# AN INVESTIGATION INTO THE ANTIMALARIAL ACTIVITY OF METAL CHELATORS

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2008

Submitted in fulfilment of the requirements for the degree of Master of Science in the School of Chemistry, University of KwaZulu-Natal, Durban.

As the candidate's supervisor I have approved this thesis/dissertation for submission.

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## FACULTY OF SCIENCE AND AGRICULTURE

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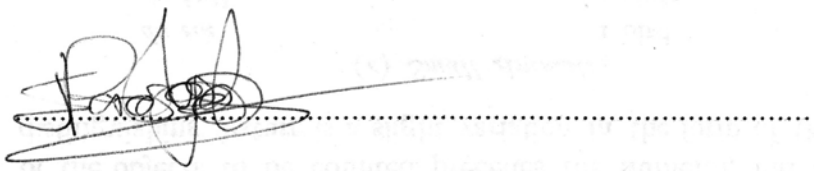

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# ABSTRACT

Malaria remains one of the greatest problems facing developing nations, especially in sub-Saharan Africa. Part of the problem stems from increased resistance to current treatments hence there is a large drive to develop novel antimalarial compounds. Several chelating compounds, including 8-hydroxyquinoline (8-HQ), 1,10-phenanthroline (1,10-phen) and 2,2:6,2-terpyridine (terpy), have disputed activities (8-HQ and 1,10-phen) or are untested (terpy). Furthermore the mechanism(s) by which these ligands and/or their complexes with metal ions exhibit their toxic effect is unknown. In order to resolve these issues, a study of the antimalarial activities of the free ligands, the ligands complexed with metal ions ($Au^{3+}$, $Cu^{2+}$, $Fe^{3+}$, $Pd^{2+}$ and $Pt^{2+}$), and the ligands with free metals in solution were measured. The ligands, complexes and metals were also tested for their ability to inhibit β-haematin formation, the mode of action ascribed to the most widely used antimalarial, chloroquine. The background toxicity levels of the various metal ions (previously unreported) were also measured and are reported here. None of the ligands were found to have particularly high activity (all approximately 1 μM). In general the metals in were found to have no beneficial effect on activity whether complexed or freely available in solution. None of the ligands were found to inhibit β-haematin formation. The complexes however, with the exception of those of $Cu^{2+}$, all inhibited β-haematin formation. Upon further investigation it was found that the each of the metal ions with the exception of $Cu^{2+}$ had an innate ability to inhibit β-haematin formation. Thus the mode of action of the ligands and the complexes is likely to be *via* different mechanisms. In an attempt to enhance the activities of the ligands they were modified by covalently linking them to nutrients essential to the malaria parasite (adenosine and pantothenic acid). These six novel compounds however, showed no improvement in activity.

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# LIST OF ABBREVIATIONS

| Abbreviation | Full Meaning |
|---|---|
| µl | microlitre |
| 1,10-phen | 1,10-phenathroline |
| 8-HQ | 8-hydroxyquinoline |
| A | adenine |
| ACT | Artemisinin-based combination therapy |
| ADA | adenosine deaminase |
| AIDS | acquired immune deficiency syndrome |
| ATR | attenuated total reflection |
| BOC-Gly | 2-(tert-butoxycarbonylamino)acetic acid |
| C | cytosine |
| conc. | concentrated |
| COSY | correlated spectrscopy |
| CT-DNA | Calf thymus deoxyribonucleic acid |
| DDT | dichlorodiphenyltrichloroethylene |
| decomp. | decomposition |
| DMF | *N*,*N*-dimethyl formamide |
| DMSO | dimethyl sulfoxide |
| DMSO-$d_6$ | deuterated dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| DPPA | diphenylphosphoryl azide |
| EDC | 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride |

| | |
|---|---|
| EDTA | ethylenediaminetetraacetic acid |
| ESI | Electron Spray Ionization |
| FIC | Fractional Inhibitory Concentration |
| FV | food vacuole |
| G | guanine |
| gly | glycine |
| HBTU | *o*-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate |
| HET | 2-hydroxyethanethiol |
| HIV | human immunodeficiency virus |
| HPLC | high performance liquid chromatography |
| HSQC | heteronuclear single quantum correlation |
| hTrxR | human thioredoxin reductase |
| Hz | Hertz |
| $IC_{50}$ | Concentration at which 50% growth inhibition occurs |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| IR | infra red |
| iRBC | infected red blood cell |
| LC-MS | liquid chromatography mass spectrometry |
| M | Molar |
| MHz | megahertz |
| min | minute |
| μM | micromolar |
| mmol | millimole |
| Mp | melting point |

| | |
|---|---|
| mRNA | messenger ribonucleic acid |
| NADH | dihydronicotinamide |
| NBMPR | nitrobenzylthioinosine |
| nM | nanomolar |
| NMR | nuclear magnetic resonance |
| no. | number |
| NPP | new permeation pathways |
| *P.f.* | *Plasmodium falciparum* |
| PEMS | parasitophorous vacuolar membrane-enclosed structures |
| *Pf*ENT1 | *Plasmodium falciparum* endogenous nucleoside transporter 1 |
| *Pf*NT1 | *Plasmodium falciparum* nucleoside transporter 1 |
| Pol I | Polymerase I |
| PPM | parasite plasma membrane |
| ppm | parts per million |
| PTSA | *para*-toluenesulphonic acid |
| PVM | parasitophorous vacuole membrane |
| RBC | red blood cell |
| RBCM | red blood cell membrane |
| RBM | Roll Back Malaria initiative |
| RNA | ribonucleic acid |
| SHAM | salicylhydroxamic acid |
| T | thymine |
| t | time |
| *T. cruzi* | *Trypanosoma cruzi* |
| *t*-BOC | Di-tert-butyl dicarbonate |

| | |
|---|---|
| TEA | triethylamine |
| terpy | 2,2':6',2" terpyridine |
| TFA | trifluouroacetic acid |
| THF | tetrahydrofuran |
| TLC | thin layer chromatography |
| TOF | time of flight |
| TrxR | thioredoxin reductase |
| TVM | tubovesicular membrane |
| UV/VIS | ultra violet/visble |
| WHO | World Health Organization |

# CHAPTER 1

# Introduction to Malaria

## 1.1 The History of Malaria

The human malaria parasite almost certainly developed at the same stage as early man, some two to ten million years ago, having evolved from the species that afflicted early apes approximately 30 million years ago.[1] This new, human malaria, which at one stage was found almost everywhere on the globe is believed to have been spread by the migration of early Neolithic groups from central Africa to the Nile valley, Mesopotamia, India and South China. The disease would later move from the Nile valley to Mediterranean Europe, as human settlements moved. Of course the greatest aid to the proliferation of malaria has been the conquests of various nations during early history, from the Greeks to the Chinese, and in later history by those of the Spanish and Portuguese, who are most likely responsible for introducing malaria to the new world.[1, 2]

Specific written records of the disease can often only be inferred from the symptoms described in ancient texts. Mesopotamian tablets, dating back to 2000 BC and written in cuneiform, make mention of deadly periodical fevers which afflicted large parts of the population.[1] Early civilizations in the Indus valley have left texts which also make reference to deadly fevers dating back to the Vedic (1500–800 BC) period, while Chinese scripts dating back as far as 2700 BC mention fevers with an associated enlargement of the spleen, symptoms typical of malarial infections.[1] The ancient Greeks also made reference to what is undoubtedly malaria. Hippocrates made the insightful connection between malaria and marshes, and noted that there were different types of malaria, distinguishable by the periodicity of their fevers.[1]

As is typical of such early civilizations the cause of this affliction was often attributed to demons (Indian and Chinese), vengeful gods (Babylonian) or some other mythological reason, upset humours (Greek).[1, 2] The association of malaria with marshes and swamps

would later lead to the name *mal'aria* from the Latin meaning "bad air".[2] Ever since these ancient times, malaria developed a reputation for destroying armies, ending sieges and would continue to plague the armies of the world, during the 11th and 12th centuries. This would even occur as late as the 20th century, when the U.S. army became involved in Vietnam.[1, 2] It has even been suggested that a nations plans for war can be seen by the medical research its army pursues.[2]

Although man has been afflicted by this parasite for many years, centuries passed before humans would finally see the *Plasmodium* parasite and determine the role of the *Anopheles* vector[a,3] in its life cycle. In November 1880, the Frenchman, Charles-Louis-Alphonse Laveran, became the first recorded person to see malaria parasites in the blood. By analyzing a sample of blood from an infected soldier he saw not only gametocytes[b,3] but also parasites in their ring stage form. Soon he would identify four forms of the parasite: the male and female gametocytes, trophozoite[c,3] and segmenter[d] stages of the parasite.[4] Laveran would later report his findings to the French Academy of Medicine, but his claims were doubted, even scoffed at, and would continue to be treated thus for several years. Only by 1895, with the aid of improved microscopes, and the work of Machiafava and Celli in Italy and Councilman and Abbott in the United States, would the life cycle of the parasite within the blood stages be fully understood.[5]

By 1886, the organisms found by Laveran were generally accepted as being the cause of malaria, however the life cycle of the parasite was still unresolved. Basil Danilewsky, a Russian zoologist-physician, had been studying the blood of birds between the years of 1884 and 1889.[2] In this blood he found remarkably similar parasites to those seen in human blood infected with malaria. This finding sent many down the road of trying to link bird malaria to human malaria. The reason for this was mostly because of an inability to see the parasites clearly since methylene blue, the most commonly used stain provided only a vague image of the parasite. As is typical of science, a serendipitous

[a] A carrier of the malaria parasite.
[b] The sexual forms of the malaria parasite.
[c] The feeding stage form of the parasite.
[d] The stages during which cell division takes place.

event would solve the problem. In 1890-1891, while studying infected blood, Dr D. L. Romanowsky, a Russian army pathologist, left a bottle of methylene blue unstopperred, allowing it to grow mouldy.[2] During this process he had in fact allowed the methylene blue to oxidize to a new compound, azure blue. Despite the mould he continued to use his stain.[2] This new stain gave never before seen detail of the parasites, and thus paved the way for better characterization of the various stages of the parasite's life cycle. A year later, in 1892 with the aid of this new stain and more powerful microscopes, a team of Italian scientists, Amigo Bignami and Ettiore Marchiafava, proved that the various forms of malaria that had been seen, crescents, rings and gametocytes, were all stages of the life cycle of a single parasite. This parasite was to be finally named *Plasmodium falciparum* (*P.f.*).[2] Finally the blood stages of the parasite inside man were understood however the tissue stages were yet to be discovered while its transmission remained a mystery.

Captain Ronald Ross an officer of the British Army and a newly qualified physician, would be the one to solve the problem of malaria's transmission. Ross himself was a disbeliever of Laveran's work, and only became convinced when another Englishman, Patrick Manson, also a qualified physician showed him the parasites in the infected blood.[2] It was Manson's belief that the mosquito was crucial to the spread of malaria, and as he became a mentor to Ross, Ross became bent on proving this connection.[2] After embarking to India, Ross fed mosquitoes on the blood of infected people containing sexual stage parasites for two years, expecting to see the development of spores within the mosquito. Every time for two years the gametocytes would simply die in the mosquito's gut. Over these two years, 1895-1897, Ross had the opportunity to view and dissect hundreds of mosquitoes, increasing not only his skills, but allowing him to become extremely familiar with the insects' anatomy. In 1897, by chance a servant would bring him ten mosquitoes of a type Ross had never seen before. He fed these mosquitoes on infected blood, and began dissecting some each day for four days. On the fourth day Ross made his great discovery: on the exterior of the stomach wall of the mosquito was a cyst. The following day he dissected his last mosquito, and found that not only were the cysts larger but they were alive and growing. These mosquitoes are believed to have been *Culex fatigans*,[2] a vector for bird malaria. It must have been

pure coincidence that Ross had been delivered mosquitoes that had fed on infected birds.[2] Although Ross's research would be interrupted by a new assignment from the British Army, his friend Manson managed to have him reassigned in order to allow him to continue his research. A year later in 1898 Ross began to focus his work on avian malaria and by the end of June that year Ross had observed all stages of the malaria parasite in the mosquito. The connection between the anopheline mosquito and human malaria transmittance would be made by the Italian Giovanni Battista Grassi, within a year of Ross's work.[2] However due to scientific politics, Ross alone would collect the Nobel Prize in 1902. The riddle of malaria's transmittance was solved. The world could now embark on a mission to eradicate malaria forever.

After the discovery that malaria was transmitted by mosquitoes there still remained the mystery as to the slow onset of infection in mosquito-bitten human. In 1903 claims were made by Fritz Schaudin that he had seen sporozoites[e,3] of *Plasmodium vivax* penetrating red blood cells.[1] Due to his stature in the scientific community his claims would remain mostly unchallenged for the next 20 years.[4] However during this time scientific evidence that the erythrocytic[f,3] stages were not the only stages capable of multiple fission, was building.[4] The same Grassi who was overlooked for the Nobel Prize had, in 1900, postulated that during the incubation period, a third developmental stage of the parasite should take place. His beliefs were later to be given support by the findings of a Brazilian scientist, H. de Beaupaire Aragao, who after examining birds infected with *Haemoproteus*,[g] found forms of malaria, capable of dividing, that developed in the endothelial[h,3] cells of the blood vessels in the lungs.[6] de Beaupaire Aragoa would later show that the schizonts[i,3] produced by these dividing forms were responsible for the invasion of erythrocytes and indirectly developed into gametocytes.[4] These findings were however, largely ignored. By 1938 the identification of the exo-erythrocytic[j] stages of the malaria parasite, in birds (only one stage is found in the human forms of malaria), was becoming more common.[4] In 1944 Huff and Coulston

[e] Minute form of *P.f.* responsible for the invasion of cells (both red blood cells and liver cells).
[f] The stages within the red blood cell (erythrocyte).
[g] A parasite of the family Haemosporidia which infects the red blood cells of birds.
[h] A thin layer of cells lining the blood vessel.
[i] Cells that divide by schizogony (asexual reproduction by repeated division of the cell nucleus).
[j] Stages which occur outside the red blood cell.

published the development from the sporozoites to the erythrocytic stages of *Plasmodium gallinaceum*.[7] It was only as late as 1948 that Shortt, Garnham and their colleagues discovered the liver stage of parasites in rhesus monkeys infected with sporozoites of *Plasmodium cynomolgi*. By taking liver tissue from human volunteers infected with *Plasmodium vivax*, they confirmed that a similar stage occurred in the human form of malaria. Shortly there after they confirmed that the same was true for *Plasmodium falciparum* and described the full life cycle of the parasite.[6]

With a better understanding of malaria (both the human stages and vector transmittance), governments around the world would embark on attempts to eradicate the parasite from their territories. Various strategies and/or combinations thereof were adopted including: extensive prophylaxis (mostly using quinine); the use of various larvicides (e.g. petroleum oil and Paris green dust); the drainage of marshes and swamps; the introduction of cattle to farmland (cattle becoming the mosquito's food source); the introduction of natural mosquito predators (e.g. frogs, fish and even dragonflies); and the use of mosquito screens and bed nets.[1, 2] In some countries these methods would prove extremely fruitful, as was the case in most European countries, while others would have only temporary or limited success. A "natural" recession of malaria in northern Europe, as well as the USA, during the mid-19$^{th}$ century was probably the result of several factors, including the conversion of swamps and marshes into farmland, improved sanitation and greater access to medication. The difference between success and failure has been related to, though not exclusively affected by, financial resources. When financial backing has been adequate for third world countries, eradication attempts have been undermined by lack of infrastructure and organization, especially in countries, such as those in Africa, where rural populations dominate the landscape. Methods that work well in more developed countries are often much harder to implement or sustain in the underdeveloped countries of Asia and Africa.

In 1939, the discovery of the action of dichlorodiphenyltrichloroethylene (DDT), as an insecticide,[8] would add a new weapon to the anti-malaria arsenal. The compound, first

synthesized some 65 years earlier,[9] was seen as a Godsend to end malaria. There were several reasons for this belief: the cost of producing DDT was low; it had high toxicity to the insects with low toxicity towards man; it was toxic by contact; and the half life after spraying was extremely long. Scientists later concluded that an application of one gram per square metre would continue to have an insecticidal effect for over 10 months.[10] However it was not to be. Mosquitoes would soon develop resistance to DDT by evolving enzymes to detoxify the compound and by avoiding house walls after the blood meal.[2] The physiological resistance to DDT, may have been a result of the excessive use of DDT by farmers, as opposed to its use against mosquitoes in eradication programmes.[2] Although DDT did not lead to the world wide eradication of malaria it is still used as a vector controlling agent in various third world countries, this despite its being outlawed in several European countries and states in the US due to its toxicity and accumulation in the food chain.

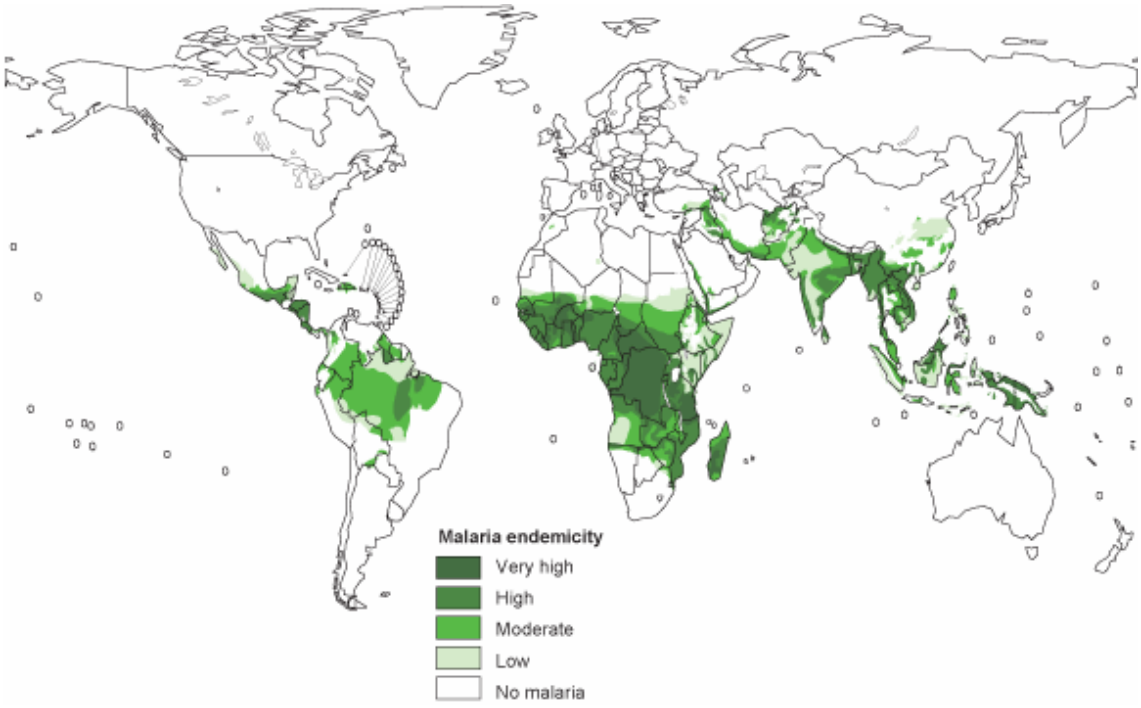
The understanding of the malaria life-cycle would also be a step forward to developing drugs to target the parasite selectively. For many years malaria had been treated by qinghao (in China) and cinchona bark (in Europe). While neither of these remedies was initially used as an antimalarial their effectiveness to treat fevers was identified in the 16$^{th}$ and 18$^{th}$ centuries respectively, without any knowledge of the malaria life-cycle. In 1596 the qinghao herb was first recommended for the treatment of fever by Li Shizhen. Only in 1972, through efforts by the Chinese government to investigate traditional remedies, was the active ingredient identified, qinghaosu (essence of qinghao).[11] The bark of the cinchona tree was brought to Europe by Jesuit priests who had observed native Peruvians using it to stop shivering. The bark was then tested on patients suffering from malaria and was found to treat the symptoms effectively.[11] In 1820 Pierre Pelletier and Joseph Caventou successfully isolated the active compound from the cinchona bark, quinine.

