2FNO_2$: C, 45.83; H, 2.31; N, 5.35; Cl, 27.06; F, 7.25. Found: C, 45.88; H, 2.34; N, 5.37; Cl, 27.06; F, not performed by lab.

F. 2,6-Dichloropyridine-N-oxide (IV). [23]

2,6-dichloropyridine (III), (2.254 g, 15 mmol), trifluoroacetic acid (29.769g , 259 mmol), and 35% hydrogen peroxide (3.4 mL) were added to a 100-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. The reaction vessel was heated on a boiling water bath for 4 h. During this time, the solution turned from colorless to pale orange. The solution was allowed to cool to room temperature, then was poured into distilled water (215 mL). This caused a precipitate to form (unreacted 2,6-dichloropyridine). This was collected and the filtrate was evaporated to a small volume using a rotary evaporator while keeping the temperature of the filtrate under 30 °C. Once this was accomplished, the remainder of the aqueous solution was added to chloroform (75 mL). Potassium carbonate was added to the mixture until the evolution of carbon

dioxide ceased. The layers were separated. The organic layer was dried using anhydrous sodium sulfate and the solvent was evaporated leaving a white, crystalline solid, IV, (2.03 g, 81%), mp 137-139 °C (lit. 139-140 °C).[23]

G. 2,6-Dichloro-4-nitropyridine-N-oxide (V).[23]

2,6-Dichloropyridine-*N*-oxide (IV) (4.250 g, 25.9 mmol), concentrated sulfuric acid (110 mL), and fuming nitric acid (55 mL) were added to a 500-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. The mixture was heated on a boiling water bath. During heating, the solution changed from light yellow to orange-yellow in color. After 2 h, the solution was allowed to cool and then it was poured on ice (approx. 250 g). This changed the color of the solution to yellow-green. The solution was neutralized with 30% ammonium hydroxide while keeping the solution under 30 °C. This was achieved by placing the solution in an acetone-ice bath. Once neutralization was achieved, a light yellow precipitate formed. To maximize crystallization, the mixture was placed in a refrigerator for 14 h. The product was recovered using vacuum filtration and recrystallized from water, affording light yellow crystals, V, (4.124 g, 76%), mp 175-177 °C (lit. 177-178.5 °C).[23]

H. 4-Amino-2,6-dichloropyridine (VI).[47]

2,6-Dichloro-4-nitropyridine-*N*-oxide (V) (4.125 g, 19.7 mmol) and glacial acetic acid (55 mL) were added to a 250-mL round-bottom flask equipped with a water condenser and a magnetic stirring bar. Iron powder (2.487 g, 44.6 mmol) was added slowly through the condenser. Once this was done, a few milliliters of glacial acetic acid were used to wash any excess iron from the condenser wall. The reaction was heated on a boiling water bath. During heating, the reaction changed from light yellow (before the addition

of iron) to black (upon addition of the iron and the beginning of the reaction) to burnt orange (at the end). After 1 h, the thick orange mixture was basified using 3 N sodium hydroxide, causing the color of the solution to change to red-brown. Ether (4 x 100 mL) was used to extract the product from the aqueous solution. The organic extract was washed with brine, dried using anhydrous sodium sulfate, and the solvent was evaporated to afford an ivory precipitate. The crude product was recrystallized from water yielding a white product , VI (2.863g, 89%), mp 171.5-172 °C (lit. 172-173 °C).[23]

I. Attempted Preparation of N-(3,5-Dichloro-4-pyridyl)succinamic acid. [1]

Note: The following procedure was the same as for section D (reaction 1).

4-Amino-2,6-dichloropyridine (0.890 g, 5.46 mmol), succinic anhydride (0.548 g, 5.44 mmol), and toluene (30 mL) were placed in a 100-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. Unlike in section D, no precipitate formed when the mixture was heated. After the reaction was cooled to room temperature, however, a large amount of fluffy, white precipitate formed. The flask was immersed in an ice bath to maximize crystallization. The product was collected using vacuum filtration (0.987 g, 69%). The melting point was 106-110 °C, with some sublimation. This product was determined to be a mixture of products in which the succinimide formed along with other unidentified compounds.

J. N-(3,5-Dichloro-4-pyridyl)succinimide (II).

1. Method 1. [1]

Note: The following procedure was the same as for section E (reaction 2).

N-(3,5-Dichloro-4-pyridyl)succinamic acid (0.987 g, 3.75 mmol), sodium acetate (0.1026 g), and acetic anhydride (7 mL) were placed in a 100-mL round-bottom flask

fitted with a water condenser and a magnetic stirring bar. Upon heating the solution turned black, and when the solution was poured into water, a black oil formed. Upon scratching, a gray, granular precipitate formed. Water-ethanol (1:1 mixture) was used for recrystallization, and the ivory rods (0.268g, 29%) had a melting point of 47-63 °C; TLC showed two spots with neither spot having the correct R_f value in 100% EtOAc.

2. Method 2. [49,63]

Note: The following procedure was the same as for method 1 in this section until specified.

4-Amino-2,6-dichloropyridine (1.036 g, 6.36 mmol), succinic anhydride (0.617 g, 6.11 mmol), and toluene (35 mL) were added to a 100-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. Once the solid was collected, the succinimide was extracted with hot methylene chloride (3 x 100 mL). The extract was dried with anhydrous sodium sulfate and the solvent evaporated to afford white prisms (0.945 g, 63%). Purification by column chromatography; mp 171-172.5 °C, TLC showed one spot (100% EtOAc); ^{1}H NMR (chloroform-d/DMSO-d_6) δ 3.1 (s, 2H), 6.5 (s, 1H); ^{13}C NMR δ 29 (methyl C), 107, 150, 157, 171 (aromatic C); mass spectrometry, m/z 247.

3. Method 3. [24]

Note: The copper catalyst was prepared by dissolving cupric sulfate (5 g) in distilled water (60 mL) then adding zinc powder (1.29 g) slowly while keeping the solution in an

ice bath. An immediate brown precipitate formed. This was collected using vacuum filtration and washed with acetone.[64]

2,6-Dichloro-4-iodopyridine (0.262 g, 0.95 mmol), succinimide (0.055 g, 0.56 mmol), and copper catalyst (0.100 g) were mixed in a 25-mL round-bottom flask equipped with a long glass tube and a magnetic stirring bar. The mixture was heated for 12 h at 150 °C. Long, white needles formed high on the inside surface of the tube (0.100 g, 74%), mp 149-152 °C; TLC showed one spot (100% EtOAc, and 80/10/10 $CHCl_3$, toluene, and EtOAc); ^{1}H NMR (DMSO-d_6) δ 2.8 (s, 2H), 7.7 (s, 3H); ^{13}C NMR δ 30 (methyl C), 108, 132, 151, (aromatic C). Since NMR gave results that were inconsistent with the structure desired, no further examination was done.

K. 4-Iodo-2,6-dichloropyridine (VII). [25]

Note: Preparation of 4-amino-2,6-dichloropyridine was performed by the methods described in sections F, G, and H.

4-Amino-2,6-dichloropyridine (0.522 g, 3.2 mmol) was added to concentrated sulfuric acid (10 mL). This mixture was cooled to 5 °C in an acetone-ice bath. Another mixture containing finely powdered sodium nitrite (0.276 g, 4.0 mmol) and concentrated sulfuric acid (5 mL) was also cooled to 5 °C. The sodium nitrite was added to the cold sulfuric acid slowly to assure that nitrogen oxides were not produced. Once both solutions were cooled, the sodium nitrite solution was added to the 4-amino-2,6-dichloropyridine solution, which turned the mixture light golden brown. 85% Phosphoric acid (12 mL) was slowly added using a separatory funnel while keeping the mixture in the ice bath. Phosphoric acid was added to liberate nitrous acid from the nitroslysulfuric acid present.

the mixture was allowed to warm to room temperature and then it was allowed to sit for 2 h. During this time, the solution turned from golden brown to orange in color. The solution was poured into ice water (250 mL), treated with urea (6.5 g) to destroy excess nitrous acid, and filtered. The filtrate was treated with a solution of potassium iodide (0.618 g, 3.7 mmol) in distilled water (12 mL). This caused an immediate brown precipitate to form, along with gas (presumably nitrogen). The mixture was heated until the evolution of gas ceased. Sodium bisulfite was added to remove any iodide present. The remaining precipitate was collected using vacuum filtration and washed with water, yielding a light brown solid (0.536 g, 61%), mp 149.5-153°C. The product was not recrystallized.

L. 4-Nitropyridine-N-oxide (XII). [26]

Pyridine-*N*-oxide (9.65 g, 104.9 mmol), concentrated sulfuric acid (30 mL), and fuming nitric acid (9 mL) were added to a 100-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. The mixture was heated (120-130 °C) for 3.5 h. The solution turned from pale yellow to bright gold. Once the time elapsed, the solution was added to ice (100 g) and then neutralized using sodium carbonate. Once sodium sulfate began to form, neutralization was stopped and the precipitate was filtered and dried. The filtrate was extracted with chloroform (3 x 125 mL) and the solvent was evaporated affording yellow crystals. The two solids were combined and recrystallized from acetone yielding light yellow crystals , XII (9.305 g, 64%), mp 157-160 °C (lit. 159 °C).[26]