Methylene blue, often used to stain parasites, became the first synthetic compound to be used to treat a disease, when in 1891, Paul Ehrlich, used the dye to cure two human patients of malaria. This discovery led to Bayer, then mostly a dye company, becoming

one the biggest pharmaceutical companies worldwide. Through its efforts Bayer developed plasmoquine, which prevented relapses of vivax malaria and mepacrine (atebrine) a compound effective against *Plasmodium falciparum*. These two compounds became the most widely used during World War II after supplies of natural quinine became scarce when the Japanese captured Java (at one stage responsible for 97% of the world's supply).[11] Due to the fighting in Africa as well as the Pacific, antimalarials formed an important part of the war effort, leading to collaboration between British, American and Australian scientists to develop better drugs. In 1934, a compound called resochin was developed by H. Andersag, an employee for Bayer. The compound showed high activity against malaria but was ultimately abandoned due to the perceived toxicity to humans. Although the compound was shelved by the Germans, it was reinvestigated by the Allies during the war who ultimately found that it was suitable for use, and renamed it chloroquine. Chloroquine became the most effective and widely used antimalarial ever.[11] Several other antimalarial drugs also resulted from Allied research programs during the war. The British developed proguanil, while American research brought the world primaquine (used to prevent relapses in *Plasmodium vivax*). Other Allied discoveries became the precursors to some modern day drugs, including mefloquine, and atovaquone. However at the beginning of the 1960s it became evident that chloroquine was losing its efficacy (probably due to its widespread use as a prophylactic) against *falciparum* malaria. Today due to the increase in resistance of parasites to various antimalarials, drugs are often administered in combinations, in an attempt to ensure that the parasite is completely killed within the host, preventing the build-up of further resistance.[11]

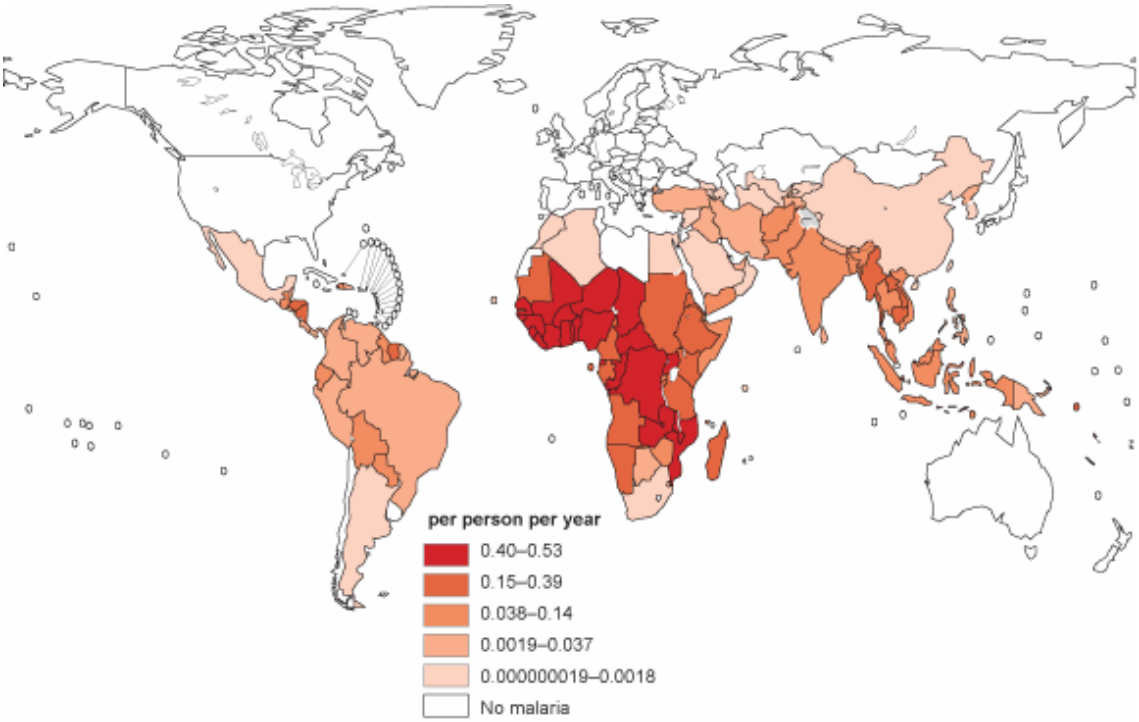
In 1998 the Roll Back Malaria (RBM) initiative was launched by the World Health Organization (WHO). This initiative has renewed efforts to minimise the effects of malaria worldwide, their goals being to halve the burden by the year 2010 and to again halve the effect by 2015.[12] As expected, the resistance of the malaria parasite to low cost drugs, together with the mosquito vector's resistance to insecticides, has been a substantial setback. This has brought more focus to preventing infection (such as the use of insecticide treated nets) and the use of combinations of drugs as opposed to single drug treatments, the two most prominent programs now endorsed by RBM.[13]

As of 2004 malaria was endemic in 107 countries with approximately 3.2 billion people exposed to infection (see Map 1.1.1 and Map 1.1.2). It is estimated that between 300 and 500 million clinical cases occur per year, caused primarily by *Plasmodium falciparum* and *Plasmodium vivax*. *Plasmodium falciparum* is directly responsible for more than one million deaths (mostly African children under the age of five) annually, and may contribute to even more deaths in combination with other illnesses. More than 80% of these deaths take place in sub-Saharan Africa. The mortality effect of malaria in Africa is amplified by the high rate of HIV/AIDS infections as well as the inability of public health systems to deal with such high infection rates.[13]




**Map 1.1.1 Areas currently at risk of malaria transmission (2003).[14]**

As with all diseases, prevention is better than cure, thus there has been a large drive by the WHO to distribute insecticide treated bed nets. The nets provide a barrier which serves to stop the mosquito vector from transmitting the disease to people. This has become a key part of the WHO's strategy to fight malaria.[15]




**Map 1.1.2 Estimated clinical cases of malaria (any species 2004).**[14]

Unfortunately there are still of course many cases of infection, which must be treated with the drugs to which the parasite is growing evermore resistant. It is now the recommendation of the WHO (since 2001) to supplement the most commonly used drugs, chloroquine and sulfadoxine-pyrimethamine, with some form of artemisinin derivative. These approaches are referred to as Artemisinin-based combination therapies (ACTs) and are considered the most effective treatments in countries where resistance (to either drug) has been observed.[15] However while many countries have "adopted" these strategies they are often not practised. As of 2004 only nine of the 42 countries which had adopted the strategy were actually practicing it.[15] It is hoped that this combination approach therapy will help to maintain control of the pandemic while new drugs are developed.

It is estimated by the report[13] that an annual sum of US$ 3.2 billion would be required to control malaria in the 82 worst affected countries and while funding has recently increased it is still short of the desired target. Most funding comes directly from

governments, which in Africa means finances are significantly short of that which is required for an effective antimalarial campaign. Thus the RBM initiative is ever dependent on private donations to help it to achieve its goals.

## 1.2 The Life Cycle of the Malaria Parasite: *Plasmodium falciparum*

The malaria parasite undergoes a significantly intricate life cycle, requiring both human and mosquito hosts to proliferate. Thus it is easier to look at the life cycle in these three parts: sexual development (taking place mostly in the mosquito-vector); exo-erythrocytic development; and erythrocytic development (both taking place in the human body).

### Exo-erythrocytic Development

In the case of *P. f.* a mere ten sporozoites is sufficient to cause an infection in the human host. Within a period of 15-45 minutes all the sporozoites (Figure 1.2.1) will have invaded the relevant cells and disappeared from circulation.[16] The sporozoites invade the parenchymal[a,3] cells of the liver (Figure 1.2.2) and then proceed to undergo schizogony[b,3] forming merozoites.[c,17] In some cases hypnozoites[d,17] are formed as opposed to merozoites, and it is these hypnozoites which are believed to be responsible for relapses of malaria in patients.[16] Hypnozoites do not occur in *Plasmodium falciparum*, but do in the remaining three forms of human malaria (*P. vivax, P. ovale* and *P. malariae*). As in the case of the oocyst,[e, 3] once the schizonts have matured the liver cell bursts releasing the merozoites into the bloodstream, where after they proceed to invade red blood cells.

[a] The functional cells of the liver (or any other organ).
[b] Asexual reproduction by repeated division of the cell nucleus.
[c] Cells formed by schizogony (asexual reproduction).
[d] Dormant parasites which live in the liver.
[e] A small cyst containing a fertilized egg (zygote, see sexual stages of reproduction).

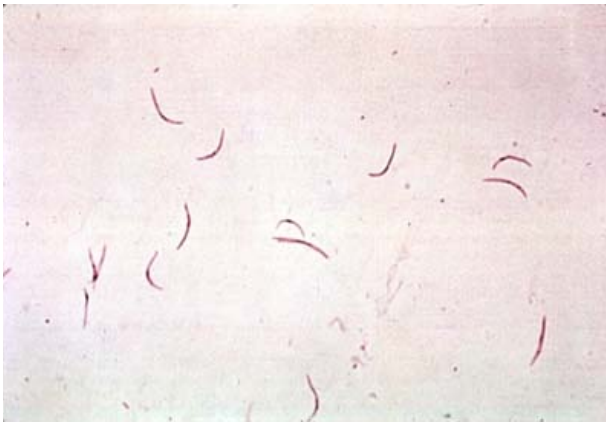


**Figure 1.2.1 *Plasmodium falciparum* sporozoites.[18]**

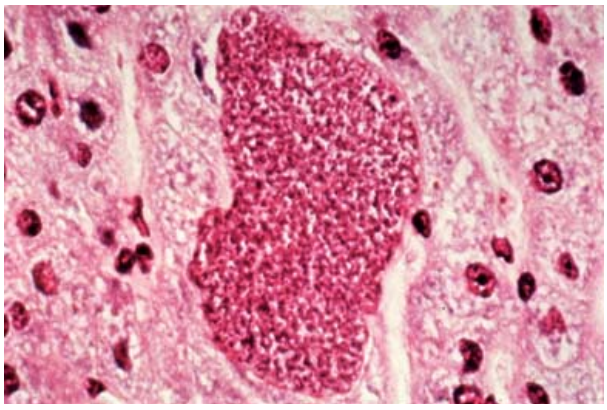


**Figure 1.2.2 *Plasmodium falciparum* liver stage of infection.[19]**

## Erythrocytic Development

In the case of mammalian *Plasmodium*, merozoites are only capable of invading red blood cells (RBCs). The merozoite is elongated in shape and goes through several steps to invade the RBC[16]:

1) attachment to the RBC;

2) reorientation of the apical end;

3) apical junction formation;

4) formation of the vacuole;

5) movement of the merozoite into the vacuole and

6) sealing of the vacuole.

It appears that the merozoite has the ability to attach itself at any point of contact between itself and the erythrocyte. Once this has been achieved, the merozoite reorients itself so that the apical end is perpendicular to the RBC surface. This allows the parasite to begin with active invasion of the cell. Initially a junction is formed between the merozoite and the RBC, following which the merozoite empties the contents of its rhoptries[f,3] into the RBC membrane. This begins the formation of the parasitophorous vacuole membrane (PVM). The merozoite then proceeds to migrate into the RBC, while the PVM surrounds the parasite, and is finally sealed once the merozoite is completely within the RBC.[16, 20]

At this stage the parasite looks like a minute circlet with a dot (the nucleus). This is commonly known as the ring stage (Figure 1.2.3). The parasite then begins to consume haemoglobin and grows into a mature trophozoite (Figure 1.2.4).

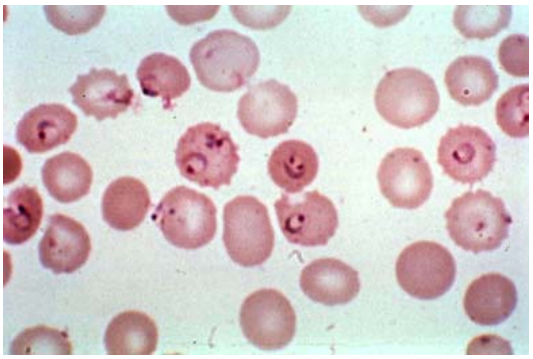


**Figure 1.2.3 *Plasmodium falciparum* early trophozoite (ring) stage.[21]**

[f] Specialised secretory organelles belonging to the parasite.

During the consumption of haemoglobin a toxic by-product, haem, is produced.[22] This by-product is crystallized out as haemozoin[g] (a haem dimer)[23] ensuring that it has no effect on the parasite.

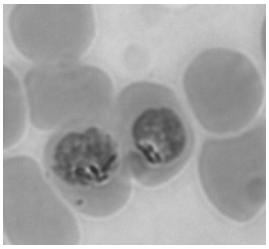


**Figure 1.2.4 *Plasmodium falciparum* mature trophozoite stage.[24]**

Once it occupies approximately half the volume of the RBC, the parasite once more begins to undergo schizogony forming between eight and 24 merozoites (Figure 1.2.5) which are released into the bloodstream and restart the invasion cycle (Figure 1.2.6).[2, 16]

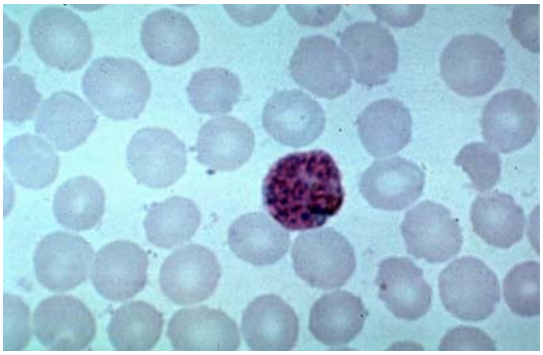


**Figure 1.2.5 *Plasmodium falciparum* fully matured schizont stage.[25]**

This cycle recurs approximately every 48 hours in cases of *P. f.* (but is different in other species) and as reported earlier is responsible for the periodic fevers experienced by malaria sufferers. Occasionally the trophozoites will develop into gametocytes, instead of merozoites. If this occurs, a mosquito vector can be infected upon taking a blood

[g] This precipitate is often seen in iRBCs as black spots and is referred to as malaria pigment. Synthetically it is known as β-haematin.

meal and the sexual stages of development are started. A summary of the life cycle is shown in Figure 1.2.7.

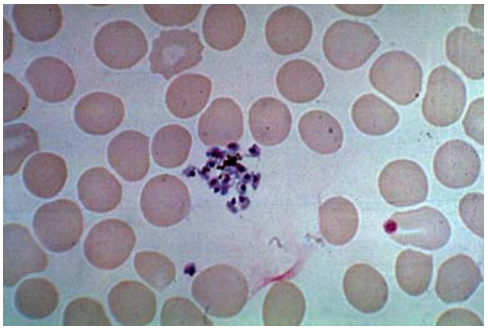


**Figure 1.2.6 *Plasmodium falciparum* ruptured merozoites free from red blood cell.[26]**

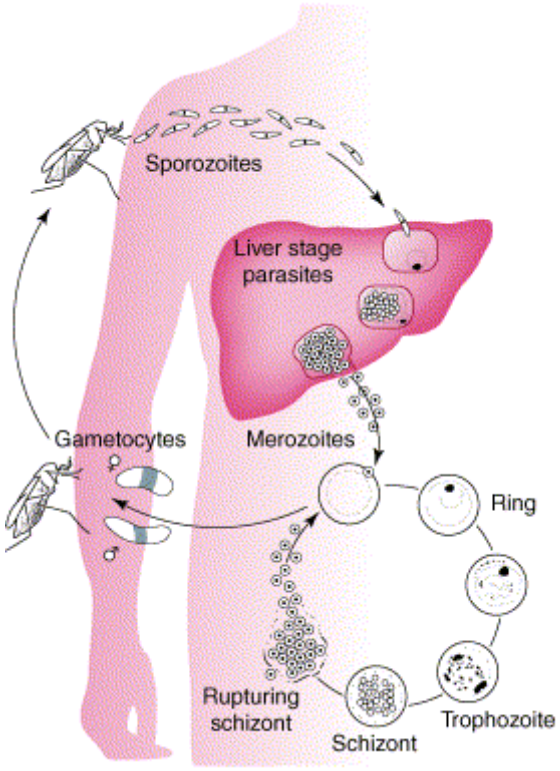



**Figure 1.2.7 Summary of the life cycle of the malaria parasite.[h27]**

[h]Reprinted from Trends in Parasitology Vol. 18, Kumar *et al.*, *A multilateral effort to develop DNA vaccines against falciparum malaria,* pp. 129-135, Copyright (2002), with permission from Elsevier.

## Haemozoin (β-Haematin)

The parasite consumes haemoglobin while it occupies an host erythrocyte in order to gain access to many purine residues which it is incapable of synthesizing.[22] As a result a toxic by-product, haem, is produced and thus the parasite must remove this toxicity. This toxin is removed when it forms a dimer which precipitates from solution, haemozoin (synthetically called β-haematin).[23] The dimer is formed when the iron centre of one haem molecule coordinates to the propionate group on another. This coordination is then reciprocated when the propionate group of the first haem molecule coordinates to the iron centre of the second. These dimers are then hydrogen bonded to one another, as seen in Figure 1.2.8. It is generally accepted that haem is capable of spontaneous dimerization under acidic conditions[28] and the polymerization has recently been reported to be catalyzed by lipids.[29] It is believed that chloroquine prevents the formation of this dimer by sterically blocking coordination,[28] through interaction with the haem molecules, allowing the toxic haem to kill the parasite.[30, 31]

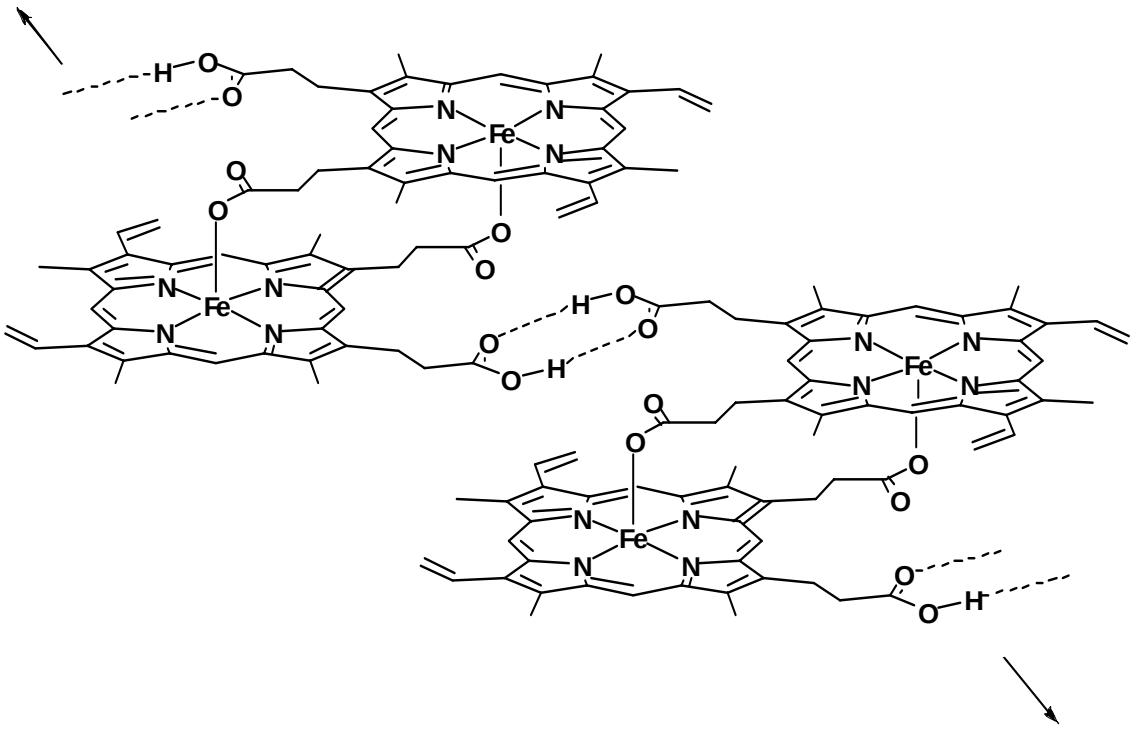



**Figure 1.2.8 Structure of haemozoin (β-haematin)** [23]

## Sexual Development

The sexual development of (*P. f.*) begins inside the human body, when, in the last stage of the life cycle, (a period of 48 hours usually culminating in the production of merozoites) gametocytes as opposed to merozoites are produced. In *P. f.* asexual reproduction dominates the first ten days of infection following which, there is a surge of gametocytaemia.[i,3] After this initial wave, gametocyte production becomes lower and less synchronized, (directly correlating with the periodicity of fevers in an infected person).[32] These gametocytes have been shown to have a half-life of up to two days (*in vitro*) or two and a half days (*in vivo*) but may persist at levels allowing infection of the mosquito for up to 22 days.[33] Infection of the mosquito vector occurs if gametocytes are taken up during a blood meal.

Once in the mosquito's stomach the gametocytes are released from the erythrocytes and converted into eight microgametes (from each male gametocyte Figure 1.2.9) and one macrogamete (from each female gametocyte Figure 1.2.10).[16] To compensate for this imbalance it is typical for more macrogametocytes to be in circulation in the human host. The microgametes are expelled with significant force during a process known as exflagellation (Figure 1.2.9).

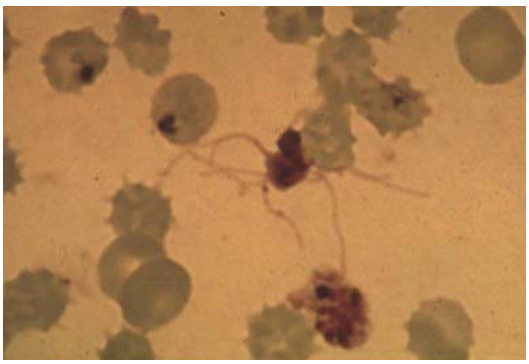


**Figure 1.2.9 *Plasmodium falciparum* male microgametocyte exflagellation.[34]**

[i] Production of sexual stages of the parasite.

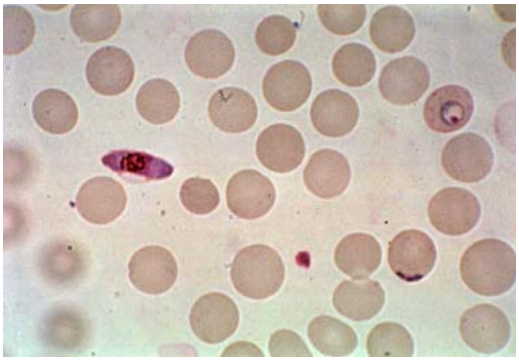


**Figure 1.2.10 *Plasmodium falciparum* female macrogametocyte.[35]**

The microgamete then fuses with a macrogamete to form a zygote[j,3] (fertilization).[16] Within 18-24 hours the zygote becomes a mobile ookinete[k,17] and breaches the stomach of the mosquito, whereupon it forms a cyst like body (oocyst) on the outer surface of the stomach wall. Inside the oocyst, asexual reproduction takes place producing thousands of sporozoites (similar to schizonts). Once the sporozoites have matured the oocyst bursts, releasing them into the haemocoele.[l,3] Following this the sporozoites migrate to the salivary glands of the vector.[16, 22] When the mosquito next takes a blood meal approximately 10% of the incumbent sporozoites are transferred to the human bloodstream and proceed to invade the liver cells.

[j] A fertilized egg.
[k] A zygote of the parasite capable of spontaneous movement.
[l] Spaces between the organs of the insect usually filled with a combination of blood, lymph and interstitial fluid.

# 1.3 Nutrient Transportation in the Infected Red Blood Cell

## General Transportation Properties

The longest part of the malaria parasite's life cycle is spent in the red blood cells (almost the full 48 hours of each life cycle in the case of *P.f.*), thus it is this stage which is the most suited for targeting by drugs. However, because the parasite has invaded the host erythrocyte, an extremely complicated system of membrane transport is established.[36-38] The nature of this membrane transport has been the source of intense study[39-66] and debate[47, 54, 67-72] (since the suggestion of a "parasitophorous duct").[67] Pouvelle *et al*[67] (and later others[68, 70, 71]) proposed the existence of a duct linking the parasite directly with the blood stream, in contrast to the more conventional idea of a sequential crossing of the three membranes (red blood cell membrane (RBCM), parasitophorous vacuole membrane (PVM) and parasite plasma membrane (PPM) see Figure 1.3.1). Due to the parasites' rate of replication it requires an immense quantity of nutrients (increasing approximately 24 fold in 48 hours).[36-38] In order to ensure that sufficient food is obtained, the parasite exploits both endogenous RBC transport pathways and induces new permeation pathways (NPP).[36-38] The fine details as to how the parasite gains access to exoerythrocytic nutrients may remain unresolved but it has been firmly established that infected red blood cells (iRBCs) accumulate more nutrients than normal RBCs. Not only are nutrients accumulated/transported at a faster rate[37, 44, 45, 48, 61, 63] but there are also certain nutrients which are exclusively transported into iRBCs and essential to the survival of the parasite.[37, 59, 61, 62, 66] These transport pathways are superb targets either by blocking them or using them as routes for drug delivery.[43, 65, 73-76] Thus a literature investigation was performed to identify substrates which are either exclusively or preferentially accumulated by the iRBC over a normal RBC.