M. 4-Chloropyridine-N-oxide (XIII). [26]

4-Nitropyridine-*N*-oxide (2.076 g, 14.8 mmol) and acetyl chloride (11 mL) were added to a 50-mL round-bottom flask equipped with a water condenser and a magnetic stirring bar. The mixture was heated at 50 °C. During heating, clumps of solid floated around in the acetyl chloride. After 30 min, the mixture was poured into ice water (150 mL) and basified using sodium carbonate. The product was extracted using chloroform (3 x 100 mL). Evaporation of the solvent afforded white crystals, XIII (1.12 g, 58%), mp 151 °C (dec.) (lit. 169.5°C (dec.),[26] 152.5-153.5°C (dec.)[55]); ^{1}H NMR (chloroform-d) δ 7.1-7.3 (m, 1H), 8.1-8.3 (m, 1H). The two melting points are probably due to polymorphism; crystal structures are unknown.

N. N-(4-pyridyl-N'-oxide)succinimide (XI).

1. Method 1.[24]

Note: For preparation of the copper catalyst and specific reaction conditions, refer to method 4, section J.

4-Chloropyridine-*N*-oxide (0.185 g, 1.43 mmol), succinimide (0.047 g, 0.47 mmol.), and copper catalyst (0.078 g) afforded long, white needles (0.008 g, 9%) with a melting point of 160-165 °C; TLC gave three spots (with such a low yield, it was of little use to determine if the product was formed with three spots appearing); mass spectrometry: m/z 553.2.

2. Method 2.

Note: This was the same procedure used in method 3, section J.

4-Aminopyridine-*N*-oxide (0.375 g, 3.4 mmol), succinic anhydride (0.233 g, 2.3 mmol), and toluene (8 mL) were mixed together and heated. An off-white precipitate was collected from methylene chloride (0.174 g, 36%), mp 104-107 °C; TLC showed three spots with virtually the same diameter (i.e., the same amount of each impurity was present, difficult to determine if any of the spots were useful).

O. 4-(N-Benzoyl)aminopyridine (XIV). [33]

4-Aminopyridine (11.274 g, 119.9 mmol), and pyridine (85 mL) were added to a 200-mL round-bottom flask fitted with a magnetic stirring bar. The reactants were cooled to 0 °C using an acetone-ice bath. Benzoyl chloride (20.657 g, 147 mmol) was added slowly. Upon initial addition, the colorless solution afforded a white solid, too thick for the stirring bar to work. Once all of the benzoyl chloride was added, the mixture was allowed to sit at 0 °C. After 2 h, the solid was collected and washed with ethyl ether. At this point, the product was the hydrochloride derivative. To dissolve the hydrochloride salt, it was added to boiling water (300 mL). Once the solid was dissolved, the filtrate was cooled and neutralized with sodium bicarbonate. Upon neutralization, a white solid formed. The solid (XIV) was collected using vacuum filtration and recrystallized using water (14.264 g, 60%), mp 205-207 °C (lit. 202 °C). [33]

P. 4-(N-Benzoylamino)pyridine-N-oxide (XV).

Note: This was a combination of two oxidation procedures.[23,26]

4-(*N*-Benzoylamino)pyridine (9.940 g, 50.2 mmol), glacial acetic acid (250 mL), and 35% hydrogen peroxide (30 mL) were placed in a 500-mL round-bottom flask fitted with

a water condenser and a magnetic stirring bar. The reactants were mixed and heated on a boiling water bath. During heating, the solution turned from colorless to yellow. After 4 h, the reaction mixture was poured into distilled water (450 mL). The resulting solution was evaporated to a small volume. To the residual material, chloroform (200 mL) was added and potassium carbonate was added to basify the solution, resulting in a fluffy, white precipitate. The solid, XV, was collected using vacuum filtration (9.513 g, 88%), mp 246-248 °C (lit. 247-250 °C).[33]

Q. 4-Aminopyridine-N-oxide (XVI). [58]

4-(*N*-Benzoylamino)pyridine-*N*-oxide (9.510 g, 44.4 mmol) was added to boiling 10% sodium hydroxide solution. After 5 min, concentrated hydrochloric acid was added until the mixture was acidic and then concentrated aqueous ammonia was added until it was basic. This caused a white, almost translucent precipitate, XVI, (4.754 g, 97%), mp 112-114 °C; TLC showed one spot (100% EtOAc, and 80/10/10 $CHCl_3$, toluene, EtOAc); ^1H NMR (DMSO-d_6) δ 3.35 (s, 2H), 7.4-7.6 (m, 1H), 7.9-8 (m, 1H).

R. Benzyltriethylammonium bromide (XVII). [65]

Benzyl bromide (10.58 g, 61.9 mmol), triethylamine (6.789 g, 67.2 mmol), and 95% ethanol (15 mL) were placed in a 100-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. The mixture was heated at reflux and the yellow solution turned dark yellow. After 64 h, the solution was allowed to cool to room temperature and ethyl ether (60 mL) was added. This caused the salt, XVII, to precipitate

as a white solid (15.740 g, 93%), mp 190-192 °C with decomposition (lit. 194 °C, dec.)[66].

S. 4-Bromopyridine-N-oxide.[55]

Note: The preparation of 4-nitropyridine-*N*-oxide was described in section L.

4-Nitropyridine-*N*-oxide (2.445 g, 17.5 mmol) and acetyl bromide (20 mL) were mixed at 0 °C in a 100-mL round-bottom flask fitted with a water condenser and a magnetic stirring bar. Once the solid dissolved, the solution was heated at 50 °C for 30 min and then at 75 °C for 15 min. During this time, the solution became significantly more viscous. Once the total time elapsed, the mixture was added to ice and neutralized with chunks of ammonium carbonate. The neutralized solution was extracted with chloroform (3 x 100 mL). The chloroform solution was washed with 5% sodium bicarbonate solution (2 x 100 mL). This caused the orange chloroform solution to lose most of its color. The chloroform was evaporated to afford an orange precipitate (0.642 g, 21%). This weight, however, was inaccurate due to the inability to dry the precipitate. Due to this, a melting point was not taken.