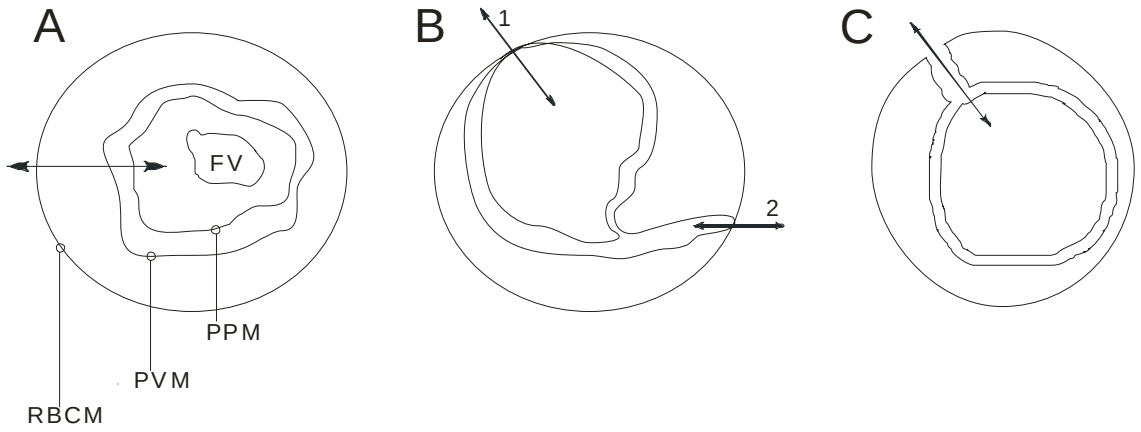



**Figure 1.3.1 Schematic showing membranes involved in trafficking of solutes in a malaria infected red blood cell.[37*]**

[*] **A** demonstrates the traditional theory of sequential transport of solutes into the parasite. **B1** and **B2** represent two different applications of the theory of a "metabolic window" which is used for transport. **C** is representative of the "parasitophorous duct" as a mode of transportation of solutes into the parasite.

Figure 1.3.1 shows the various modes of transport thought to take place in an iRBC. Diagram A demonstrates the traditional concept, of sequential transport across the three membranes, the RBCM, PVM and PPM, commonly referred to the "sequential pathway".[37] Diagram B represents two alternate pathways known as "metabolic windows". At B1 both the PVM and PPM are closely located to the RBCM to allow solute exchange directly between the parasite and the growth medium.[77] Alternatively at B2 the tubovesicular membrane (TVM) is extended out from the PVM to the RBCM. Here it is postulated that the TVM is fused with the RBCM creating a specialized junction through which solutes may pass immediately to the parasite. In both cases transport through the host erythrocyte is avoided.[53] The so called "parasitophorous duct" is demonstrated in C. Here a pathway exposing the parasite membrane to the external medium is created, and is believed to facilitate direct transport of macromolecules into the parasite.[67] This idea of the duct is heavily debated[47, 54, 68-72] and although there is significant evidence for its existence,[68, 70] there are alternative explanations for the observed experimental results.[37] While its existence has not been disproven "the duct" is generally no longer supported by scientists in this field. Figure 1.3.1 highlights the complexity associated with drug delivery into the parasite. There

may be even more membranes which need to be crossed, if for example a drug is only active in the food vacuole (FV). It is likely that drugs which exhibit their effect by inhibiting the formation of β-haematin[a] would need to locate there.

In order to gain a better understanding of parasite transportation work has also been done to identify the genetic code that expresses transporters.[55, 57, 61] Desai, Krogstad and McCleskey[46] have even isolated the parasite from its erythrocytic host and used the patch clamp method[b,78] to investigate the nature of transportation on the PVM, demonstrating that transport across this membrane is largely unselective.

Extensive work has been done in order to try to bring some understanding to the transport systems in use by the malaria parasite (including those of the iRBC). This body of work has determined various factors affecting the rate of transport of solutes including, solute size,[42, 48, 56, 63] charge[48, 56] and even counterions.[48, 49] While any or all of the above modes of transportation may be used by the parasite, the details of how the parasite takes up solutes is less important (to this study) than the actual solutes which are taken up, and how much.[36-38] In order to ensure the delivery of these nutrients the parasite makes use of the endogenous RBC channels and transporters belonging to the red blood cell as well as NPPs[39-43, 63] and accumulates and transports far more nutrients both in variety and quantity, when compared with healthy RBCs.

[a] A synthetic form of haemazoin – the crystals formed by the parasite to precipitate the toxic haem by-product which results from the consumption of haemoglobin.

[b] A technique which allows one to measure the transportation of solutes across single membranes *via* channels.

# CHAPTER 2

# Introduction to Antimalarial Agents

## 2.1 Transportation of Metal Ions into the Malaria Parasite

The transportation and accumulation of metal ions into the malaria parasite is one aspect of the organism which is of particular interest to this study. While there has been a significant amount of work performed on the transport and uptake of organic solutes, there has in contrast been very little research on the parasites preference for metals, especially for the transition metals copper, iron, gold and platinum.

Due to the often trace amounts of gold and platinum in the human body it is not surprising that there is no report of the iRBCs ability to transport these metals. With respect to copper and iron there is some, though little research, on the parasites need for these metals.

It has been shown that during the parasites life cycle it has no need to accumulate copper, since the amounts present within the RBC prior to invasion are sufficient to support the parasite.[79] It was even found that the parasite demonstrated pathways which in fact *removed* copper from the infected erythrocyte, leaving the infected parasite with approximately 66% of the amount found in a normal erythrocyte.[79] Thus it appears that the parasite has an efficient copper efflux system to remove the toxic metal which is freed during the degradation of erythrocyte proteins.[79]

In the case of iron there has been evidence to both suggest and refute the idea that iron is obtained from the serum, from the erythrocyte as free iron or from degraded

haemoglobin. Reports in favour of transport from the serum are evidence for transferrin[a,17]-independent iron transport[80] and the resistance to malaria that people suffering from anaemia[b,17] have shown.[81] Arguments against the serum as a source of iron stem from the lack of transferrin receptors on iRBCs,[82, 83] that iron supplemented diets had no effect on the course of malaria in infected patients[84] and that there was a minimal increase in the uptake radiolabelled iron in iRBCs when compared with normal RBCs.[85] The lack of activity from iron sequestration agents, when preloaded into erythrocytes, suggests that free erythrocytic iron is not required by the parasite[68] and while haemoglobin degradation might seem an obvious source of iron,[86] it has however been demonstrated by Egan *et al.*,[87] that 95% of parasite digested haem iron can be found in haemazoin. This however does not preclude the parasite from using the host erythrocyte as a source of iron, since the remaining iron (5%) might well be enough to supply the parasite. The parasite's source of iron is disputed but it has been demonstrated in the radiolabelled experiments of Scheibel *et al.*[85] that free iron is inefficiently transported and accumulated by the malaria-infected erythrocyte. They did however also prove that transport of this metal ion, and presumably others, such as copper, gold and platinum, can be mediated by ligands such as 8-hydroxyquinoline (uptake increased from 9% to 50% of serum iron in the presence of 8-hydroxyquinoline).[85]

What little research there is on the transport of metal ions (perhaps with exception of sodium and calcium) into the infected erythrocyte, suggests that in general transportation of metals into the parasite is likely to be mediated by organic ligands. This transport is exemplified by the case of 8-hydroxyquinoline and iron. It is likely therefore that the ligands used in this study, 8-hydroxyquinoline, 1,10-phenanthroline and 2,2':6',2" terpyridine might well facilitate the transport of metal ions (e.g. iron, copper, gold and platinum) into the iRBC.

[a] A protein responsible for the transport of iron into cells.
[b] A condition in which a person has a low number of red blood cells, in this case caused by an iron deficiency.

## 2.2 The Use of 8-Hydroxyquinoline as an Antimalarial Agent and its Mode of Action

The first investigation into the efficacy of 8-hydroxyquinoline (8-HQ) as antimalarial appears to have been carried out by Scheibel, Adler and Stanton in a series of papers from 1980 to 1986 (The Antimalarial Activity of Selected Aromatic Chelators).[85, 88-90] Their reason for investigating this compound was that it had similar characteristics to other compounds which had shown antimalarial activity. These characteristics are the ability to inhibit metalloprotein oxidases and high binding constants for transition metal ions. Both of these properties are possessed by 8-HQ.[88] After the work by Scheibel *et al.*, 8-HQ appears to have been largely ignored by the malaria scientific community, although it was recently reported to have extremely high activity (ten fold higher than chloroquine),[91] while another paper reported only mild (μM) activity.[92] The author of the former paper has produced no further reports on the activity of 8-HQ since his short communication.

The initial study done by Scheibel and Adler[88] revealed that this compound had an $IC_{50}$ of slightly less than 6.89 μM after one life cycle. Later publications would report the activity found in this study to be as high as 8.3 nM,[93] however this activity could only be attributed to 8-HQ after two parasite life cycles (96 hours), a practice not commonly used to measure the $IC_{50}$ of antimalarials. After the initial investigations the authors attempted to elucidate the possible mode of action of this compound.

Their ideas for the mechanism by which 8-HQ kills the parasite came largely from work led by Adrien Albert in the late 1940s and early 1950s.[94-97] This series of papers investigated the antibacterial activity of 8-HQ. A range of 8-HQ related compounds (some of which can be seen in Figure 2.2.1) were first investigated to see which had higher activity. The results of this study were:

1) only compounds with an hydroxyl substituent at the 8 position demonstrated activity (2-, 3-, 4-, 5-, 6-, and 7-hydroxyquinolines were all inactive);
2) substitution at the 2 position reduced activity (as in the case of 2-methyl-8-hydroxyquinoline, **2** );
3) hydrophilic groups reduced activity and
4) replacing the hydroxyl group with an ether (8-methoxyquinoline, **3**) removed activity entirely.

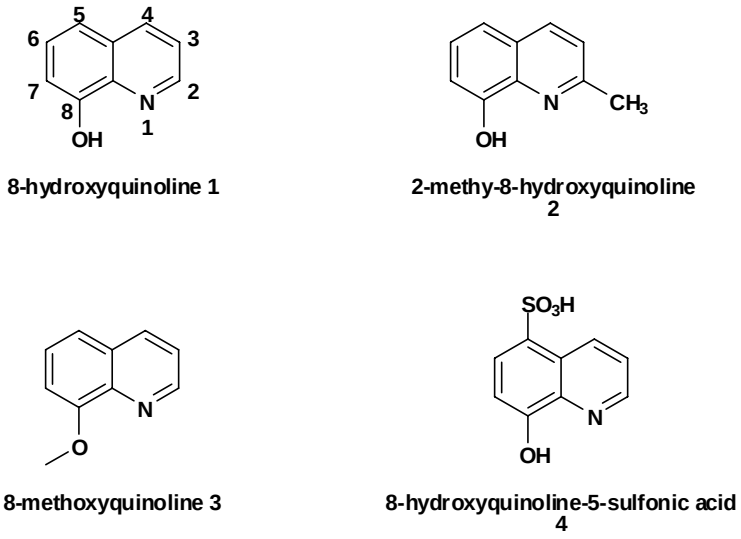



**Figure 2.2.1 Selected compounds tested by Albert *et al*.[94]**

These results suggested that chelation was an absolute necessity for activity.[94] Furthermore the addition of various divalent metals ($Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Cu^{2+}$ and $Zn^{2+}$) to the growth medium were found to prevent the activity of 8-HQ. Other chelators tested showed low activity, suggesting that a strong chelator would not necessarily demonstrate good antibacterial activity and that the molecular structure was important too.[94]

In 1950 Albert, Gibson and Rubbo did more in depth studies into the bactericidal action of 8-HQ.[95] In this study they at looked at how subtle changes in the growth medium affected the activity of 8-HQ. This time the results were far more interesting:

1) the activity of 8-HQ was reduced or even halted when trace metals were removed from the growth medium;
2) activity was restored upon the addition of cupric or ferric salts to a depleted growth medium (for Gram-positive organisms);
3) cobalt was uniquely antagonistic to the action of 8-HQ;
4) lowering the oxidation potential of the medium below -0.06 V stopped the action of 8-HQ;
5) the presence of red blood cells (intact only) at 20% of the medium was found to lower the activity approximately 30 fold (something which might well hinder any potential antimalarial activity) and
6) the action of 8-HQ was different in Gram-positive and Gram-negative bacteria, and differed from species to species within these two groups.

The authors believed that these results point to the activity being due to an 8-HQ complex involving either copper or iron, which is responsible for the oxidation of a susceptible group associated with a metabolic process essential to the survival of the bacterium. This is a direct conclusion of results 1 and 2 above. It is also suggested that the protection afforded by cobalt is due to its ability to protect this group from oxidation. This was concluded since nickel which has a higher affinity for 8-HQ afforded no such protection. Both the nickel and cobalt 8-HQ complexes were shown to be non-toxic.[95] Hence it would appear that the action of 8-HQ appears to be *via* some sort of Fenton mechanism.[a,98]

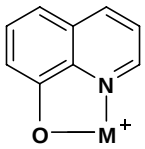
Later the same team of authors investigated what the active complex might be.[96] To do this they made a variety of growth media with different ratios of metal ions ($Cu^{2+}$, $Fe^{2+}$ and $Fe^{3+}$) and 8-HQ. The results of this study indicated the following:

1) the action of 8-HQ was dependent on the presence of one or more of $Cu^{2+}$, $Fe^{2+}$ and $Fe^{3+}$;

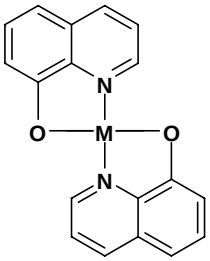
[a] The general group of reactions in which $Fe^{3+}$ reacts with $H_2O_2$ to form an hydroxyl radical and an hydroxide ion.

2) when 8-HQ was present in large excess compared with the metal ions the activity dropped rapidly (activity was quickly restored upon the addition of at least half an equivalent of one of the metal ions) and
3) when the metal concentration was increased beyond four equivalents was found to have an adverse effect on activity (though not as extreme as the effect caused by excess 8-HQ).

These results have suggested that the nature of the 8-HQ-metal complex is important, i.e. whether a 1:1 complex, **5**, or a 2:1 complex, **6**, is present (see Figure 2.2.2). The results above, in fact, suggest that a 1:1 complex is the cytotoxic species, since an excess of metal ions still results in some activity. The authors' final postulate is that the mechanism is a result of both the 1:1 and 2:1 complexes. They describe a mechanism in which a lipophilic 2:1 complex is formed, facilitating transport across the cell membrane. Once inside, an 8-HQ group dissociates, forming a 1:1 complex and freeing sites on the metal to allow the oxidation of sensitive groups (such as –SH) on enzymes or metabolites.[96] The suggestion that a lipophilic complex is required for activity is supported by the behaviour of the complex formed with **4**, which has identical affinities for metal ions to those of 8-HQ, but lacks activity.[94]



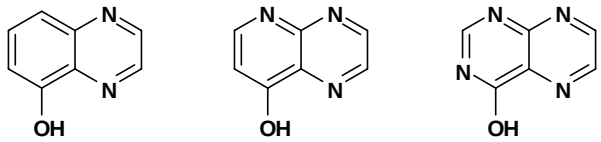

**1:1 8-HQ:M hydrophilic complex (M = $Cu^{2+}$ or $Fe^{2+}$)**
**5**




**2:1 8-HQ:M lipophilic complex (M = $Cu^{2+}$ or $Fe^{2+}$)**
**6**

**Figure 2.2.2 Proposed complexes responsible for bactericidal activity of 8-HQ.**

In order to further support the argument for the requirement of a lipophilic 2:1 complex, the authors synthesized a series of 8-HQ derivatives, both less and more lipophilic, and tested them for their bactericidal activity. The more lipophilic compounds (determined using the oleyl alcohol:water partition coefficients) were found to have similar but not increased activity. However the more hydrophilic compounds, **7, 8** and **9** , made by the addition of one or more nitrogen atoms into the aromatic rings of 8-HQ, were found to have markedly reduced activity. Examples of some of the mono-, di-, and triazo compounds designed are shown in Figure 2.2.3.

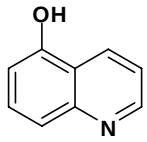



Monoazo-8HQ 7 Diazo-8HQ 8 Triazo-8HQ 9

**Figure 2.2.3 8-HQ species derivatised to increase hydrophilicity.**

The activity was found to decrease with an increase in the number of azo groups in the rings (**7** > **8** > **9**).[97] Thus these results supported the authors hypothesis from 1953.[96] The results of these antibacterial studies would be the basis for the work done by Scheibel and co-workers when investigating the possible mode of action of 8-HQ on malaria parasites.[85, 88-90]

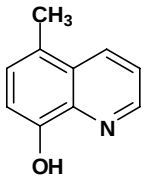
In 1980 Scheibel and Adler reported their results for the antimalarial activity of 8-HQ ($6.89 \times 10^{-6}$ M) and two of its analogues, 2-methyl-8-hydroxyquinoline, **2**, ($6.28 \times 10^{-4}$ M) and 5-hydroxyquinoline (> $6.89 \times 10^{-4}$ M). These findings suggest that the activity of 8-HQ is derived from its chelating ability, since **2** showed reduced activity (possibly due to steric hindrance from the 2-methyl group), while 5-hydroxyquinoline (Figure 2.2.4) showed almost no activity (likely due to its inability to chelate).[88]



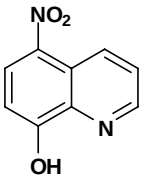

**5-hydroxyquinoline**
**10**

**Figure 2.2.4 Structure of 5-hydroxyquinoline.**

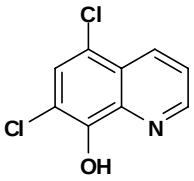
Following this, Scheibel and Adler investigated further derivatives (Figure 2.2.5) of 8-HQ in 1981[89] and 1982.[90] The aim behind this was to investigate the effect of lipophilicity on activity. Furthermore the authors also investigated the effect of adding 1 equivalent of cobalt, or 1 equivalent of copper/iron upon the activity of the 5-methyl species, **11**, (see Figure 2.2.5). Based on the findings of Albert, Rubbo and Gibson[95], it was expected that cobalt would antagonize the activity of the 8-HQ derivatives while iron would potentiate it. Upon testing it was found that neither transition metal had any effect on activity. The change in lipophilicity was also found to have little effect, and in



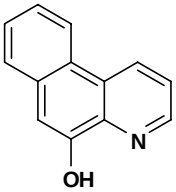

**5-methylquinolin-8-ol**
**11**



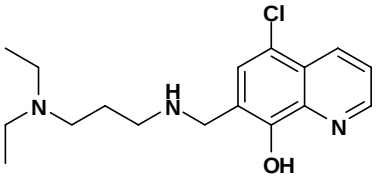

**5-nitroquinolin-8-ol**
**12**




**5,7-dichloroquinolin-8-ol**
**13**




**benzo[*f*]quinolin-5-ol**
**14**




**KAN-322**
**15**

**Figure 2.2.5 8-HQ derivatives tested by Scheibel and Adler.[89, 90]**

fact the only compound with comparable activity to 8-HQ itself was KAN-322 ($IC_{50}$ = 1.5 x $10^{-6}$ M). The authors suggest that the lack of activity of benzo[*f*]quinolin-5-ol, **14**, may be due to steric hindrance incurred when coordinating to the receptor site (which

may be different in the malaria parasite compared with those in the bacterial species studied by Albert *et al*.).[94-97]

A series of results[85] led the authors to later believe that the mechanism of action as an antimalarial was different from that found in the antibacterial studies:[94-97]

1) the addition of copper or iron did not potentiate the effect of 8-HQ;
2) no concentration quenching was observed at concentrations of 8-HQ which far exceeded those of iron or copper;
3) increasing lipophilicity did not necessarily increase activity and
4) neither cobalt nor EDTA antagonized the activity of 8-HQ.

In light of this the authors decided to investigate whether in fact iron was carried into the parasite by 8-HQ. This was done by adding $^{59}FeCl_3$ to the growth medium. The results showed that accumulation of $^{59}Fe$ into the parasite was far greater and occurred at a much quicker rate in the presence of 8-HQ. It was also found that approximately 30% of the iron taken into the red blood cell (55% of the total in the growth medium) was located in the parasites. Surprisingly KAN-322 was also found to transport $^{59}Fe$ into the red blood cell (35% of the total in the growth medium) of which an impressive 65% was located in the parasite itself.[85] The increased accumulation of the $^{59}Fe$-(KAN-322) complex inside the parasite may be due to pH trapping made possible by the secondary and tertiary amines located on the side chain of KAN-322. Following the results the authors suggest that saturating the metals (iron or copper) with 8-HQ prior to addition to the growth medium may provide a better understanding of the mechanism. Presumably this would help to determine exactly which complex is responsible for the antimalarial activity of 8-HQ (i.e. a 1:1, 2:1 or 3:1 complex). However there appear to be no further reports from these authors on the mode of action of 8-HQ.

The next report in the literature of 8-HQ with respect to its antimalarial activity is by Egan, Ross and Adams, in a paper in which the authors investigate the ability of

compounds to inhibit spontaneous β-haematin[b] formation *in situ*.[28] The main aim of this paper was to demonstrate a quick assay to determine this action. It was suggested by Egan *et al.* that chloroquine acts by directly preventing the formation of haemozoin crystals (naturally occurring β-haematin), which normally removes toxic haemin (a by-product from the digestion of haemoglobin) from solution.[28, 30] In this paper[28] 8-HQ is not attributed any antimalarial activity (although it is not tested in this report) and does not prevent the formation of β-haematin. Hence it seems likely that this is not the mode of action of the compound. Other authors have proposed that chloroquine acts on an enzyme responsible for the formation of haemozoin[31, 99] and has even been suggested to inhibit DNA replication[100].