IV. NMR Spectra

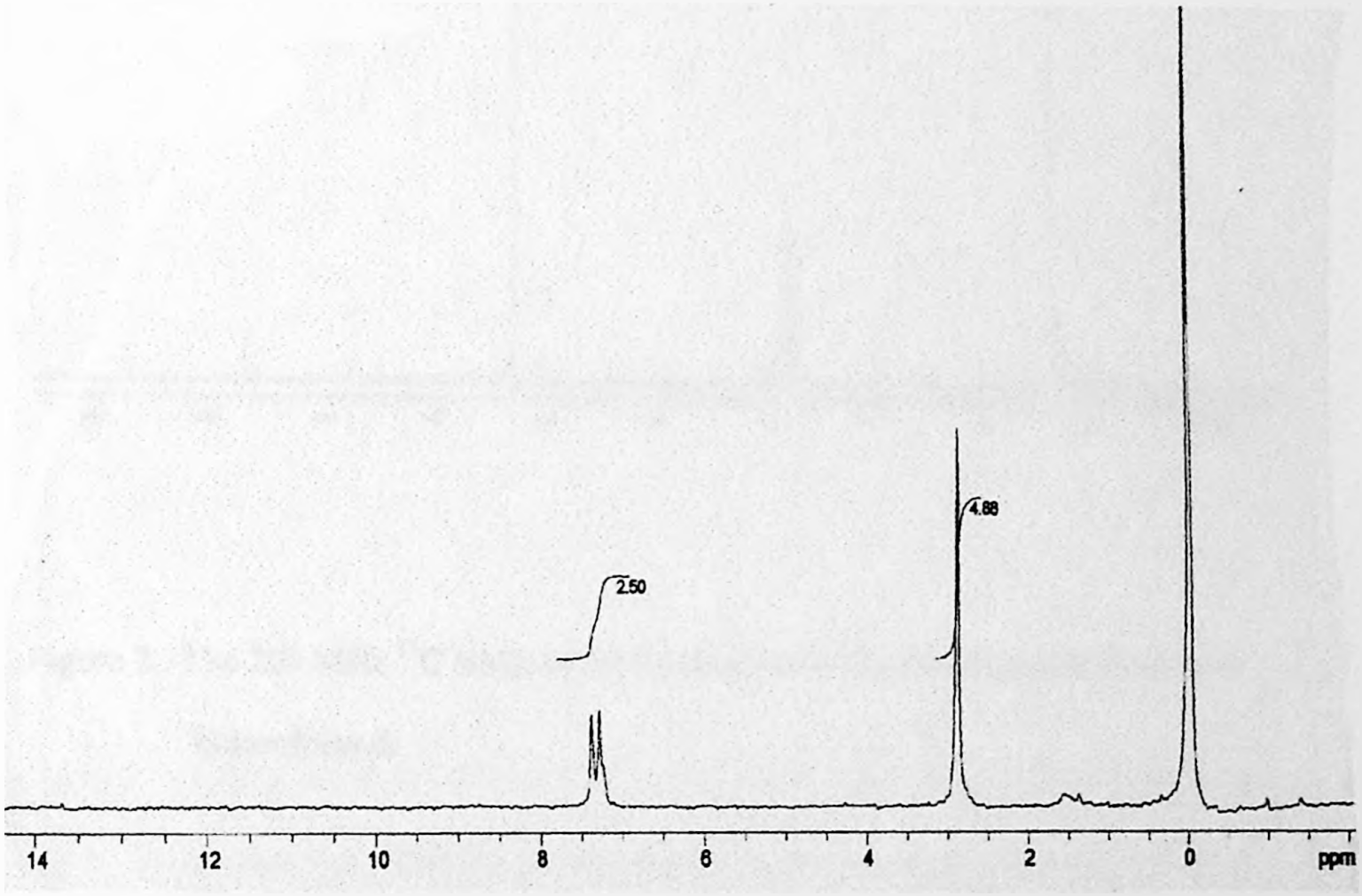


Figure 1. The 60 MHz ^{1}H NMR of *N*-(3,5-Dichloro-4-fluorophenyl)succinimide in acetone-d_6.

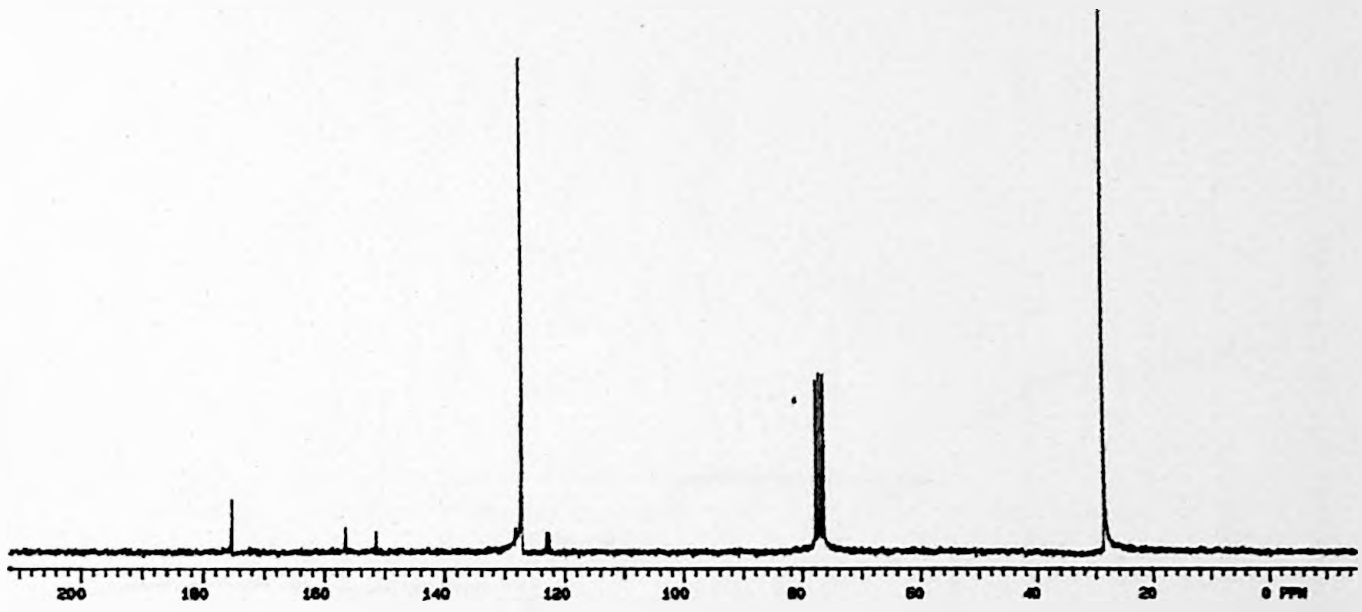


Figure 2. The 200 MHz ^{13}C NMR of *N*-(3,5-dichloro-4-fluorophenyl)succinimide in chloroform-d.