In 2004 Roberts *et al.*[92] tested the ability of 8-HQ to inhibit the proliferation of malaria parasites. The objective of their paper was to demonstrate the presence of a previously unidentified alternative oxidase common to apicomplexan parasites[c,17] (the investigation included *Toxoplasma gondii* and *Plasmodium falciparum* but concentrated on *Cryptosporidium parvum*). The authors demonstrated that both salicylhydroxamic acid (SHAM) and 8-HQ inhibited the growth of *P.f.* (and in fact all three parasites) at similar concentrations. The authors suggested that the inhibition of this alternative oxidase may be the mode of action of these compounds.

In 2003 Biswas[91] reported an investigation into the activity of 8-HQ. In this paper an $IC_{50}$ of between 1 and 10 nM against both chloroquine-susceptible and chloroquine resistant strains of the parasite was reported. The $IC_{50}$s for chloroquine in this report are between 0.41 μM (chloroquine susceptible strains) and 3.45 μM (chloroquine-resistant strains), activity much lower than 8-HQ. These results appear to be in contradiction with almost all previously published data on the antimalarial activity of 8-HQ as well that reported by Roberts *et al.*[92] (activity of 11 μM and 8.6 μM against a chloroquine resistant and chloroquine susceptible strain respectively). To date no further details on this high activity have been reported by Biswas.

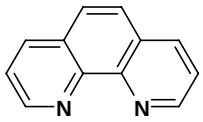
[b] The synthetic form of haemozoin. Chloroquine is believed to act by inhibiting the formation of this crystal.

[c] Parasites which have an organelle known as the apical complex, believed to be used during cell invasion.

Clearly there has been some confusion over the antimalarial activity of 8-HQ,[28, 88, 91, 93] and while the mode of action against bacteria seems well studied,[94-97] there remains some question as to how the compound acts against the malaria parasite. The first aim of this study is to determine, independently, the activity of 8-HQ against *P.f.*, and to either confirm or refute one or more of the previously reported activities.[88, 91] Second, the role of metal ions in the activity of this ligand is also to be investigated, *i.e.* are ions such as $Fe^{3+}$ or $Cu^{2+}$ essential for 8-HQ to exhibit its toxic effect (as they are for 8-HQ's antibacterial activity), and if so what is in fact the active species? Third, some investigations will be done to gain insight as to the possible mode of action of this compound (it should be noted however, that mechanistic studies are extremely complicated due to the nature of the parasite). Thus it is the overall aim of this study to bring some clarity to the presently unclear data on the antimalarial action of 8-HQ.

## 2.3 The Use of 1,10-Phenanthroline as an Antimalarial Agent and its Mode of Action

The compound 1,10-phenathroline (1,10-phen, Figure 2.3.1) has been shown to cleave DNA in a Fenton-type mechanism similar to that believed to be the antibacterial mode of action of 8-HQ.[101] When the ability of 1,10-phen to inhibit *Escherichia coli* DNA Polymerase I (Pol I)[a,98] was first discovered, it was attributed to this ligands affinity for $Zn^{2+}$ and the sequestration of this ion from the polymerase. This was demonstrated by dialysis of the holoenzyme[b,3] against 1,10-phen, and subsequent lack of activity thereafter.[102] However Sigman and coworkers would later show, over several years, that this mode of action was unlikely, and that a $(1,10\text{-phen})_2Cu^+$ complex was responsible for a Fenton-mediated destruction of DNA, the products of which facilitate inhibition of Pol I.[103-108]



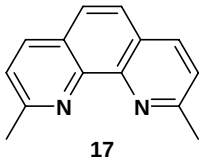

**16**

**Figure 2.3.1 The structure of 1,10-phenanthroline.**

After confirming the inhibitory effect of 1,10-phen on Pol I, D'Aurora *et al.*[103], demonstrated that this activity was dependent on a 2:1 ratio of 1,10-phen:$Cu^{2+/+}$ and a thiol (it would later be shown that dihydronicotinamide, NADH, could also facilitate the single electron reduction of $Cu^{2+}$ to $Cu^+$, although at a much slower rate)[109] is used to facilitate the reduction of $Cu^{2+}$ to $Cu^+$. In this paper it was further demonstrated that

[a] One of several enzymes which catalyzes the formation of DNA.
[b] A combination of the protein component of an enzyme (apoenzyme) and the functioning part of an enzyme (coenzyme).

other metals ($Co^{2+/3+}$, $Zn^{2+}$, $Fe^{2+/3+}$ or $Cd^{2+}$) did not have the ability to facilitate cleavage of poly (dA-T), used in place of Pol I to measure DNA cleavage ability. Following this the same authors showed by a series of experiments that the 2:1 complex must be the active form of the ligand.[104] This was done by demonstrating that 9-phenanthroic acid (a non-chelator with the same skeletal structure) did not demonstrate any inhibitory ability when used in place of 1,10-phen. However 2,9-dimethyl-1,10-phenanthroline, a chelator specific for $Cu^{+}$, totally prevented inhibition at a ten fold excess, implying the necessity of $Cu^{+}$ ions for activity. The lack of activity of 2,9-dimethyl-1,10-phenanthroline suggested that steric hindrance might prevent it from approaching the mechanistically important locus in DNA or poly(dA-dT).




17

**Figure 2.3.2 The structure of 2,9-dimethyl-1,10-phenanthroline.**

A year later in 1979 Sigman *et al.*[105] demonstrated two important aspects of the activity of the $(1,10\text{-phen})_2Cu^{+}$ complex. The first was an absolute necessity for $O_2$ (far lower activity was observed under anaerobic conditions) and secondly that it is in fact the products of the DNA cleavage by $(1,10\text{-phen})_2Cu^{+}$ which are responsible for the inhibition of Pol I. To prove the latter point, the authors cleaved poly(dA-dT) under normal conditions,[105] quenched the reaction by adding of 2,9-dimethyl-1,10-phenanthroline (20 fold the concentration of 1,10-phen) and took an aliquot of the quenched reaction to add to a matrix containing Pol I. This solution containing Pol I and the aliquot had much lower activity when compared with a control matrix containing no aliquot. The rate of the inhibition reaction was also shown to be fast (occurring within one minute of the addition of reagents) further indicating that the products of cleavage are responsible for activity and not sequestration of zinc from the polymerase, as previously suggested.[102]

Graham *et al.*[106] would go on to show that superoxide is generated during the reaction of the complex with DNA, but that superoxide itself is not responsible for cleavage, since other superoxide generators had no cleaving effect. The same group later discovered that hydrogen peroxide could be used in place of molecular oxygen, that the complex showed no preference for any of the four nucleic bases (A, T, C or G) and that there is an almost absolute preference for double stranded DNA.[107] Pope and Sigman would later show that the complex would preferably cleave B-DNA (only 10-20% of A-DNA is cleaved while no cleavage of Z-DNA is observed).[110] This result implies that there is a preference for cleavage of DNA over RNA since RNA occurs only in the A form.

Thedarahn *et al.*[108] would go on to postulate the mechanistic scheme for the reaction of $(1,10\text{-phen})_2Cu^+$ with DNA. Their conclusions were essentially a direct result of the identification of the cleavage products,[110, 111] the work done by Sigman and his coworkers described above[104-107] and that of other scientists[112]. The simplistic mechanism involves the formation of the $(1,10\text{-phen})_2Cu^{2+}$ complex, which is then reduced in a single electron transfer reaction to $(1,10\text{-phen})_2Cu^+$. This species is then reversibly bound to DNA and oxidized by hydrogen peroxide to generate the copper-oxo species responsible for the DNA cleavage products. This non-covalent bonding is believed to occur non-intercalatively, in the minor groove of the DNA. In line with this postulation derivitization at the 5 position was found to have no steric effect on DNA scission, suggesting that this position could be exploited to target scission more specifically. The mechanistic scheme can be seen in Figure 2.3.3.

$$(1,10\text{-phen})Cu^{2+} \overset{+1e^-}{\rightleftharpoons} (1,10\text{-phen})Cu^+$$

$$DNA + (1,10\text{-phen})Cu^+ \rightleftharpoons DNA\text{-}(1,10\text{-phen})Cu^+$$

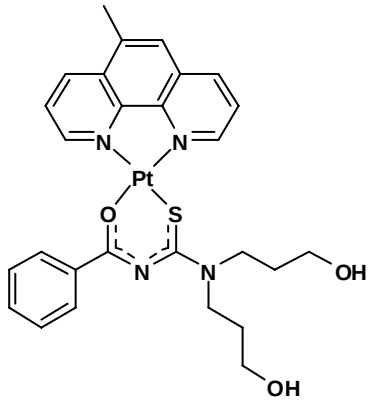
$$DNA\text{-}(1,10\text{-phen})Cu^+ \xrightarrow{H_2O_2} \text{scission products}$$

**Figure 2.3.3 Mechanistic scheme for DNA scission by 1,10-phenanthroline-copper complex.**

The most common use of 1,10-phen with regards to malaria appears to be in classifying proteases as metalloproteases.[113-115] However more recently the compound has been

incorporated into inorganic complexes and tested[116], as a protease inhibitor during various stages of parasite development[117, 118] and has been derivatized and tested against malaria.[119-121] At the conception of this project it was apparent that 1,10-phen itself had not been tested for its antimalarial activity (although some derivatives were tested as early as 2000).[119] Since then however at least three articles have been published on the antimalarial activity of this compound and its derivatives.[116-118]

The use of 1,10-phen in a preformed inorganic complex, **18**, as an antimalarial by Egan *et al.*[116] appears to be the earliest use of this ligand. The ligand was used to form platinum (II) complexes an example of which can be seen in Figure 2.3.4.




**18**

**Figure 2.3.4 An example of a 1,10-phenanthroline complex tested as an antimalarial.[116]**

These complexes were designed to facilitate π–π stacking, and expected to act by preventing β-haematin formation. Most of the compounds were found to have a strong ability to inhibit β-haematin formation, but no correlation between β-haematin inhibition and activity was found, possibly due to the cationic nature of the compounds.[116] In fact the compounds with the weakest β-haematin inhibition had the highest *in vitro* antimalarial activity ($IC_{50}$ of 0.141 μM). All of the ligands themselves were shown to have no ability to inhibit β-haematin formation, however their antimalarial activities, as free ligands, were not investigated.

In 2005 Torres *et al.*[117] investigated the effect of protease inhibitors during gametogenesis and the early zygote stages of parasite development. Amongst the inhibitors used was 1,10-phen. The ligand was found to totally inhibit gametogenesis at concentrations > 500 μM (whereas the $IC_{50}$ is approximately 200 μM). This effect was shown to be markedly reduced (a mere 4.93% inhibition using a concentration of 600 μM) with the addition of $Zn^{2+}$, an effect often observed due to the ligand's affinity for this cation. Once this had been determined the ability to prevent various stages of parasite development from gametogenesis to ookinete development was investigated. This was done by introducing the inhibitor to gametocyte-infected blood cultures (ranging from t = 0 to t = 120 min). The inhibitory effect of 1,10-phen (1 mM) was found to reduce almost linearly with time, and had almost no effect at 120 minutes, suggesting that it is only active during gametogenesis (t = 0 min) and the early zygote stages. However this only applies to the vector-based stages of the life cycle and has no relevance to the stages inside the human host.

A year later (2006), Kitjaroentham *et al.* appear to have been the first group to test 1,10-phen against malaria *in vitro*.[118] In their work the authors have made a strong case to suggest that the action of this ligand is *via* its ability to inhibit the invasion of red blood cells by the parasite. The authors identify three steps which are involved in their invasion inhibition assay: schizont development; merozoite release from the red blood cell; and merozoite invasion of a new red blood cell. To rule out a mode of action which acts during the first step, parasites were isolated at the schizont stage of development and exposed to 1,10-phen. In a second experiment parasitophorous vacuolar membrane-enclosed structures (PEMS) containing merozoites (merozoites contained in a vacuole free of the red blood cell) were treated with 1,10-phenantholine. The $IC_{50}$s were found to be similar (25 μM and 29 μM), suggesting that neither schizont development nor escape from the red blood cell was inhibited. To further demonstrate that merozoite release is not affected, 1,10-phen was compared with E64, a compound previously shown to inhibit release.[122] When parasite proliferation is inhibited and the mode of action is prevention of merozoite release, a far greater number of schizont infected red blood cells are observed, which was the case for E64, but not for 1,10-phen. This article does well to suggest that the mode of action of 1,10-phen may be *via*

inhibition of invasion, but there is no evidence in this paper to suggest that 1,10-phen does not inhibit other metalloproteases or even act by an entirely different mechanism, during the earlier stages of parasite development. An alternative mode of action would be suggested if a lower $IC_{50}$ was found when testing 1,10-phen over a longer period of the parasites life cycle.

Prior to the work described above there has been an investigation into the activity of some 1,10-phen derivatives.[119] The investigations included the 1,10-phen derivatives shown in Figure 2.3.5. Of these the methyl-quarternized species, **20,** was found to be the most active, 0.19 μM, a ten fold increase on the activity of the parent molecule, 2.4 μM. The fact that this compound exhibited the highest activity suggests implicitly that the mode of action is not due to metalloproteases inhibition, since steric hindrance prevents the quarternized 1,10-phen species from coordinating a metal ion. Recently Yapi *et al.*[120] reported again that quarternization of the nitrogen at the 10 position enhanced the antimalarial activity of a 1,10-phen derivative (also ten fold of that of the parent molecule 3.29 μM to 0.35 μM). This article also reported *in vivo* antimalarial results, but only looked at the most active *in vitro* compound (**20**), no comparison was made between its *in vivo* activity relative to the other 1,10-phen derivatives.

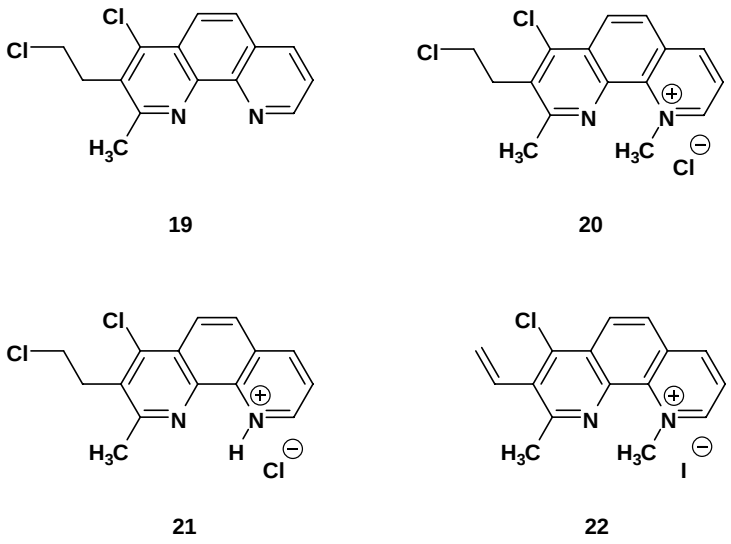



**Figure 2.3.5 1,10-phenanthroline derivatives previously tested for antimalarial activity.[119, 120]**

Wijayanti *et al.*[121] have gone on to test a series of quarternized 1,10-phen compounds *in vivo* (mouse model, *Plasmodium berghei*). The compounds, **23** - **28,** are shown in Figure 2.3.6. The *N*-benzyl derivatives were found to be more active than the *N*-alkyl derivatives, reportedly due to the more lipophilic nature of the *N*-benzyl derivatives. The activity of the salts was found to be I > Cl > Br (active in the same order of magnitude, however) while **28** was found to have reduced activity, which the authors propose may be due to the extra bulkiness introduced by the para-methoxy group. Compared with chloroquine however these compounds have a ten fold lower activity and a much lower therapeutic index (toxicity towards malaria divided by toxicity towards human cell lines).

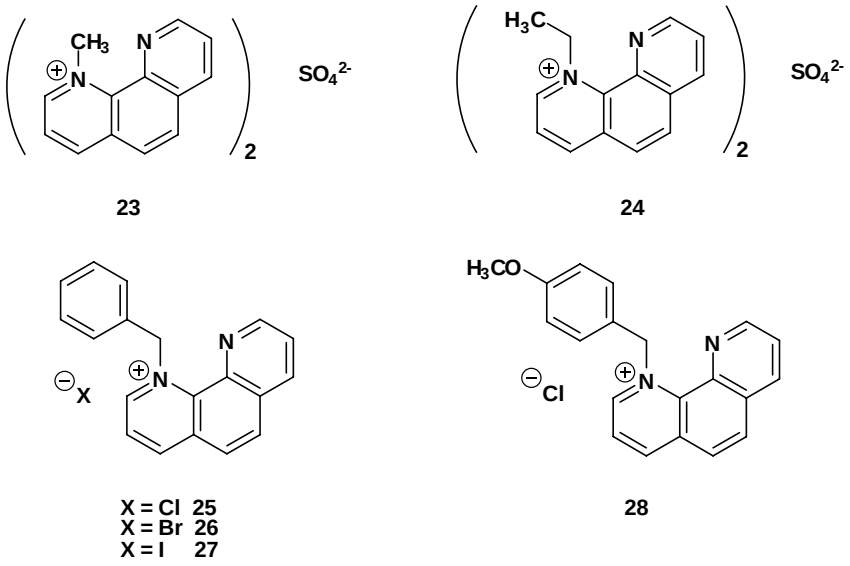


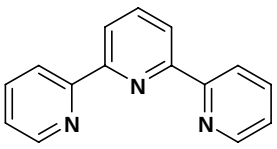

**Figure 2.3.6 1,10-Phenanthroline derivatives tested *in vivo* against *Plasmodium berghei* infected mice.[121]**

This concludes the extent to which the antimalarial effect and mode of action of 1,10-phen has been investigated to date. While 1,10-phen has been well established as a DNA intercalator,[101] its antimalarial effect has been ascribed to different modes of action, including β-haematin formation inhibition (as a complex),[116] inhibition of metalloproteases (as a free ligand)[118] and an unknown mechanism (for quarternized 1,10-phenanthrolines).[119] It is quite possible that each of these types of compounds

(complexes, the free ligand or quarternized ligands) act *via* a different mechanism. With respect to 1,10-phen therefore it is intended to, as far as is possible, investigate whether the ligand's activity is different when used alone or when combined with free metal ions (*e.g.* $Cu^{2+}$) in solution, and to gain any insight as to its mode of action.

## 2.4 The Use of 2,2':6',2'' Terpyridine and its Mode of Action

Around the same time that Sigman and his group were working on the action of 1,10-phen, another researcher, S.J. Lippard began his extensive study into the ability of 2,2':6',2'' terpyridine (terpy, Figure 2.4.1) complexes to bind to DNA. Lippard demonstrated that platinum (II) terpy complexes, especially those ligated with a thiol compound, were capable of intercalating DNA.[123] It has been demonstrated that several other complexes of terpy ($Au^{3+}$ or $Cu^{2+}$)[124, 125] are also capable of intercalating into DNA and that the binding constant can be adjusted by changing the accompanying ligand.[123, 126, 127] Only recently have some terpy complexes been tested for their antimalarial activities in a patent released by Lowe.[128] However neither the free ligand nor its complexes with several transition metals, Au, Cu or Fe, has been tested for antimalarial activity. In light of paucity of literature on the antimalarial activity of terpy compounds, a brief review of terpy anticancer studies has been done. This should give some insight on the potential modes of action and types of terpy compounds which have been studied for bioactivity.




29

**Figure 2.4.1 Structure of 2,2':6',2'' terpyridine.**

## The Modes of Action of 2,2':6',2'' Terpyridine and its Complexes

In 1974 Jennette *et al.*[123] demonstrated through several techniques, that platinum (II) complexes of terpy, the chloride, **30**, and 2-hydroxyethanethiol species, **31**, in particular (Figure 2.4.2), intercalated into DNA. The results of various forms of analysis suggested that intercalation was taking place, and that it was of a similar nature to that observed for ethidium bromide.[123] However it was apparent that the binding observed for **30** was weaker than **31**, attributed (by the authors) to the lability of the chloride ligand.[123, 126] Using X-ray fiber diffraction patterns Bond *et al.*[129] demonstrated that the interaction with calf thymus DNA (CT-DNA) is intercalative and further that it is *via* the neighbour exclusion binding model (where platinum is found in every other interbase pair site). Reports have also shown that complexes with a net +2 charge have stronger intercalative binding constants,[127] *e.g.* 4-picoline-Pt-terpy complex **32** (Figure 2.4.2). This complex was found to have an intercalative binding constant two orders of magnitude higher ($10^6$ *vs.* $10^4$ $M^{-1}$) than **31.**

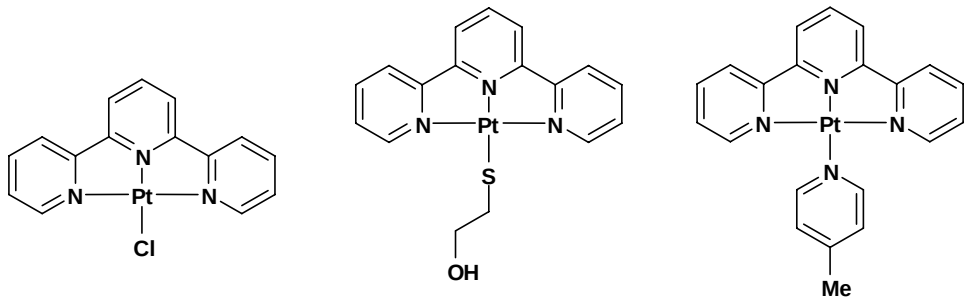
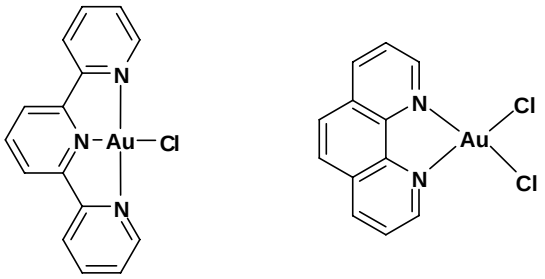



**Figure 2.4.2 Terpyridine species shown to intercalate DNA by Jennette *et al.*[123] and 4-picoline-terpy-Pt(II).[127]**

While the DNA intercalating ability of Pt-terpy species has been known for many years, it has also been shown that **32**, in particular, is capable of platinating the nitrogen atoms on some nucleosides, in a way similar to that of cisplatin.[130] Using NMR studies Lowe and Vilaivan showed that the nucleosides adenosine and guanosine (and their 2'-deoxy

derivatives) were capable of displacing the 4-picoline ligand, resulting in platination of one or more nitrogen atoms on the nucleosides ($N^7$ for the two guanosine species and both $N^1$ and $N^6$ in the two adenosine species). This is the mode of action of cisplatin and is a possible alternative mode of action for Pt-terpy complexes in general.