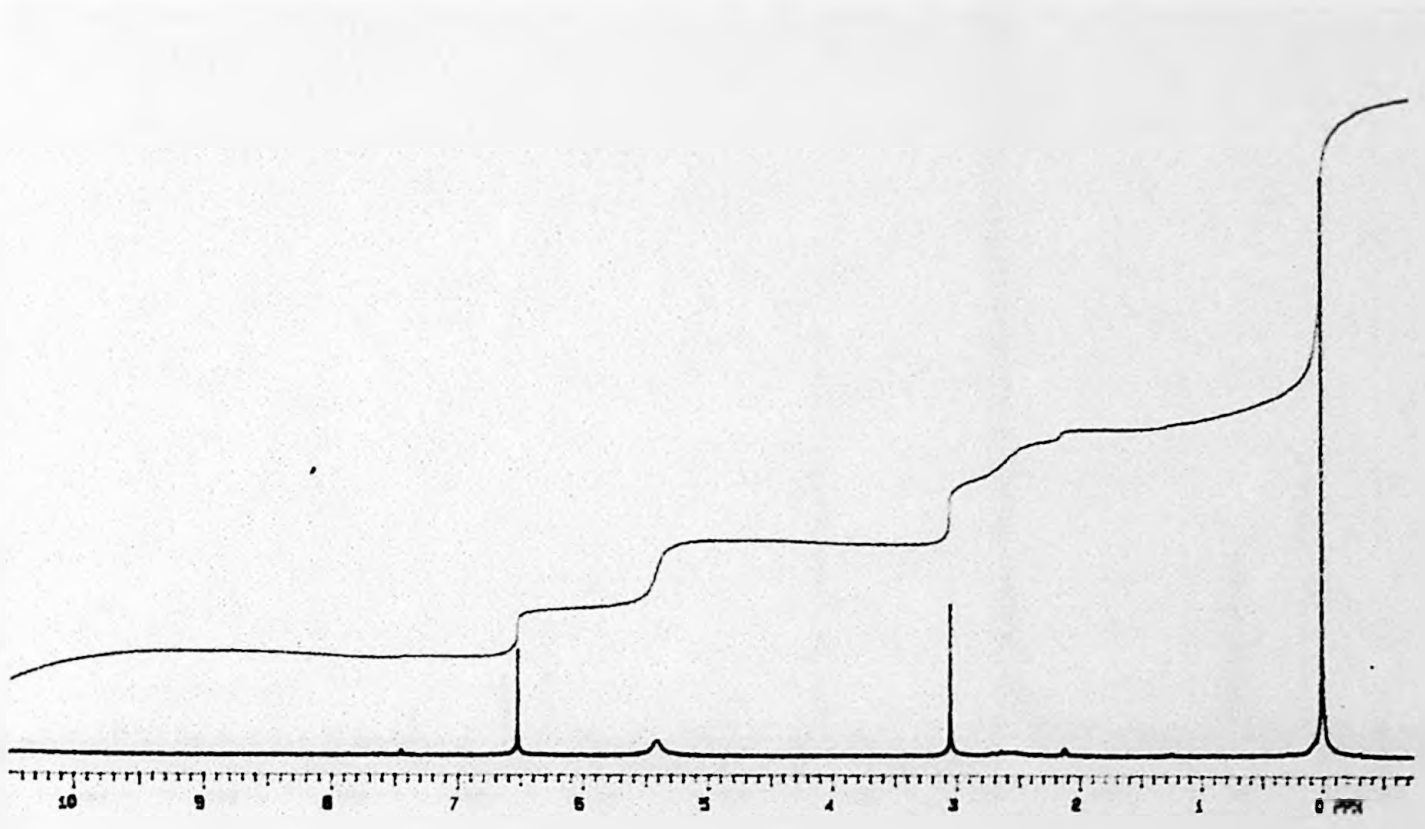


Figure 3. The 200 MHz ^{1}H NMR of *N*-(3,5-dichloro-4-pyridyl)succinimide in a mixture of dimethyl sulfoxide-d_6 and chloroform-d.

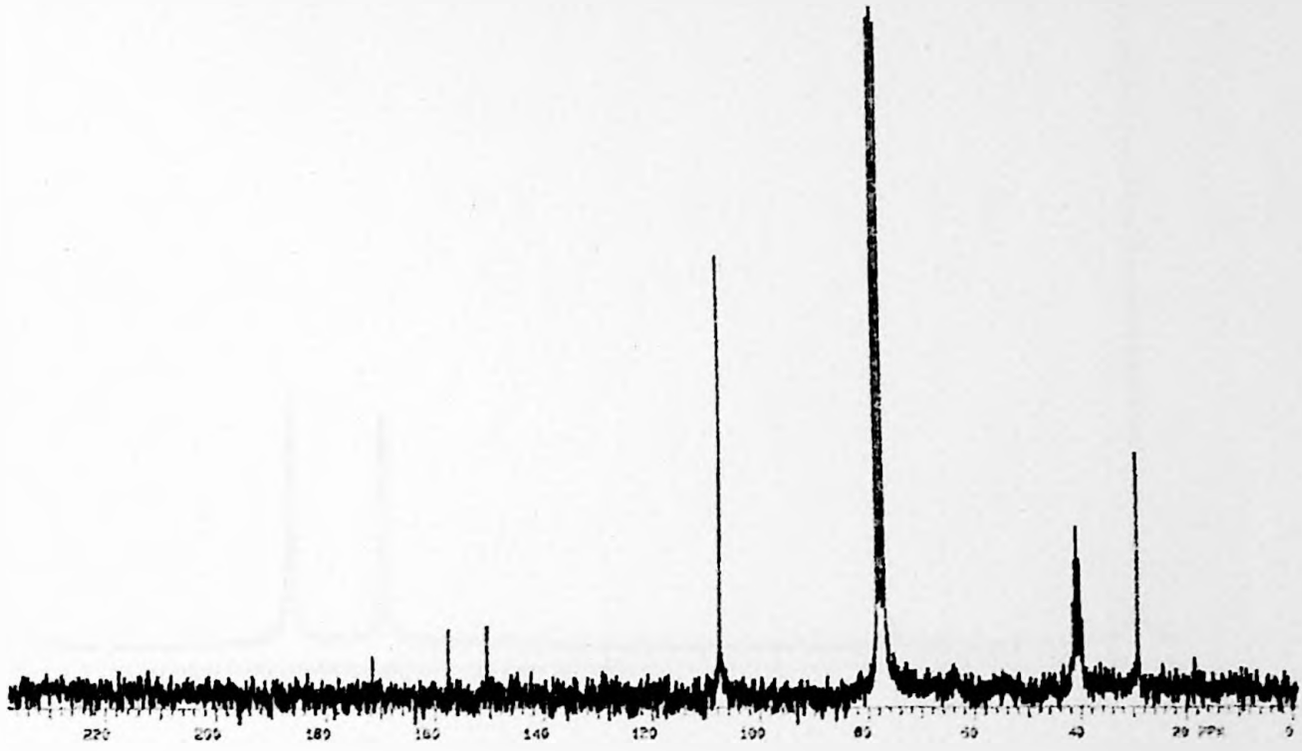


Figure 4. The 200 MHz ^{13}C NMR of *N*-(3,5-dichloro-4-pyridyl)succinimide in a mixture of dimethyl sulfoxide-d_6 and chloroform-d.