In 2001 Messori *et al.*[125] reported an investigation into the binding of several gold(III) complexes with DNA among which were those seen in Figure 2.4.3. All of the binding was weak and reversible, and the authors suggest that it was mostly electrostatic in nature. From their evidence the authors concluded that Au-terpy initially binds intercalatively and slowly switches to a coordinative mode of binding.[125] The nature of the interaction of these compounds with DNA is implied as the mode of action for their anticancer activity, reported earlier.[131]




**[Au(terpy)Cl]$Cl_2$** **33**

**[Au(phen)$Cl_2$]Cl** **34**

**Figure 2.4.3 Gold(III) complexes investigated for their and DNA binding affinity[125] anti-tumor activity.[131]**

Recent results have suggested that the different terpy complexes (*e.g.* the $Au^{3+}$ and $Pt^{2+}$ complexes) have different rates of intercalation.[132] In these studies the authors found that [Au(terpy)Cl]$Cl_2$ only showed evidence of retardation after three hours while [Pt(terpy)Cl]Cl demonstrated retardation of DNA within one hour (electrophoresis with CT-DNA used in all cases). The authors here believe that the action of the $Au^{3+}$ complex is due to an initial electrostatic interaction which through slow kinetics converts to a coordinative interaction. [132]

In 2006 a series of phenyl-terpy gold derivatives were tested for DNA binding capabilities and anticancer activities.[133] Both the free ligands and the gold complexes were tested. While the gold complexes, **35-37** (Figure 2.4.4), were shown to bind DNA no evidence was found to suggest that the free ligands were capable of any binding. The authors also demonstrated that intracellular DNA was bound to the complexes by measuring the gold content in extracted DNA using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The highest binding ratio was one Au atom *per* 4900 nucleotides.[133]

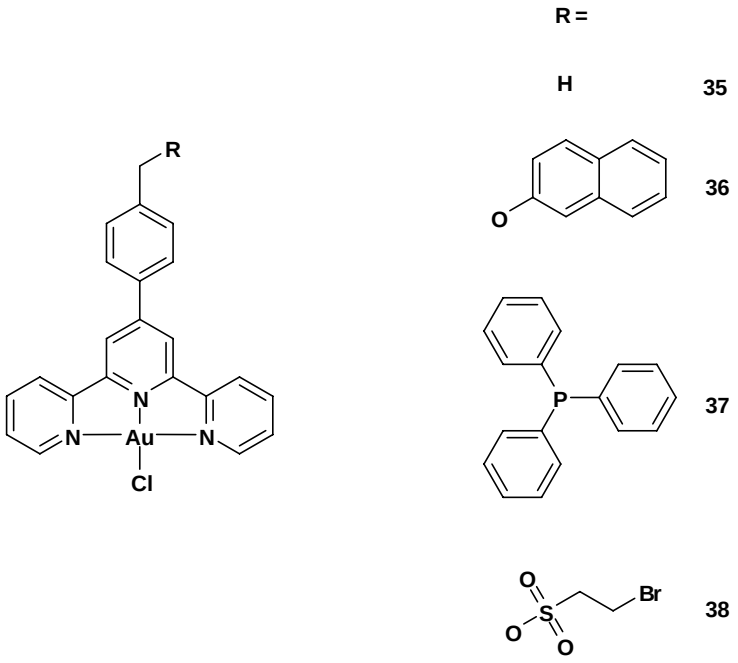


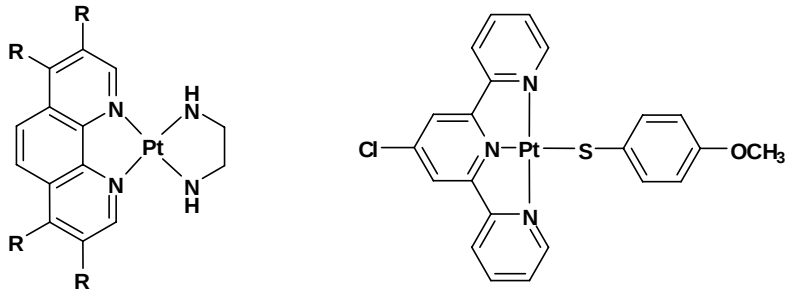

**Figure 2.4.4 Gold(III) complexes of and ligands (without Au centre) investigated for both DNA binding affinity and antitumor activity.[133]**

The interaction of some Cu-terpy derivatives with DNA has also recently been studied.[124, 134] These derivatives were found to bind intercalatively to DNA but had only moderate binding constants ($10^4$ $M^{-1}$). It was also demonstrated that the complexes were capable of DNA scission in the presence of hydrogen peroxide (similar to the case of Cu-phen species in the presence of hydrogen peroxide as demonstrated by Sigman).[101] These appear to be the only studies on the behaviour of Cu-terpy complexes with DNA. In light of the ability of terpy complexes to intercalate and platinate DNA they would appear to be good candidates for anticancer therapy (or even

for the treatment of malaria), since they should help to localise drugs within the DNA of tumour (or parasitic) cells.

## The Biological Activity of 2,2':6',2'' Terpyridine and its Complexes

The earliest investigation into the biological activity of terpy was reported in 1985 by McFayden *et al.*.[135] The authors investigated the *in vitro* and *in vivo* (mouse model) anticancer activity of a series of 1,10-phen and terpy platinum(II) intercalating complexes, among others. The highest activities were for the two 1,10-phen complexes **39** and **40** seen in Figure 2.4.5 ($IC_{50}$s of 0.7 μM and 2 μM, *in vitro*). For the terpyridine series complexes with thiolato counter ligands had the highest activity, *e.g.* the terpy complex **41**. [Pt(terpy)Cl]Cl had the lowest activity of terpy series, $IC_{50}$ of 450 μM. The terpy ligand, when tested uncomplexed to a metal, had activity higher than any of its associated complexes It has been suggested that the activity of the free ligands could be attributed to a metal sequestration mechanism, or the formation of toxic metal complexes[135]. None of the complexes showed any significant activity *in vivo*.



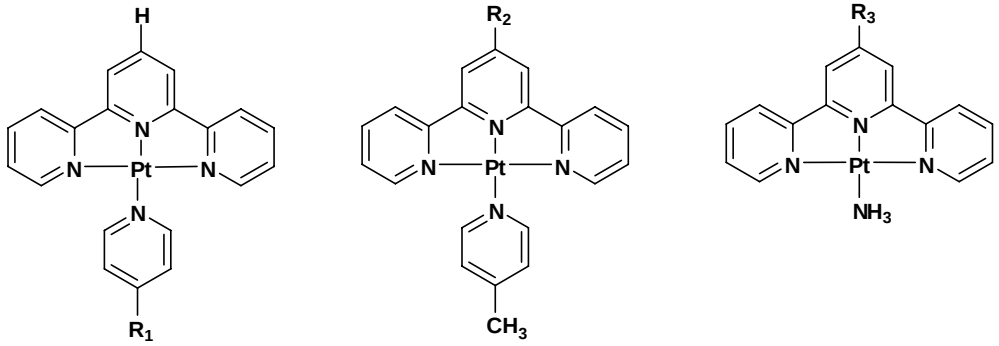

**R = H 39**
**R = $CH_3$ 40**

**41**

**Figure 2.4.5 Complexes active against Leukemia L1210.[135]**

Following these results, the most in depth study of the activity of terpy complexes has been by a group under Lowe[128, 136-140] Their initial work was an investigation into the cytotoxicity of terpy-platinum complexes against the protozoa *Leishmania donovani*,

*Trypanosoma cruzi* and *Trypanosoma brucei*.[136] Most of the complexes tested had a pyridine-type counter ligand (see Figure 2.4.6), as these have higher DNA intercalation binding constants (~ $10^6$ – $10^7$ $M^{-1}$).[136] However complexes with more labile ligands, Pt(terpy)$Cl^+$, Pt(terpy)$H_2O^{2+}$ and Pt(terpy)$NH_3^{2+}$, did in some cases exhibit higher antiparasitic activity than complexes designed with pyridine-type counter ligands. The authors also investigated the effect of derivatising the four position on terpy. From these results they discovered that a *chloro* or *para*-bromophenyl group enhanced activity the most. Complexes with these derivatives and an ammonia counter ligand, **47** and **48**, gave the highest (**47**) antiparasitic activity. While these two complexes gave significant activity in all three species of protozoan, the activity of each complex was different for each species of protozoan.




$R_1$ = $CH_3$ **42**
$R_1$ = Br **43**

$R_2$ = *p*-$BrC_6H_4$ **44**
$R_2$ = Cl **45**
$R_2$ = Br **46**

$R_3$ = *p*-$BrC_6H_4$ **47**
$R_3$ = Cl **48**

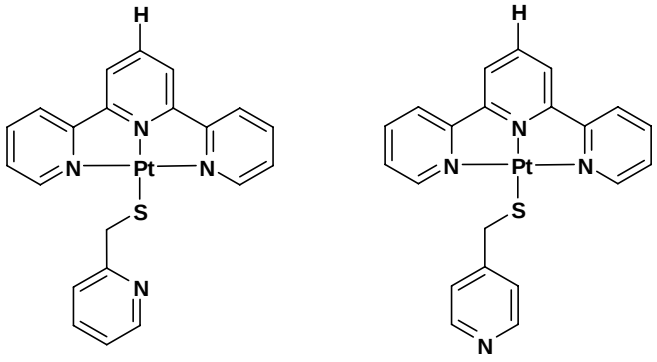
**Figure 2.4.6 Pt(terpy) complexes tested against the protozoa *L. donovani, T. cruzi* and *T. brucei*.[136]**

The mechanism of action here could be due to either DNA intercalation or DNA platination.[136] Attempts to discern which mechanism might be occurring, by using a terpy platinum complex with a thiol ligand did not provide any clarity as to the mode of action of these compounds. Shortly after this, Lowe *et al.*[138] published anticancer results for a similar series of terpy complexes, most of which were the same as those used in the earlier study. [136] The activity against several human ovarian cancer cell lines was tested. The activities of the free ligands were not reported in either study.

Bonse *et al.*[137] (2000) have reported that the most active platinum-terpy complexes (i.e. **47** and **48**) of their earlier work[136] are capable of inhibiting *T. cruzi* trypanothione[a,17] reductase. This inhibition was irreversible, strongly suggesting that this is the target of platinum-terpy complexes, and suggests that enzyme inhibition is a further possible mode of action for these compounds. Interestingly Pt-terpy complexes have been shown to bind, reversibly, to a cysteine residue on recombinant human serum albumin.[b, 17, 139] Ross *et al.*[139] suggest that this ability might be the transport mechanism which platinum-terpy complexes use to localise into cancerous cells. Furthermore it implies that the mode of action of these drugs could be *via* the irreversible binding to cysteine residues found on other important enzymes.[139]

In 2001, Becker *et al.*,[140] demonstrated exactly this. The authors showed that the platinum-terpy complexes were capable of inhibiting human thioredoxin reductase (hTrxR) irreversibly under *in vivo* conditions. This enzyme is responsible for DNA synthesis (transcription), autocrine growth stimulation and antioxidant defence, and is upregulated in tumour cells. Due to the affinity of platinum for thiol groups, it is likely that the selenocysteine residue is the site of attack by the complexes.[140] This work has been buoyed by the recent *in vivo* studies by Becker *et al.*[141] who demonstrated that the same mechanism occurs in rat models, treated with platinum-terpy complexes. They found that in rats treated with the complexes **49** and **50** (Figure 2.4.7), TrxR activity was reduced in several organ tissues (*e.g.* kidney, liver and skin) as well as in tumour cells. The compounds were found to be effective against established tumours and had higher anticancer activity than that observed for cisplatin.

[a] An enzyme which reduces thioredoxins (proteins which act as antioxidants).
[b] A protein with several functions (mostly transportation) and which is the most common protein in the blood plasma.



49 50

**Figure 2.4.7 Complexes tested in rat models for TrxR inhibition.[141]**

Around the same time that Lowe and his group were researching the activity of Pt-terpy complexes another group, under Messori were looking at Au-terpy complexes. In 2000 they reported the antitumor activity of a series of gold(III) complexes (Figure 2.4.3).[131] While the report on the antitumor activity of these complexes shows that the terpy ($IC_{50}$ 0.1 – 0.3 μM) and 1,10-phen ($IC_{50}$ 2.7 – 3.7 μM) complexes are the most active. It is interesting to note that the ligands themselves (terpy and 1,10-phen alone) were as toxic as their corresponding complexes. This activity is loosely ascribed to the formation of toxic metal complexes (*e.g.* Cu-phen) or metal sequestration.[131]

The antitumor activity of the complexes in Figure 2.4.4 was reported in 2006.[133] The most active species was **36**, 80% inhibition at 10 nM, approximately $10^5$ greater activity than cisplatin. Interestingly each of the corresponding ligands has slightly better activity than its associated gold complex. In the cases of the complexes the DNA binding affinity was found to correspond to the activity.[133] No explanation is given for the higher activity observed for the ligands themselves. While it is perhaps unintended the authors have demonstrated that, as seen before, terpy-type ligands often have as good as or better activity than the metal complexes thereof, which brings into question the necessity of transition metals for activity.

In 2006 the anticancer activity of the terpy ligand itself was tested.[142] Zhao *et al.*[142] prepared several terpy compounds (Figure 2.4.8), being various combinations of 2, 3 and 4 substituted pyridines. None of the compounds, with the exception of 2,2':6',2" terpyridine showed any reasonable activity (the other terpyridine compounds were 1 000 fold less effective than 2,2':6',2" terpyridine). The activity of terpy was better than or as good as the activity of current anticancer drugs (doxorubicin and camptothecin) in almost all the cell lines investigated. It also reported that terpy has no topoisomerase I[c,3] inhibitory activity, implying that this is not the mode of action of this compound. This paper however has demonstrated, as seen before,[133] that ligands themselves are capable of high anticancer activity, although the mechanism by which ligands and complexes act may be and probably are, very different.

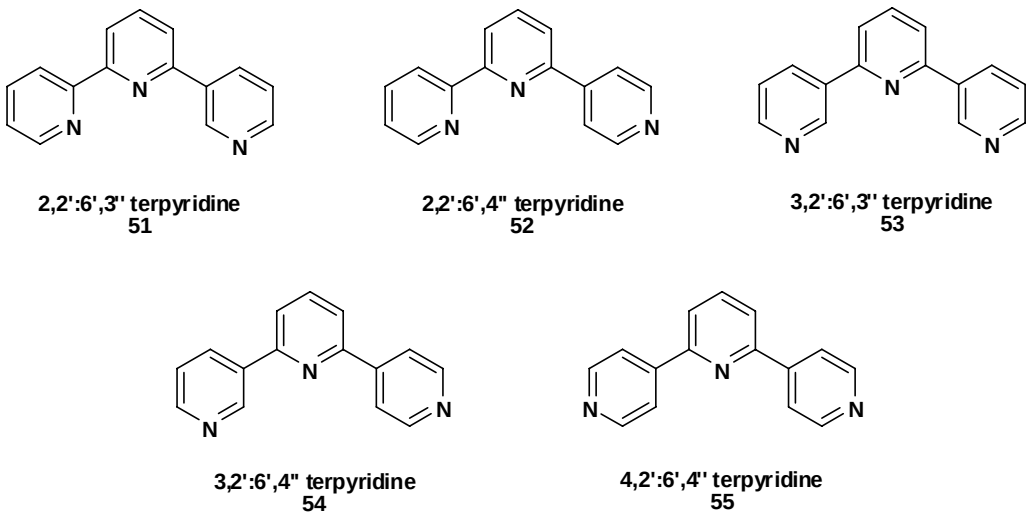


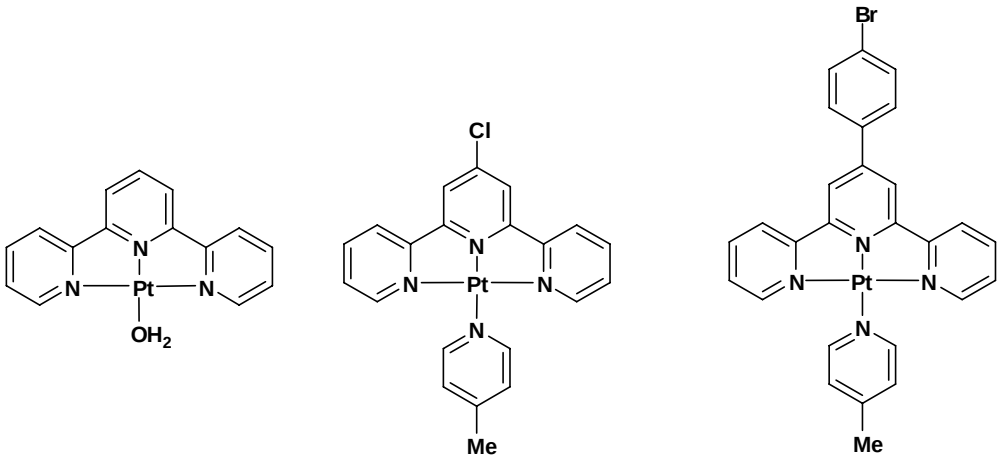

**Figure 2.4.8 Terpyridine compounds screened against several human cancer cell lines, all lacking activity.[142]**

## The Use of 2,2':6',2'' Terpyridine as an Antimalarial Agent

As stated earlier the only reported use of terpy (or complexes thereof as antimalarials) has come from Lowe in a patent.[128] The three compounds investigated, **56, 57** and **58**

[c] An enzyme responsible for cutting and rejoining a single DNA strand, altering the topology of the DNA.

(see Figure 2.4.9), had $IC_{50}$s of 2.7 μM, 20.6 μM and 2.4 μM respectively. No discussion on these results is presented in the patent.




**56** **57** **58**

**Figure 2.4.9 Terpyridine species tested by Lowe[128] (all as $BF_4$ salts, and the only terpy species to have been tested for antimalarial activity to date).**

Unlike 8-HQ and 1,10-phen, very little investigation into the antimalarial activity of terpy has been done. For this reason it is the aim here to determine whether terpy and its complexes, which have demonstrated good anticancer activity, have similarly good antimalarial action (as one patent has reported).[128] And while the behaviour of terpy complexes with DNA is well documented, there is no guarantee that its interaction with DNA is responsible for any activity which might be observed in antimalarial studies. Hence a study of the antimalarial action of both terpy alone, as well as the complexes of terpy with several metal ions, including $Pt^{2+}$, $Au^{3+}$, $Cu^{2+}$ and $Fe^{3+}$ will be undertaken. As in the case with both 8-HQ and 1,10-phen, there are many possible mechanisms by which the ligand and its complexes might exhibit activity (*e.g.* metalloprotease inhibition, β-haematin formation inhibition, DNA intercalation or platination or metal sequestration). Therefore efforts will also be made to gain insight into the mode of action of the free ligand and its complexes to determine whether they are in fact the same or different.

## 2.5 Summary

The literature which covers the antimalarial activity of the three ligands, 8-HQ, 1,10-phen and terpy, is slightly different in each case. However the questions surrounding the activities and mode of action of each of the ligands are very similar. An extensive study including all three ligands in the same study has also not yet been performed. Each of the ligands has been demonstrated to have a significant amount of activity in an alternative area of biochemistry: 8-HQ as an antibacterial; 1,10-phen as a DNA scission agent; and terpy as an anticancer agent. In each of these applications it has been shown, or sometimes assumed, that the activity is dependent on the presence of metal ions, whether in a premade complex or by complexation *in situ*. It is necessary therefore to investigate what the role of metal ions in the action of these compounds is. In order to do this several experiments must be performed:

1) the toxicity of free metal ions must be investigated as these have to date never been reported;
2) the toxicities of the free ligands should be investigated to provide a base activity;
3) the effect of free metals at high, but non-toxic levels in the growth medium, on the activities of the free ligands should be investigated;
4) the toxicities of premade ligand-metal complexes should be investigated and
5) the ability of each compound to inhibit haemazoin formation should be investigated.

These five points describe the work which should bring clarity and add to the current literature. Point 1 will provide insight as to the toxicity of various metal ions to the parasite. Point 2 will bring clarity to the disputed activity of 8-HQ and 1,10-phen and will also be the first report in open literature of the antimalarial activity of terpy. Points 3 and 4 will help to determine the importance and role of metals in the mode of action of these compounds, whether they act by formation of a toxic complex, metalloenzyme inhibition or metal sequestration. Point 5 will be useful in determining whether

haemozoin formation inhibition might be the mode of action of the metals, ligands or their complexes.

Thus it is the aim of the work here to provide a solid foundation on the antimalarial activities of the metals ($Cu^{2+}$, $Fe^{3+}$, $Au^{3+}$ and $Pt^{2+}$) and ligands (8-HQ, 1,10-phen and terpy). The secondary aim is to investigate role(s) that these metal ions play in each of ligands' mode(s) of action.

# CHAPTER 3

# Targeting Agents

## 3.1 Nutrients Selective to *Plasmodium falciparum* Infected Red Blood Cells

### The Exploitation of Parasite Transport Systems

There has been a substantial amount of work covering the transport into and accumulation of solutes in the malaria-infected RBC. Important to our study however is the iRBCs requirement for two solutes, pantothenic acid (which is exclusively transported by the iRBC)[59, 62, 64, 66] and L-adenosine (which is accumulated to a much higher extent in the iRBC than the normal RBC).[50]

In the treatment of any illness, drug delivery is extremely important. From Figure 1.5.1 (Chapter 1) it is apparent that in malaria it is even more complicated than usual because the parasite "hides" in the human erythrocyte. A drug must therefore cross at least three membranes to reach the parasite (and even more to reach organelles or specifically the food vacuole). Thus it may be possible, by selecting solutes which are exclusively or preferentially, transported or accumulated by iRBCs to enhance the activity and/or selectivity index of a drug when attached to a carrier molecule.[38, 43, 53, 74] Four different ways of exploiting the parasite and iRBCs NPP have been described[38]. The most obvious is to use compounds to block the nutrient transport pathways, essentially killing the parasite through starvation.[143-145] The second strategy which has been employed involves a combination approach. By using a toxic compound together with a compound which inhibits a normal RBC channel, the toxic compound is selectively accumulated by the iRBC.[65, 74] This selectivity is due to the parasites use of both endogenous transport pathways and the NPP (see Figure 3.1.1). A third approach is to develop compounds that are only activated when metabolised and ensure that they are

selectively metabolised by the parasite. The final approach is to exploit the parasites requirement for a particular nutrient by using the NPP to deliver a toxic compound with the nutrient,[43, 53, 65] sometimes referred to as a Trojan horse or magic bullet strategy.[38] These methods have been exploited by Gero *et al.*[65] who developed an L-nucleoside derivative which was selectively metabolised by the parasite, but was otherwise inactive. In this report Gero *et al.*[65] made use of both of these final two strategies and developed an intricate drug, one selectively transported and selectively metabolised by iRBCs.