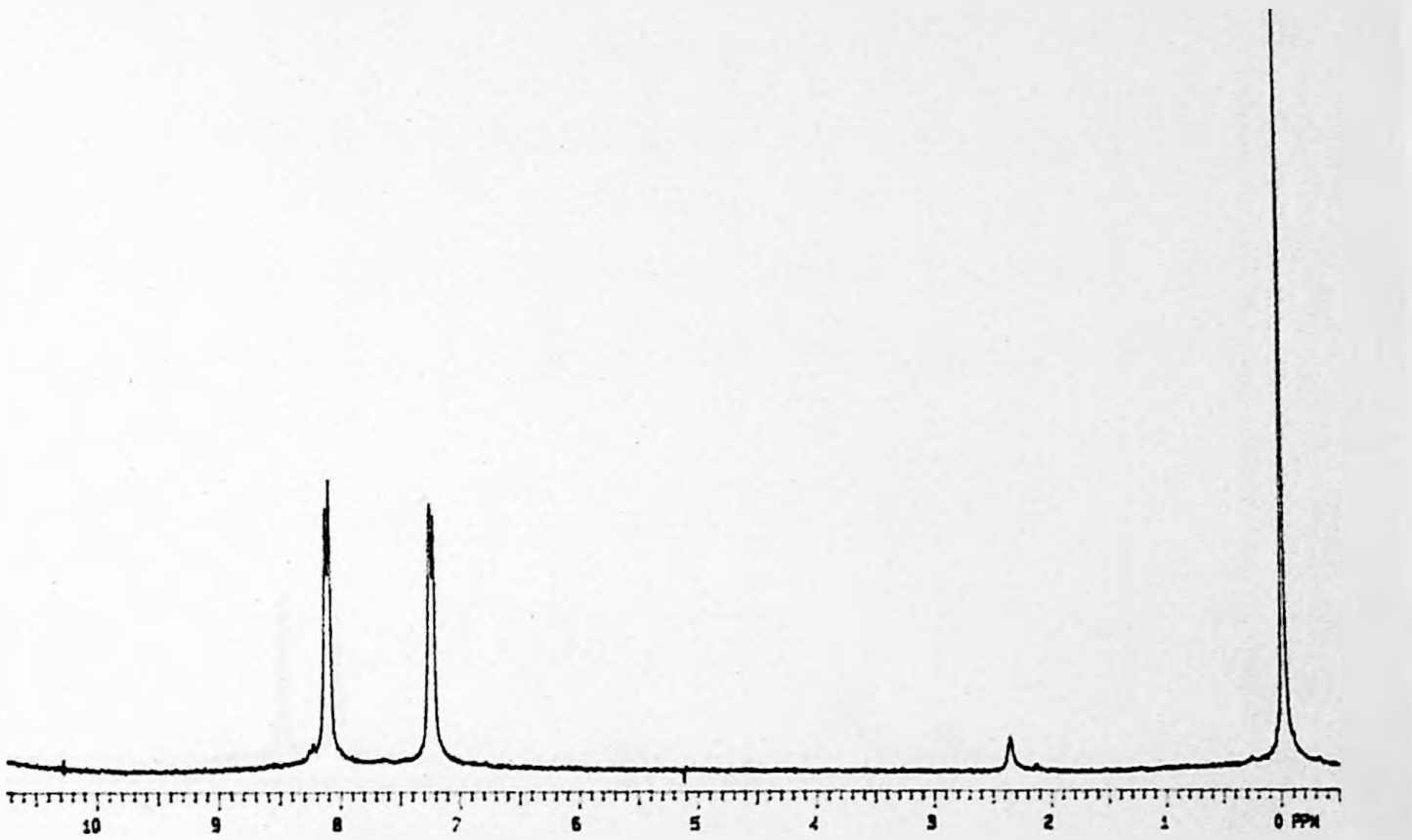


Figure 5. The 200 MHz ^{1}H NMR of 4-chloropyridine-*N*-oxide in dimethyl sulfoxide-d_6.

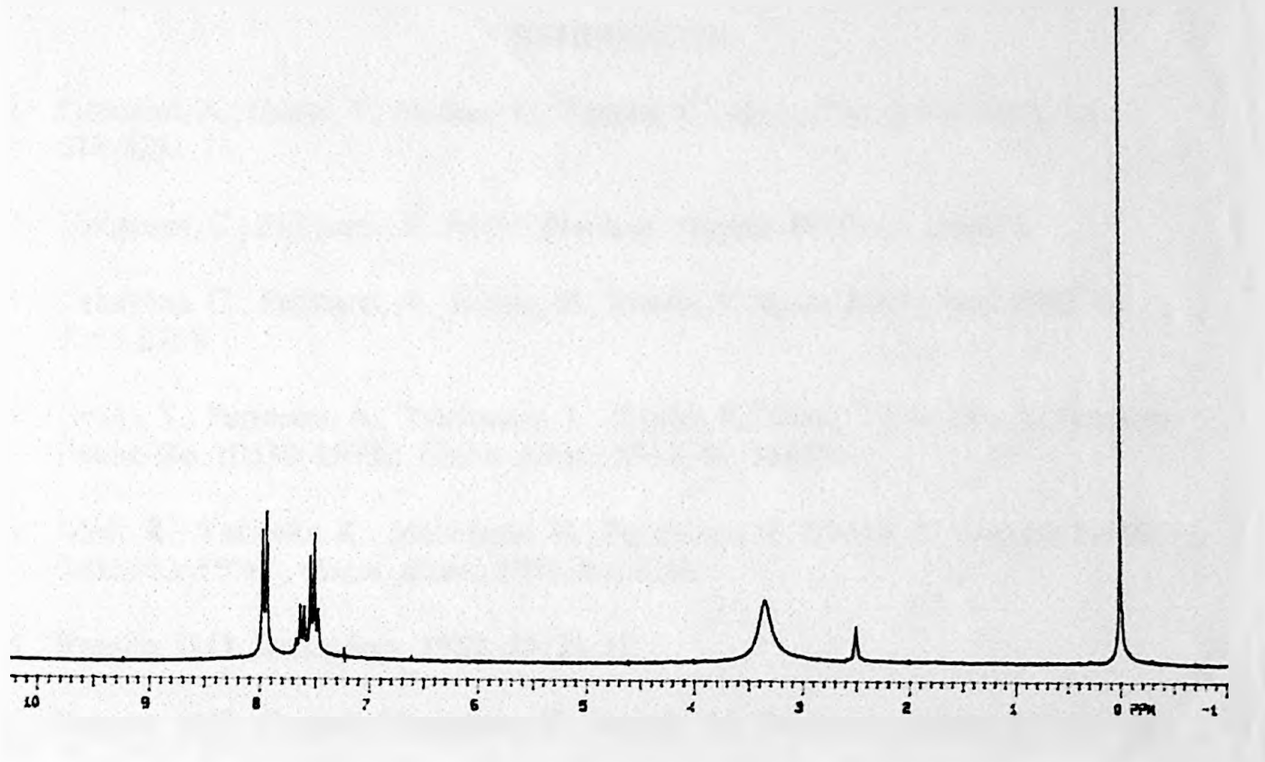


Figure 6. The 200 MHz ^{1}H NMR of 4-nitropyridine-*N*-oxide in dimethyl sulfoxide-d_6.

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