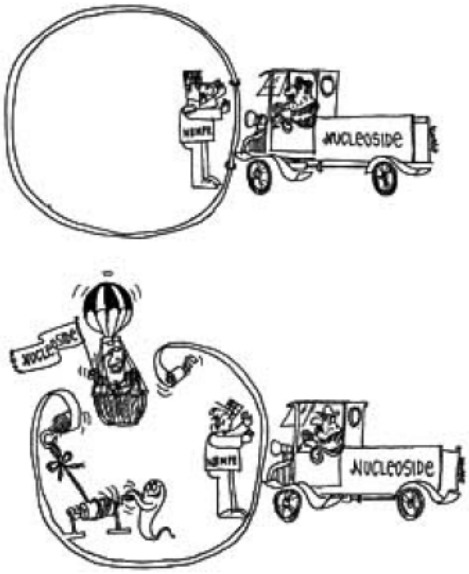



**Figure 3.1.1 Nucleoside transport in a normal (top) and infected (bottom) RBC when using a dual treatment approach.**[a65, 74]

## Pantothenic Acid

Pantothenic acid (Figure 3.1.2) has been identified as one the few solutes which is absolutely essential to the survival of the parasite *P.f.*.[59, 146] Saliba *et al.*[62] showed that

[a] Figure appears in Current Pharmaceutical Design Vol. 9 Iss. 11 pp. 867-877 (2003). Reprinted from Parasitology Today Vol. 8, Gero and Upston,, *Altered Membrane-Permeability - a New Approach to Malaria Chemotherapy*, pp. 283-286, Copyright (1992), with permission from Elsevier.

this transport is via the NPP expressed by the iRBC, and that the normal RBC expresses no active transporter for pantothenate. Furthermore pantothenate was shown to undergo

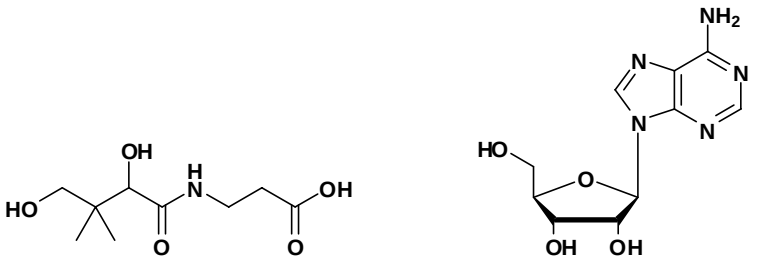



**Figure 3.1.2 Targeting agents for *Plasmodium falciparum*.**

phosphorylation within the parasite (rate of phosphorylation *P.f.*/RBC = 10), and not in the host erythrocyte implying that transport of this solute is indeed across all three membranes (RBCM, PVM and PPM).[62] It is likely that transport across the PVM is easily achieved due to its unselective nature (previously described by Desai, Krogstad and McCleskey[46]) and Saliba and Kirk[64] have demonstrated that transport across the PPM is mediated by $H^+$. The transport of pantothenate across this membrane was found to be dependent on $H^+$ concentration and independent of $Na^+$ concentration (in contrast with mammalian cells). The authors also discovered that the parasite's pantothenate kinase has a much higher affinity for pantothenate than the mammalian kinases. The kinase is efficient at phosphorylating and thereby trapping this substrate, at high concentrations, inside the parasite cytosol.[64] Previously it had been demonstrated that the *P.f.* trophozoites could accumulate pantothenate to up to 150% of the external concentration within 20 minutes. Following these findings Saliba and Kirk have moved in the direction of identifying analogues of pantothenate which exhibit antimalarial action by either blocking transport through the NPP or inhibiting the parasite pantothenate kinase.[146-149]

## Adenosine

A second nutrient for which the parasite has an exclusive affinity (when compared with normal erythrocytes) is L-adenosine (Figure 3.1.2).[50] This is most probably as a result of the parasite's high requirement for nucleosides, as it is incapable of *de novo* purine synthesis.[22] The endogenous erythrocyte nucleoside transporter is selective for the D-isomer of this compound and does not show any appreciable transport of the L-isomer.[50] This finding was the result of several studies in which Gero and coworkers[44, 45, 50] first demonstrated that the uptake and incorporation into polynucleotides of adenosine by iRBCs was almost unaffected by nitrobenzylthioinosine (NBMPR), a known inhibitor of the erythrocyte nucleoside transporter.[44] The conclusion from this result was that the parasite must be making use of the NPP, which ones specifically remained unknown, either new nucleoside trafficking routes had been developed, or the RBC transporter had been sufficiently modified to render it insensitive to the inhibitor.[44] Thus it became apparent that it may be viable to treat malaria using two drugs: an erythrocyte nucleoside transport inhibitor, and a toxic nucleoside.[44] It was in fact shortly thereafter demonstrated that this hypothesis held true (at least *in vitro*), and that NBMPR ($IC_{50}$ 4.2 μM) and the toxic nucleoside tubercidin ($IC_{50}$ 0.51 μM) were shown to have a synergistic effect used together [activity was at concentrations lower than would be ascribed to a purely additive effect, determined by Fractional Inhibitory Concentration (FIC) values].[45] Upston and Gero[50] discovered that the iRBC was in fact transporting L-adenosine as well as the D-isomer. In this study[50] they established that the transport which was occurring was active (*i.e.* not simple diffusion), non-saturable and that the L-adenosine was metabolised into L-inosine and not incorporated ($1/10^{th}$ of the amount of D-adenosine) into nucleic acid material. Several L-adenosine transport inhibitors were also identified.[50] In 1998 Penny *et al.*[61] injected *Xenopus laevis* oocytes[b,3] with mRNA isolated from *P.f.* parasites. From this technique it was demonstrated that *P.f.* encodes its own transporters of nucleosides, nucleobases, hexoses and monocarboxylates. The uptake of D-adenosine by this transporter was stereospecific (not inhibited by high concentrations of the L-isomer), purine selective and insensitive to inhibitors of

[b] An immature egg cell of the species *Xenopus laevis* commonly used in studies involving the expression of RNA and/or DNA

erythrocyte transporters.[61] Brown *et al*[150] would go on to show that the parasite adenosine deaminase[c] (ADA) is upregulated 1000 fold, compared with that in the uninfected erythrocyte. In addition the parasite ADA alone was capable of metabolizing L-adenosine into L-inosine, an additional difference between the treatment of the L-nucleoside in iRBCs and normal RBCs.[150] Mammalian ADAs in general do not use the L-isomer.[151]

In 2000 two groups independently[55, 57] reported the identification of a *P.f.* nucleoside transporter, designated PfNT1[55] and PfENT1[57] respectively. The two proteins were identical with the exception of one base position.[57] A detailed analysis of the transport properties of this protein was made by Carter *et al.*.[55] Their findings were that PfNT1 had a high selectivity for adenosine over other nucleosides and that the transporter was selective for D-adenosine, but not stereoselective for it (both the D- and L-isomers were transported, although the rate of uptake of the D-isomer was much higher).[55] The inability for known NPP inhibitors[48] to affect the D-adenosine[55] uptake suggests that this transport pathway is not involved in NPP transport discovered by Kirk.[48] This transporter was later located at the PPM, using immunogold labelling techniques.[58] Both sets of authors indicate that the parasite's ability to transport L-isomers of nucleosides is an excellent means by which to deliver toxic compounds.[55, 57] An excellent exploitation of this transport pathway was demonstrated by Gero *et al.*[65], when they chemically bonded 2'-deoxy-L-adenosine (a carrier) with 5-fluoro-D-2' deoxyuridine-monophosphate (a cytotoxic nucleoside) *via* a phosphate linker. The compound was designed to make use of both selective transportation and the parasite ADA's ability to metabolize the L isomers of adenosine. The advantage of these drugs really lies in the lack of toxicity towards mammalian cells, (non-toxic to mice treated with doses as high as 400 mg/kg).[65] A key part to the strategy of Gero *et al.*[65], is that the compounds are non-toxic until metabolised.

[c] An enzyme responsible for the irreversible conversion of adenosine to inosine (converting an amine group to a ketone, deamination)

In this project it is hoped that compounds such as pantothenic acid and adenosine will confer increased transport and accumulation into the parasite when chemically combined (*via* a sigma bond) with the ligands. Furthermore it is not intended that the compounds should need to be metabolised for action, *i.e.* their mode(s) of action should remain unchanged in the derivatized form. It would be preferable to derivatize the ligands, 8-HQ, 1,10-phen and terpy with D-adenosine, this isomer is extremely expensive, thus derivatization with L-adenosine will be undertaken instead as proof of concept. While the idea of using these carriers for drug delivery is itself not novel, the use of pantothenic acid and adenosine to deliver the selected ligands into the parasite appears to be previously unreported. Furthermore the covalent linking of these carriers to the ligands is also unreported.

While it is clear that *P.f.* needs both these nutrients, pantothenic acid and adenosine (the D- or L-isomer), their usefulness as drug delivery pathways remains to be investigated. Whether they can increase the activity of antimalarial compounds would be of significant interest both for current drugs and for the development of new drugs. It is the intention here to link both of these compounds to the ligands already discussed (8-HQ, 1,10-phen and terpy), and to determine if any appreciable increase in toxicity is conferred on the ligands. This will make use of the ‘Trojan horse’ type strategy, by modifying the ligands from Chapter 2, according to the synthetic schemes in the Chapter 4. It is hoped that the parasite’s requirement for this solute will increase the accumulation (and possibly selectivity) of the ligands, and hence their activities.

# CHAPTER 4

# Synthetic Design and Results

## 4.1 Synthesis of Complexes

As part of the planned research it was necessary to synthesize the complexes of each of the ligands. These were synthesised according to the relevant established procedures.[152, 153]

The 8-HQ complexes of copper and iron were made according to the procedures described in by Welcher.[152] However since these complexes were not soluble in water, DMSO, DMF, acetone, ethanol, methanol or THF could therefore not be tested. As these were the two metals of most significance to 8-HQ, the synthesis of complexes was discontinued.

Similarly the 1,10-phen complexes were synthesized according to literature methods.[153] These complexes (made as the $ClO_4^-$ salts) also demonstrated a total lack of solubility (copper and iron complexes) and thus this work was also discontinued.

The terpy complexes of platinum,[154] gold,[155] copper,[156] iron[157] and palladium,[158] (Figure 4.1.1), were all synthesized according to previously established methods. Where possible the synthesis was confirmed by NMR studies, IR and high resolution mass spectrometry (the spectra may be seen in the appendix). In general the synthesis proceeded as described in the relevant literature, however the palladium terpy complex was synthesised *via* two methods. Both products gave identical NMR spectra.

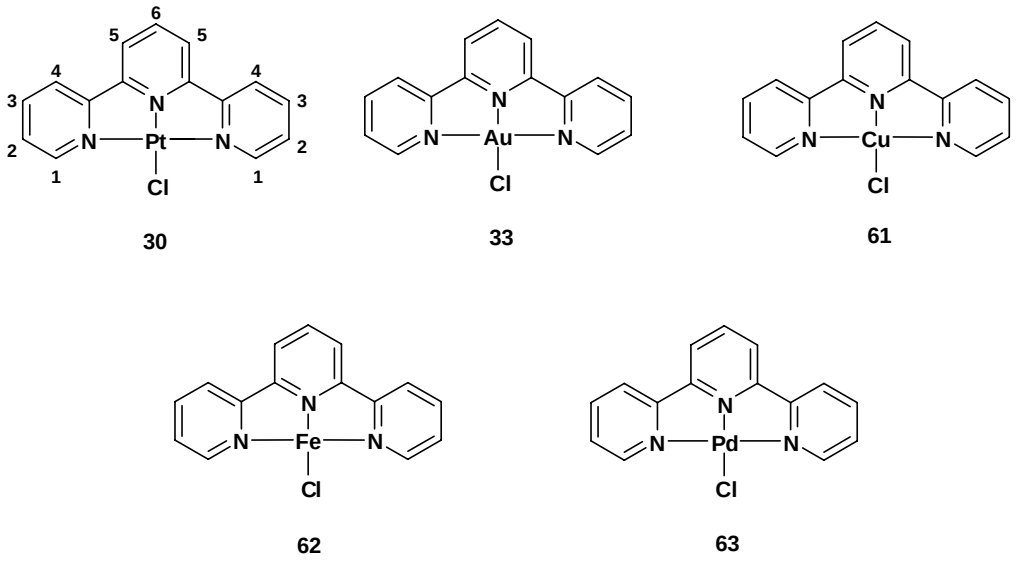



**Figure 4.1.1 Structures of terpy complexes synthesized and tested (counterions excluded from structures).**

**Table 4.1.1 $^{1}$H Chemical shifts for terpy complexes.**

| Proton | Chemical shift/ppm (multiplicity, integration)[i] | | | |
|---|---|---|---|---|
| | **29** | **30** | **33** | **63** |
| $H_1$ | 7.83 (m, 2H) | 8.89 (dd, 2H) | 8.77 (dd, 2H) | 8.72 (dd, 2H) |
| $H_2$ | 7.30 (m, 2H) | 7.95 (m, 2H) | 7.60 (m, 2H) | 7.88 (m, 2H) |
| $H_3$ | 8.68 (m, 2H) | 8.51 (td, 2H) | 8.15 (m, 2H) | 8.46 (td, 2H) |
| $H_4$ | 8.61 (m, 2H) | 8.64 (m, 2H) | 8.49 (d, 2H) | 8.64 (m, 2H) |
| $H_5$ | 8.45 (m, 2H) | 8.64 (m, 2H) | 8.71 (d, 2H) | 8.64 (m, 2H) |
| $H_6$ | 7.93 (m, 1H) | 8.64 (m, 1H) | 8.15 (m, 1H) | 8.64 (m, 1H) |

[i] $^{1}$H NMR spectra collected in DMSO-$d_6$.

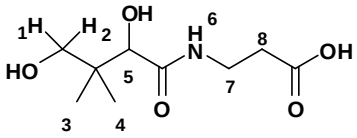
The $^{1}$H NMR spectra of the platinum, gold and palladium complexes (Table 4.1.1) all demonstrate downfield proton shifts, as one would expect, due to the donation of the nitrogen atoms lone pairs for coordinative bonding to the respective metal centres.

NMR spectra for the copper and iron complexes could not be obtained due to their paramagnetic nature.

# 4.2 The Design and Synthesis of Target Ligands

## Targeting Agents

The synthetic procedure for this work was started by preparing the two targeting agents, pantothenic acid **59** and 5'-chloro-adenosine. As will be described later 5'-chloro-adenosine did not facilitate any desired coupling and was thus replaced with the more reactive 5'-carboxylic-acid-adenosine, **66**.




**59**

**Figure 4.2.1 Numbering of pantothenic acid.**

The two targeting agents, pantothenic acid and 5'-carboxylic-acid-adenosine, were prepared from calcium pantothenate and adenosine respectively. Pantothenic acid was prepared by eluting a solution of the hemi-calcium salt through an ion exchange resin, and the acid was isolated in quantitative yield. It was found that removal of the solvent (water) had to be done by lyophilization as the use of heat resulted in the formation of unwanted impurities observed in the NMR spectra ($^{1}$H and $^{13}$C). The chemical shifts for pantothenic acid ($^{1}$H NMR in $D_2O$) are reported in Table 4.2.1, assigned from literature.[159]

**Table 4.2.1 $^{1}$H Chemical shifts for pantothenic acid, 59.**

| Proton | Chemical shift/ppm (multiplicity, integration)[i] |
|---|---|
| | **59** |
| $H_{1,7}$ | 3.39 (m, 3H) |
| $H_2$ | 3.28 (m, 1H) |
| $H_3$ | 0.81 (s, 3H) |
| $H_4$ | 0.77 (s, 3H) |
| $H_5$ | 3.87 (s, 1H) |
| $H_8$ | 2.52 (t, 2H) |

[i] $^{1}$H NMR spectrum collected in $D_2O$.

The synthetic scheme for the synthesis of **66** is seen in Figure 4.2.2. The synthesis involves the protection of the alcohol groups on the nucleoside,[160] followed by oxidation using $KMnO_4$,[161] and subsequent deprotection.

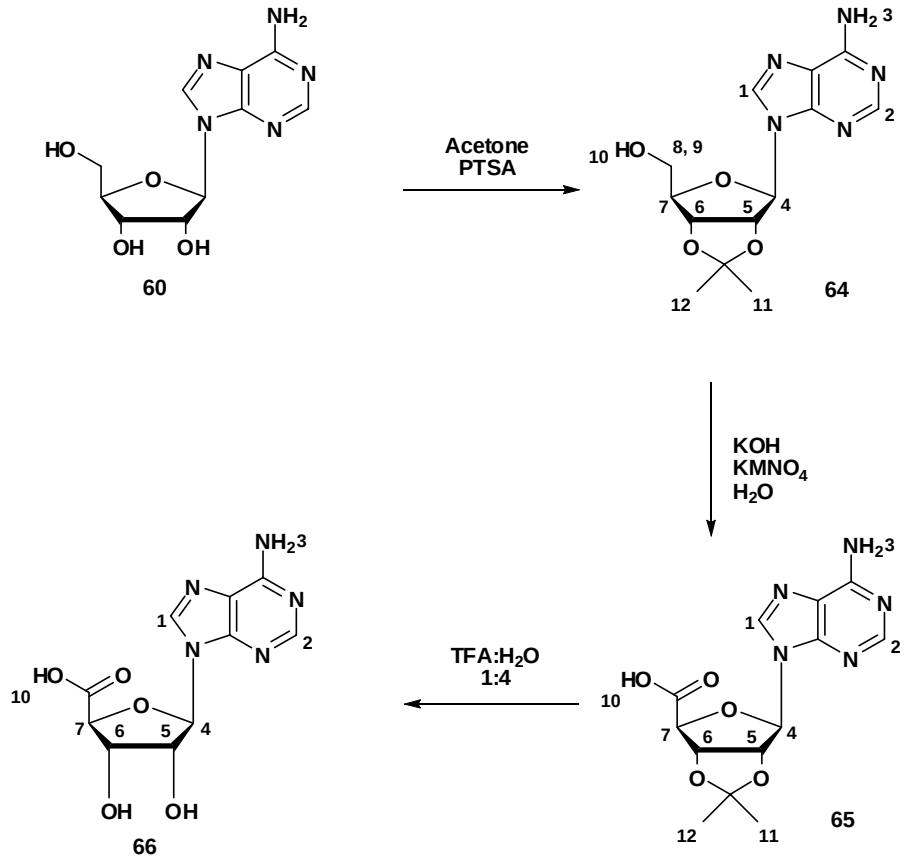



**Figure 4.2.2 Synthetic route for 5'-carboxylic-acid-adenosine, 66.**

This synthesis proceeded without any difficulties and was easily tracked by $^{1}$H NMR, see Table 4.2.2. Protection of adenosine, resulting in **64**, is easily confirmed by the appearance of the two singlets at 1.52 ppm and 1.31 ppm, integrating for three protons each, corresponding to $H_{11}$ and $H_{12}$ respectively. At the same time the signals due to the previously unprotected –OH groups (5.51 ppm and 5.24 ppm) are no longer seen (adenosine $^{1}$H spectrum not shown). Similarly the oxidation step can be confirmed by the loss of the two protons signals $H_8$ and $H_9$. The final deprotection step is confirmed by the disappearance of protons due to $H_{11}$ and $H_{12}$. Due to the untidiness of the spectrum in pure DMSO, $D_2O$ was added, resulting in the complete disappearance of the signal due to $H_3$, and any of the –OH groups. However a COSY correlation (in DMSO alone, COSY spectrum not shown) is still observed between these –OH protons and $H_5$ and $H_6$.

**Table 4.2.2 $^{1}$H Chemical shifts for 5'-carboxylic acid synthesis 66.**

| Proton | Chemical shift/ppm (multiplicity, integration)[i] | | |
|---|---|---|---|
| | **64** | **65** | **66** |
| $H_1$ | 8.33 (s, 1H) | 8.23 (s 1H) | 8.68 (s, 1H) |
| $H_2$ | 8.14 (s 1H) | 8.07 (s, 1H) | 8.41 (s, 1H) |
| $H_3$ | 7.35 (s 2H) | 7.26 (s, 2H) | Displaced by $D_2O$[ii] |
| $H_4$ | 6.11 (d, 1H) | 6.31 (s, 1H) | 6.07 (d, 1H) |
| $H_5$ | 5.33 (dd, 1H) | 5.52 (dd, 1H) | 4.52 (dd, 1H) |
| $H_6$ | 4.95 (dd, 1H) | 5.45 (d, 1H) | 4.46 (d, 1H) |
| $H_7$ | 4.20 (m, 1H) | 4.67 (d, 1H) | 4.32 (dd, 1H) |
| $H_{8,9}$ | 3.54 (m, 2H) | - | - |
| $H_{10}$ | 5.24 (t, 1H) | - | - |
| $H_{11}$ | 1.52 (s, 3H) | 1.51 (s, 3H) | - |
| $H_{12}$ | 1.31 (s, 3H) | 1.34 (s,3H) | - |

[i] $^{1}$H NMR spectra collected in DMSO-$d_6$.
[ii] $D_2O$ was added to produce a neater spectrum.

## 8-Hydroxyquinoline and 1,10-Phenanthroline Derivatives

Having identified both the ligands and the targeting agents to be studied, several initial synthetic schemes were proposed. The original synthetic design for the derivatized versions of 8-HQ and 1,10-phen is outlined in Figure 4.2.3 and Figure 4.2.4 respectively. The approach of making an aromatic amino derivative of each of the ligands (8-HQ and 1,10-phen) and then linking these directly to the targeting agents was proposed.

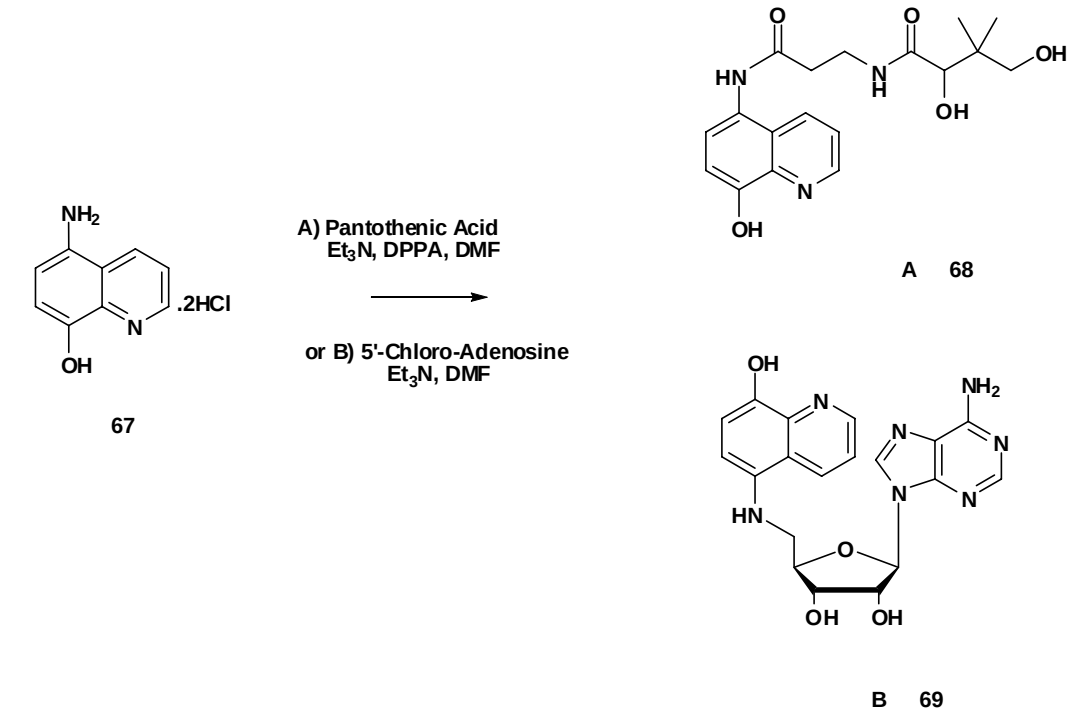



**Figure 4.2.3 Proposed synthetic scheme for derivatives of 8-HQ.**

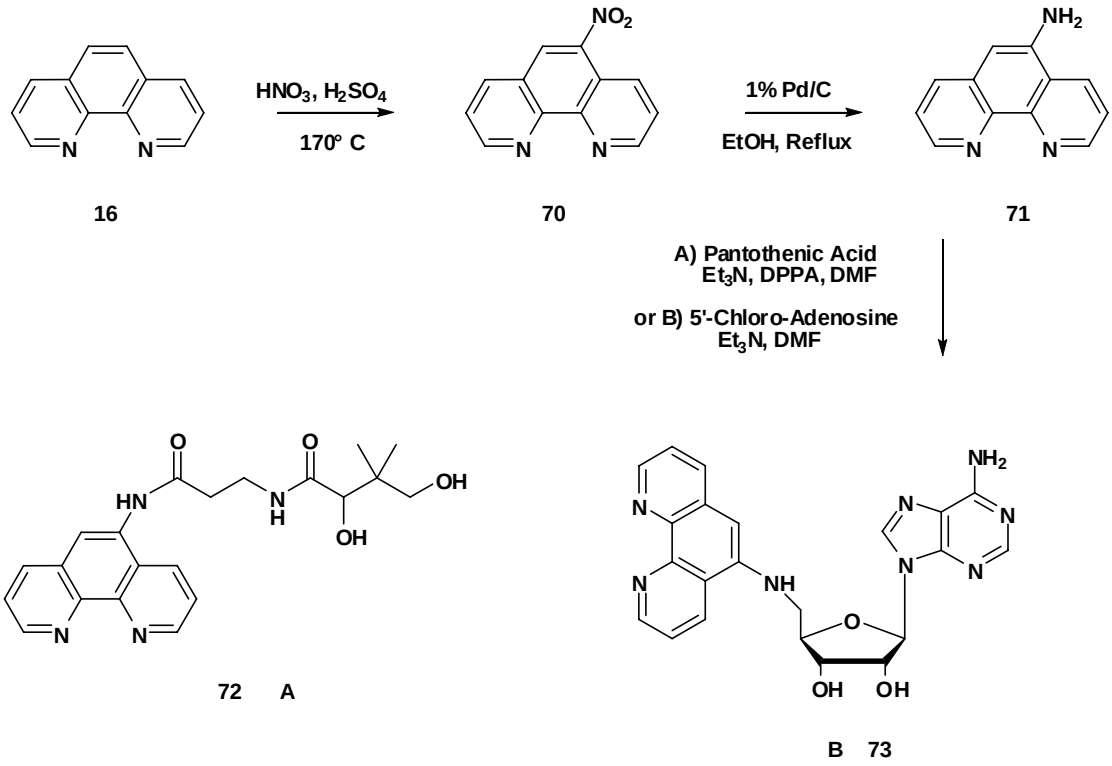


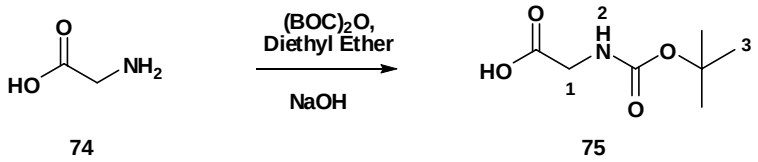

**Figure 4.2.4 Proposed synthesis for derivatives of 1,10-phen.**

In each of these synthetic schemes some of the reactions were found to render very poor yields or no desired product at all. For example the reactions of 5-amino-8-HQ **67**, and 5-amino-1,10-phen **71**, with 5'-chloro-adenosine, were both found to be non-reactive (monitored by LC-MS) under a variety of conditions (including temperature, solvents and bases). Both of these compounds also demonstrated limited reactivity with pantothenic acid as well. This is likely due to the electron withdrawing effect of ring-bound nitrogen atoms, resulting in a drop of nucleophilicity of the amine at the five position on each of the compounds.[161, 162] Previous reactions where amines have been coupled with 5'-chloro-adenosine, were achieved under extreme conditions (a bomb reactor[163] or extended periods of time).[164] The literature procedures for purification of the products were also extensive, involving both preparative HPLC, the use of an ion exchange resin and resulted in yields around 50%.

In response to the poor reactivity observed here and the limited number of reported reactions for the amine derivatized ligands, **67** and **71**, it was decided to adapt the synthetic scheme. These new schemes included a spacer (or bridge) between the original ligands and the targeting agents. This would reduce possible steric interactions that could hinder the reactivity. Furthermore a spacer would break conjugation, reducing the electron withdrawing effects of the ring-bound nitrogen atoms, increasing the nucleophilicity of the ligands. A spacer also serves to keep the targeting agent and ligand chemically and electronically separate, a characteristic which may be advantageous biologically. Several different spacers were considered including 3-bromopropylamine and glycine, **74**. Attempts to couple 3-bromopropylamine with 5'-chloro-adenosine were unsuccessful, these again involved various bases, solvents and heating methods (conventional or microwave). Similarly attempts to couple *N*-protected 3-bromopropylamine with either **67** or **71** were unsuccessful.

Ultimately glycine **74** was introduced as the spacer. Glycine was first *N*-protected using the *t*-BOC group to give **75** (Figure 4.2.5, chemical shifts in Table 4.2.3), and then reacted in large excess (16 equivalents converted to the anhydride *in situ* using DCC, a method which has only recently been reported)[165] with each of the two ligands, to give

**76** and **80** respectively. Using EDC as a coupling reagent was also successful, but separation of the coupling reagent from the product proved difficult. The glycine-nitrogen sites on derivatives **76** and **80** were then deprotected to give **77** and **81**, respectively.




74 75

**Figure 4.2.5 Linker used for 8-HQ and 1,10-phen derivatives (both glycine, 74).**

**Table 4.2.3 $^{1}$H Chemical shifts for *N*-BOC-glycine, 75.[166]**

| Proton[i] | Chemical shift/ppm (multiplicity, integration)[ii] |
|---|---|
| | **75** |
| $H_{1,E\&Z}$ | 3.93 (m, 2H) |
| $H_{2,E}$ | 6.70 (b, 0.33H) |
| $H_{2,Z}$ | 5.06 (b, 0.67H) |
| $H_3$ | 1.43 (s, 9H) |

[i] **75** exists in both E and Z conformations, and thus gives a slightly complicated proton spectrum.
[ii] $^{1}$H NMR spectrum collected in $CDCl_3$.

Coupling of the intermediate ligands **77** and **81** with 5'-chloro-adenosine, was again either low yielding or produced no desired target compound. Following these results the general reactivity of 5'-chloro-adenosine was investigated. For this purpose phenylethylamine was used as a synthetic mimic for the ligands. It was found under various conditions (conventional heating or microwave heating) and using a variety of solvents, that this compound, 5'-chloro-adenosine, had limited reactivity. Therefore it was decided to replace 5'-chloro-adenosine, with the more reactive, 5'-carboxylic-acid-adenosine **66**, the synthesis of which is described in Figure 4.2.2. The final synthetic

routes for the derivatized versions of 8-HQ and 1,10-phen are described in Figure 4.2.6 and Figure 4.2.7.

At this point each of the targeting agents was reacted with these newly functionalized ligands, giving compounds **78** and **79** (in the case of 8-HQ), and **82** and **83** (1,10-phen) respectively (Figure 4.2.6 and Figure 4.2.7). For the final steps it should be noted that tracking of these reactions was not possible using normal phase TLC plates. Hence LC-MS was the preferred method of analysis used to track the reaction of each ligand with each of the targeting agents. Coupling of the amines with pantothenic acid, **59**, was achieved using diphenylphosphoryl azide (DPPA), a general procedure well established in literature.[167-170] In the case of coupling with **66**, *o*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) was used as a coupling reagent.

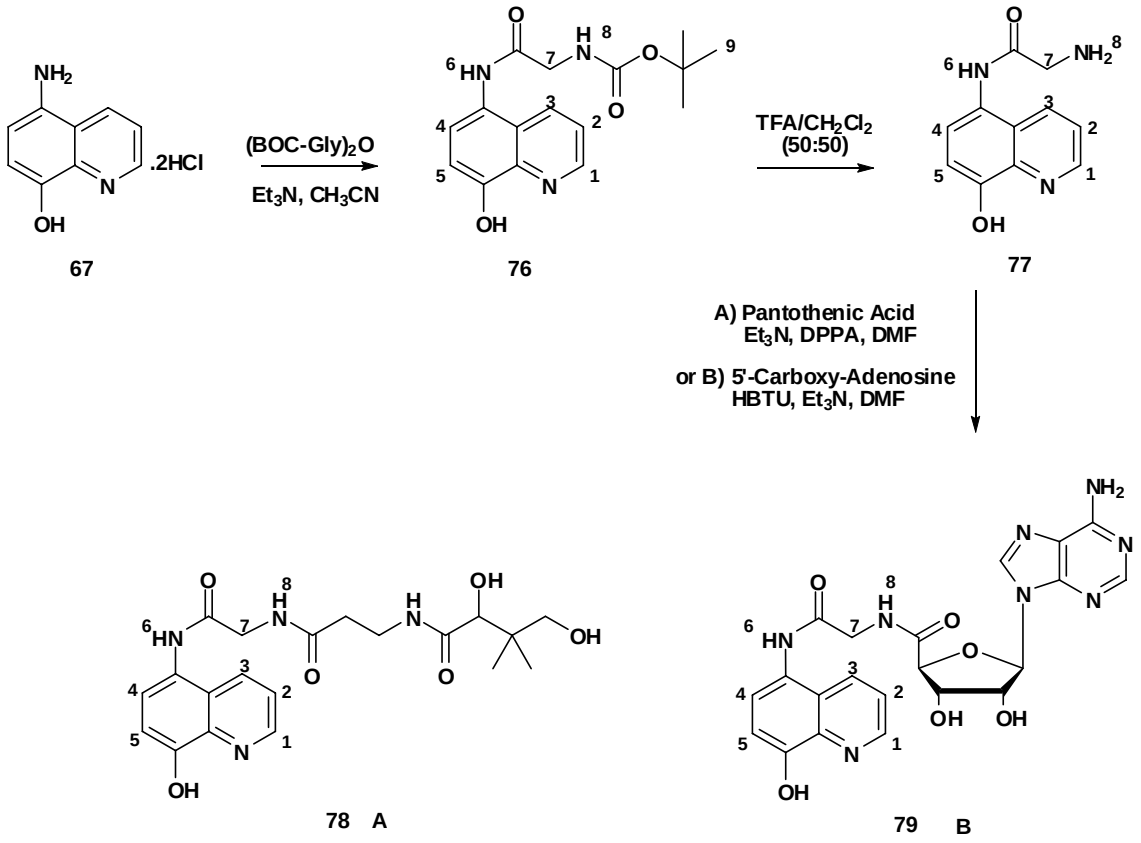



**Figure 4.2.6 Synthetic route for derivatization of 8-HQ with pantothenic acid (A) or adenosine (B).**

The $^{1}$H chemical shifts for the synthesis of the 8-HQ derivatives **76-79**, are reported in Table 4.2.4. The addition of **75** to 8-HQ is monitored by the appearance of a doublet (2H) at 3.81 ppm, due to $H_7$ ($H_2$ on **75**) and the singlet (9H) at 1.40 ppm due to $H_9$ ($H_3$ on **75**). Deprotection with TFA affords the loss of this singlet and a downfield shift for the amide $H_6$ and the amine $H_8$. The addition of either **59** or **66** results in a spectrum which appears almost as an overlap of the two spectra of the precursors (i.e. either **59** and **77** or **66** and **77**) thus the assignment of the $^{1}$H signals for **78** and **79** is fairly trivial.

**Table 4.2.4 $^{1}$H Chemical shifts for synthesis of 8-HQ derivatives.**

| Proton | Chemical shift/ppm (multiplicity, integration)[i] | | | |
|---|---|---|---|---|
| | **76** | **77** | **78** | **79** |
| $H_1$ | 8.85 (dd, 1H) | 8.93 (d, 1H) | 8.91 (s, 1H) | 8.89 (d, 1H) |
| $H_2$ | 7.56 (m, 1H) | 7.69 (dd, 1H) | 7.68 (dd, 1H) | 7.63 (dd, 1H) |
| $H_3$ | 8.31 (dd, 1H) | 8.52 (d, 1H) | 8.44 (d, 1H) | 8.41 (d, 1H) |
| $H_4$ | 7.39 (d, 1H) | 7.52 (d, 1H) | 7.44 (d, 1H) | 7.45 (d, 1H) |
| $H_5$ | 7.05 (d, 1H) | 7.16 (d, 1H) | 7.13 (d, 1H) | 7.09 (d, 1H) |
| $H_6$ | 9.76 (s, 1H) | 10.35 (s, 1H) | 9.87 (s, 1H) | 9.94 (s, 1H) |
| $H_7$ (and A-$H_7$) | 3.81 (d, 2H) | 3.92 (m, 2H) | 3.97 (d, 2H) | 4.21 (m, 3H) |
| $H_8$ | 7.12 (t, 1H) | 8.17 (b, 2H) | 8.35 (t, 1H) | 9.27 (s, 1H) |
| $H_9$/P-OH | 1.40 (s, 9H) | - | 8.14 (s) | - |
| P-$H_{1,2,7}$/A-$H_1$ | - | - | 3.22 (m, 4H) | 8.51 (s, 1H) |
| P-$H_3$/A-$H_2$ | - | - | 0.79 (s, 3H) | 8.26 (s, 1H) |
| P-$H_4$/A-$H_3$ | - | - | 0.78 (s, 3H) | - |
| P-$H_5$/A-$H_4$ | - | - | 3.69 (s, 1H) | 6.03 (d, 1H) |
| P-$H_6$/A-$H_5$ | - | - | 7.76 (t, 1H) | 4.67 (dd, 1H) |
| P-$H_8$/A-$H_6$ | - | - | 2.41 (m, 2H) | 4.46 (s, 1H) |

[i] $^{1}$H NMR spectra collected in DMSO-$d_6$, P denotes pantothenate peaks and A denotes adenosine peaks.

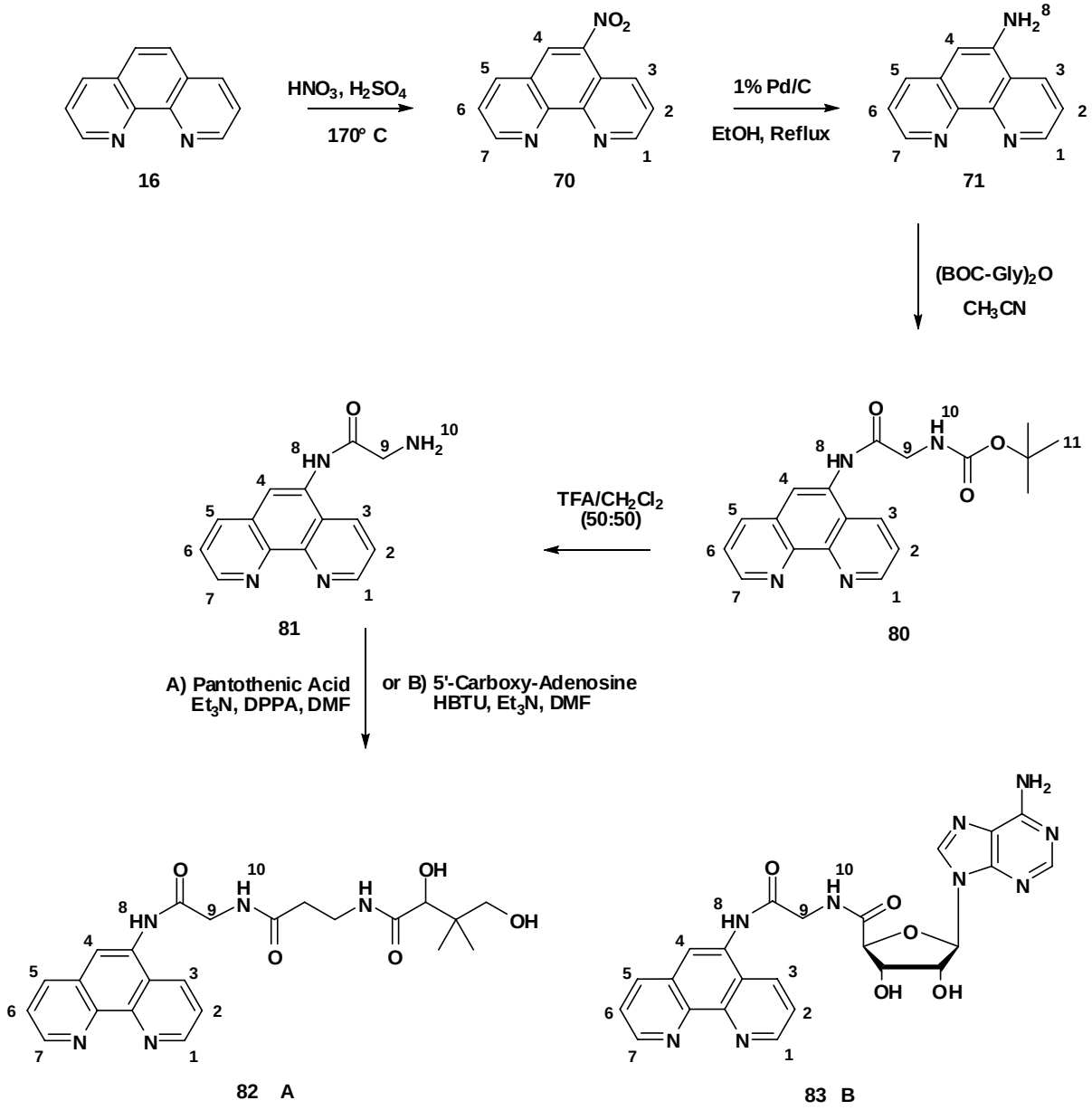



**Figure 4.2.7 Synthetic route for derivatization of 1,10-phen with pantothenic acid (A) or adenosine (B).**

The synthetic route for 1,10-phen began by conversion of the ligand to a nitro compound, which was then reduced to an amine (1% Pd/C) which then opened up synthetic routes to the intermediate and target ligands, the $^{1}$H NMR shifts of which are reported in Table 4.2.5. Conversion of 1,10-phen to **70** was done under harsh conditions (the reaction only proceeded at 160–170 °C in the presence of 98% $H_2SO_4$

**Table 4.2.5 $^1$H Chemical shifts for synthesis of 1,10-phen derivatives.**

| Proton | Chemical shift/ppm (multiplicity, integration)[i] | | | | | |
|---|---|---|---|---|---|---|
| | **70** | **71** | **80** | **81** | **82** | **83** |
| $H_1$ | 9.35 (dd, 1H) | 9.18 (dd, 1H) | 9.12 (dd, 1H) | 9.30 (d, 1H) | 9.13 (d, 1H) | 9.13 (d, 1H) |
| $H_2$ | 7.80 (m, 1H) | 7.63 (dd, 1H) | 7.80 (m, 1H) | 8.11 (m, 1H) | 7.83 (dd, 1H) | 7.83 (dd, 1H) |
| $H_3$ | 9.02 (dd, 1H) | 8.26 (dd, 1H) | 8.61 (d, 1H) | 8.98 (m, 1H) | 8.60 (d, 1H) | 8.66 (d, 1H) |
| $H_4$ | 8.69 (s, 1H) | 6.93 (s, 1H) | 7.20 (s, 1H) | 8.44 (s, 1H) | 8.12 (s, 1H) | 8.13 (s, 1H) |
| $H_5$ | 8.42 (dd, 1H) | 7.96 (dd, 1H) | 8.45 (d, 1H) | 8.98 (m,1H) | 8.46 (d, 1H) | 8.46 (d, 1H) |
| $H_6$ | 7.80 (m, 1H) | 7.48 (dd, 1H) | 7.75 (m, 1H) | 8.11 (m, 1H) | 7.76 (m, 1H) | 7.74 (dd, 1H) |
| $H_7$ | 9.29 (dd, 1H) | 8.92 (dd, 1H) | 9.03 (dd, 1H) | 9.19 (d, 1H) | 9.05 (d, 1H) | 9.04 (d, 1H) |
| $H_8$ | - | 4.25 (b, 2H) | 10.15 (s, 1H) | 10.91 (s, 1H) | 10.20 (s, 1H) | 10.30 (s, 1H) |
| $H_9$ (and A-$H_7$) | - | - | 3.93 (d, 2H) | 4.08 (d, 2H) | 4.08 (d, 2H) | 4.28 (m, 3H) |
| $H_{10}$ | - | - | 8.13 (s, 1H) | 8.29 (b, 2H) | 8.41 (t, 1H) | 9.52 (s, 1H) |
| $H_{11}$ | - | - | 1.48 (s, 9H) | - | - | - |
| P-OH | - | - | - | - | 8.15 (s) | - |
| P-$H_{1,2,7}$/A-$H_1$ | - | - | - | - | 3.25 (m, 4H) | 8.44 (s, 1H) |
| P-$H_3$/A-$H_2$ | - | - | - | - | 0.81 (s, 3H) | 8.16 (s, 1H) |
| P-$H_4$/A-$H_3$ | - | - | - | - | 0.79 (s, 3H) | 7.42 (s, 2H) |
| P-$H_5$/A-$H_4$ | - | - | - | - | 3.71 (s, 1H) | 6.03 (d, 1H) |
| P-$H_6$/A-$H_5$ | - | - | - | - | 7.76 (m, 1H) | 4.71 (dd, 1H) |
| P-$H_8$/A-$H_6$ | - | - | - | - | 2.43 (m, 2H) | 4.46 (s, 1H) |

[i] All $^1$H NMR spectra collected in DMSO-$d_6$, except **70** and **71** which were collected in $CDCl_3$, P denotes pantothenate peaks and A denotes adenosine peaks.

and 63% $HNO_3$, lower concentrations of nitric acid did not result in nitration).[171] The nitration of 1,10-phen to **70** is easily confirmed by the downfield singlet $H_4$ (1H) appearing at 8.69 ppm. Reduction of the nitro group to an amine, **71,** results in the singlet shifting to 6.93 ppm and the appearance of a broad peak (2H) at 4.25 ppm. It

should be noted that most synthetic steps (for both 8-HQ and 1,10-phen) were investigated using **71**, because of the relatively simple synthesis and low cost of reaction materials (with the exception of the catalyst, which can be recycled).

Coupling of **71** with **75** to give **80**, was (as in the derivitization of 8-HQ) monitored by the appearance of a doublet (2H) at 3.93 ppm and a singlet (9H) at 1.48 ppm. Furthermore the amide proton $H_8$ is dramatically shifted downfield from 4.25 ppm to 10.15 ppm. Deprotection of the glycine amine was confirmed by the disappearance of the singlet at 1.48 ppm, assigned to the *t*-BOC protons ($H_{11}$).

As in the case of 8-HQ the proton spectra of the final derivatized ligands of 1,10-phen appear almost as an overlay of the two precursors in each case, *i.e.* **80** with **59** or **80** with **66**. The most dramatic shift is observed for $H_8$, which moves upfield by approximately 0.7 ppm (**82**) and 0.6 ppm (**83**) in each case.

The work has resulted in the successful synthesis of four novel compounds, the adenosine and pantothenic acid derivatives of 8-HQ and 1,10-phen (compounds **78**, **79**, **82** and **83**), all previously untested for their activity against *Plasmodium falciparum*. However while the synthesis of each of these molecules was successful, their low yields (approximately 20% in each case) and the difficulty of the procedures involved in purifying them would likely make these compounds financially unviable as commercial antimalarial drugs.

## 2,2':6',2'' Terpyridine Derivatives

The synthetic route for terpy was similar to that for 1,10-phen: to synthesize an amino derivative **85** and then couple this directly with the targeting agents. The first step of this plan was abandoned, as attempts to convert the chloro-terpy derivative **84** to **85**, according to the method of Mutai *et al.*[172] were unsuccessful (Figure 4.2.8). Other

authors have previously reported synthetic difficulty with this reaction.[173] The step involving coupling with 5'-chloro-adenosine, was also abandoned due to the poor reactivity observed in the cases of 8-HQ and 1,10-phen.

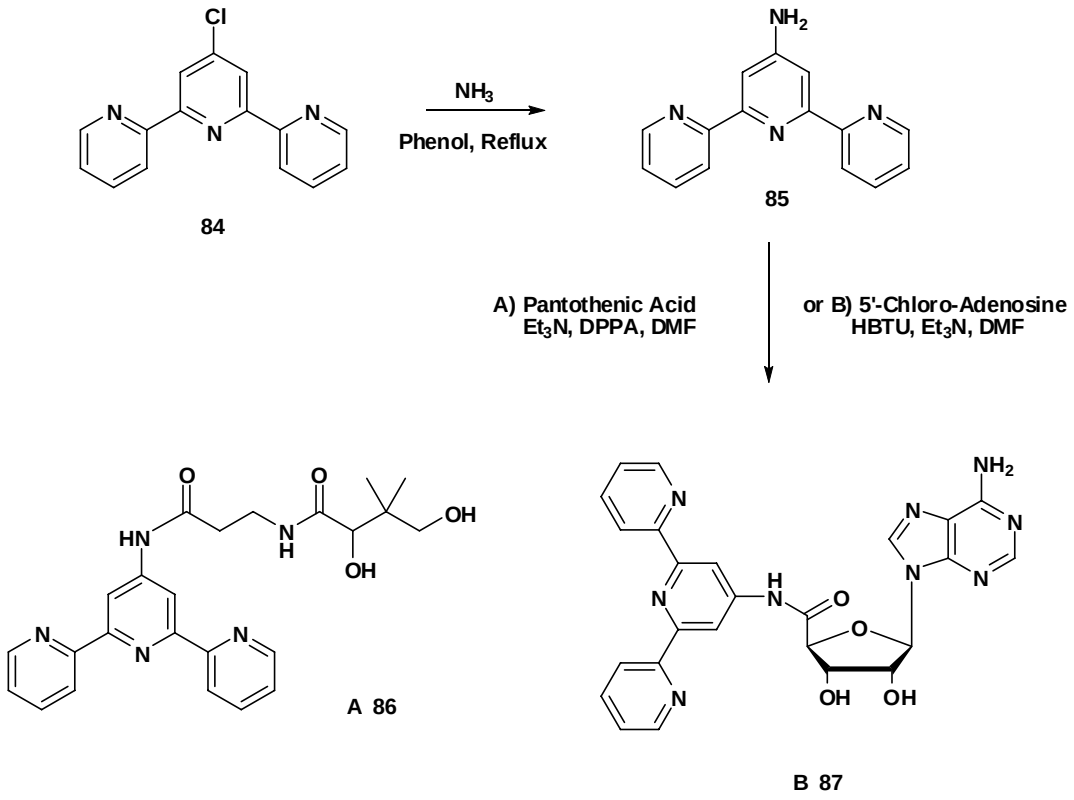



**Figure 4.2.8 Proposed synthetic scheme for derivatives of terpy.**

Thus as with 8-HQ and 1,10-phen a different synthetic route had to be established. While glycine proved to be a successful solution in the cases of 8-HQ and 1,10-phen, **84** would first have to be converted to some form of amine. The solution to this was provided by Senechal-David *et al*.,[173] who have demonstrated that 1,3-diaminopropane reacts well with **84**. For the synthetic procedure here 1,2-diaminoethane **88** (Figure 4.2.9) was used instead to give the aminoethyl-amino-terpy derivative **89**. This reaction provided both a site for coupling with the targeting agents (an amine), as well as a spacer, in one synthetic step. This derivative, **89**, was then reacted with each of the targeting agents, **59** and **66**, to give **90** and **91**, respectively (using the same conditions employed for 8-HQ and 1,10-phen) Figure 4.2.9.

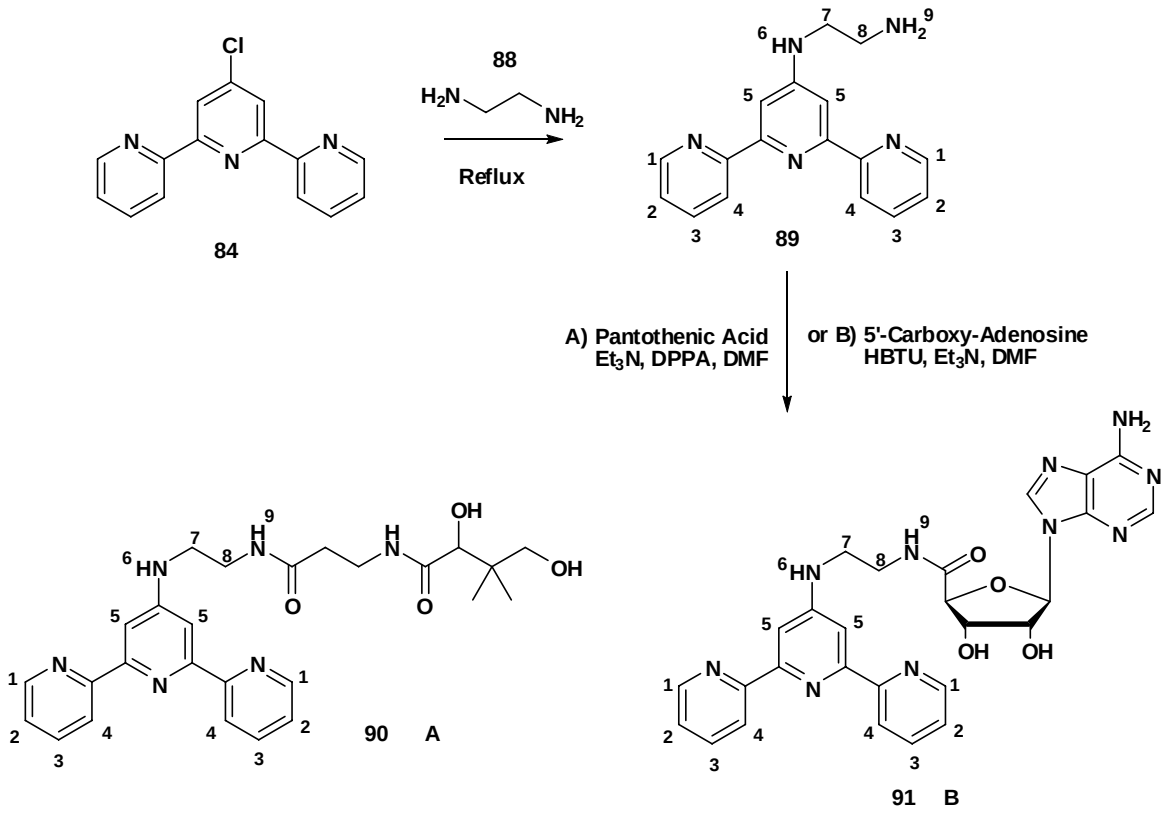



**Figure 4.2.9 Synthetic route for derivatization of terpy with pantothenic acid (A) or adenosine (B).**

A comparison of the $^1$H NMR spectra of the terpy derivatives is provided in Table 4.2.6. The successful synthesis of **89** (at a slightly higher yield of 50% compared with other target molecules) is confirmed by the appearance of the two additional signals at 3.41 ppm (2H) and 3.00 ppm (2H) due to the ethyl protons on the product. Further confirmation is the appearance of an –NH- triplet at 4.86 ppm (1H), corresponding to $H_6$. Reaction with **59** gave **90**. The $^1$H NMR spectrum of **90** is slightly more complicated than that of the 8-HQ and 1,10-phen derivatives of pantothenic acid. This is due to some overlap between $H_7$ and $H_8$ from terpy and $H_1$, $H_2$ $H_7$ of **59**, leading to multiplets in these regions, however this is easily observed due to the correspondence to both terpy carbons and pantothenic acid carbons in the HSQC spectrum. The spectrum however again, appears much like a combination of the spectra of the two starting compounds.

**Table 4.2.6 $^1$H Chemical shifts for synthesis of terpy derivatives.**

| Proton | Chemical shift/ppm (multiplicity, integration)[i] | | |
|---|---|---|---|
| | **89** | **90** | **91** |
| $H_1$ | 7.28 (ddd, 2H) | 7.15 (m, 2H) | 7.51 (m, 1H) |
| $H_2$ | 7.80 (ddd, 2H) | 7.59 (m,2H) | 8.00 (m, 2H) |
| $H_3$ | 8.65 (ddd, 2H) | 8.60 (m, 2H) | 8.73 (m, 2H) |
| $H_4$ | 8.59 (dt, 2H) | 7.99 (m, 2H) | 8.49 (m, 2H) |
| $H_5$ | 7.68 (s, 2H) | 7.99 (m, 2H) | 7.68 (s, 2H) |
| $H_6$ | 4.86 (t, 1H) | 7.33 (t, 1H) | - |
| $H_7$ | 3.41 (m, 2H) | 3.38 (m, 2H) | 3.50 (t, 2H) |
| $H_8$ | 3.00 (m, 2H) | 3.38 (m, 2H) | 2.90 (t, 2H) |
| $H_9$ | - | 8.38 (s, 1H) | 9.29 (s, 1H) |
| P-OH/ A-$H_7$ | - | - | 4.20 (s, 1H) |
| P-$H_{1,2,7}$/A-$H_1$ | - | 3.38 (m, 4H) | 8.37 (s, 1H) |
| P-$H_3$/A-$H_2$ | - | 0.82 (s, 3H) | 8.23 (s, 1H) |
| P-$H_4$/A-$H_3$ | - | 0.78 (s, 3H) | 7.41 (s, 2H) |
| P-$H_5$/A-$H_4$ | - | 3.89 (s, 1H) | 5.95 (d, 1H) |
| P-$H_6$/A-$H_5$ | - | - | 4.63 (d, 1H) |
| P-$H_8$/A-$H_6$ | - | 2.44 (t, 2H) | 4.35 (s, 1H) |

[i] $^1$H NMR spectra collected as follows: **89** in $CDCl_3$, **90** in $D_2O$ and **91** in DMSO-$d_6$, P denotes pantothenate peaks and A denotes adenosine peaks.

The spectrum of **91** (once more only a low yield of 20% could be achieved) is easily elucidated due to the lack of overlapping of proton signals due to **66** and **91**. The only problem associated with this spectrum again is the reduced signals of $H_7$ and $H_8$ due to the associated suppression of the water peak. As with the synthesis of earlier adenosine

derivatives the proton NMR spectrum is essentially a simple combination of each of the precursors.

The synthesis of all derivatives has been confirmed using NMR and MS methods. Due to the often poor reactivity of the compounds the synthetic routes were adapted to include a spacer between the ligands and the targeting agents. This ultimately allowed for the ligands and targeting agents to be covalently linked, resulting in six novel compounds (**78**, **79**, **82**, **83**, **90** and **91**). This type of structure may also confer other advantages by keeping the ligands and the targeting agents chemically separate, a property which may be important for transportation and/or uptake by the parasite.

# CHAPTER 5

# Antimalarial Testing Results and Discussion

## 5.1 Baseline Antimalarial Activities

### Activity of Transition Metal Ions

As part of examining the roles of metals in this study it was important to first determine the background activities of the transition metals of interest. The ions investigated in this study included $Cu^{2+}$ (because of its previous use with 8-HQ[95, 96] and 1,10-phen[101]), $Fe^{3+}$ (for its previous use with 8-HQ[85, 95, 96]), $Au^{3+}$ and $Pt^{2+}$ (for their previous use with terpy).[123, 133, 136] The antimalarial activities of these transition metal ions were previously unreported. The toxic affect of each of the metals was determined using their chloride salts. These results can be seen in Table 5.1.1.

**Table 5.1.1 Antimalarial activity of transition metals[i] (against strain 3D7[ii]).**

| Metal Salt | $IC_{50}/\mu M$ |
|---|---|
| $CuCl_2$ | 35.2 |
| $FeCl_3$ | $20.0 \times 10^3$ |
| $HAuCl_4$ | 23.5 |
| $K_2PtCl_4$ | 2.41 |
| $PdCl_2$ | ND[iii] |

[i] All metal salts were dissolved in deionised $H_2O$.
[ii] The 3D7 is a chloroquine susceptible strain of the malaria parasite.
[iii] $PdCl_2$ preicpitated out during testing.

With the exception of the platinum salt, the toxicities of the metals are quite low, and as one might expect in a biological system, the toxicity increases in the order $Fe^{3+} < Cu^{2+} < Au^{3+} < Pt^{2+}$. With these values established it was decided that the metals would have

minimal toxic effect at concentrations of 0.5 μM (as free metals in the growth medium). The extremely low activity of iron is likely due to the formation of iron oxides at the testing pH, approximately pH 7. Thus this concentration was used when investigating the ability of the various metals, when uncomplexed, to potentiate the activities of the each of the ligands, 8-HQ, 1,10-phen and terpy.

## Activity of Ligands

The aim of studying 8-HQ was to bring clarity to the currently published literature. Some authors have reported micromolar activity,[88] while another has reported nanomolar acvivity.[91] The role played by metals in the activity of this ligand will also be investigated.

Initial research was done on the activity of several 8-HQ derivatives, to determine if there were any simple structural alterations which might affect activity. The compounds in Figure 5.1.1 were tested (for motivation see Chapter 2). The results of these test are shown in Table 5.1.2.

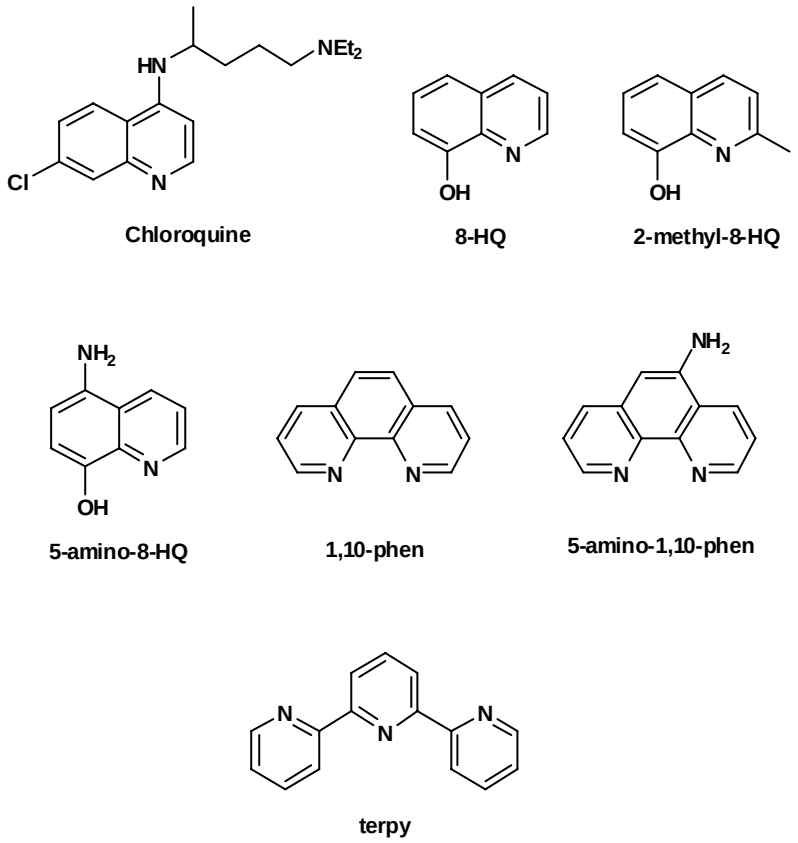



**Figure 5.1.1 Compounds tested for their ability to inhibit β-haematin formation.**

**Table 5.1.2 Antimalarial activity of selected ligands (against strain 3D7).**

| **Ligand** | **$IC_{50}$/μM** |
|---|---|
| Chloroquine | 0.022 |
| 8-HQ (**1**) | 2.4 |
| 2-methyl-8-HQ (**2**) | 47 |
| 5-amino-8-HQ (**67**) | 1.0 |
| 1,10-phen (**16**) | 0.99 |
| 5-amino-1,10-phen (**71**) | 0.94 |
| Terpy (**29**) | 0.85 |

The results obtained here are in agreement with those previously reported by Scheibel and his coworkers in their series of papers,[88] and not with those of Biswas.[91] The activity of 8-HQ (and its derivatives in general) is in the micromolar, and not nanomolar, range of activity and is less active than chloroquine (several cloroquine resistant and susceptible strains were tested, including 3D7, by Biswas[91] under the same conditions as those used in this study).

Furthermore it is evident here that the activity of 2-methyl-8-HQ is lower (by one order of magnitude), while the 5-amino-8-HQ species has comparable activity with that of 8-HQ itself. The lower activity of 2-methyl-8-HQ suggests that the mode of action of 8-HQ may require some form of complexation, exhibiting its antimalarial effect either as a toxic metal complex (as suggested in the reported antibacterial studies)[94] or by blocking the metal site on a metalloprotease (as has been suggested for 1,10-phen)[118]. It is also evident that derivitization at the five position has little effect on the activity of 8-HQ. In light of this result, 5-amino-8-HQ was used for all other studies as it would be the starting point for the synthesis of the target ligands.

Similarly the activities of 1,10-phen and 5-amino-1,10-phen were of the same order of magnitude. The activities found here, approximately 1 μM, are an order of magnitude higher than that reported by Kitjaroentham *et al.*[118] (24 μM) who proposed that the mode of action was *via* the inhibition of a metalloprotease involved in RBC invasion. Again because of the similar activities of 5-amino-1,10-phen and 1,10-phen the amino version was used for further metal-ligand studies.

Of the three ligands terpy demonstrated the highest activity, 0.85 μM (although it is of the same order of magnitude as both 8-HQ and 1,10-phen). It appears that this is the first reported testing of this ligand against malaria.

With the background of activities of both the metal ions, and the ligands established, it became possible to investigate how increased free metal concentrations might affect their activities.

# 5.2 Antimalarial Activities of Ligands with Metals

## Activity of Ligands with Metal-Enhanced Growth Medium

The second part of this study was aimed at determining whether increasing the metal concentration in the growth medium would potentiate the activity of any of the ligands. With the baseline toxicities of the metals determined, a background concentration of 0.5 μM was used. These results are reported in Table 5.2.1.

**Table 5.2.1 Activities of ligands in presence of free metals.**

| Ligand (Metal Ion) | $IC_{50}$/μM |
|---|---|
| 5-amino-8-HQ ($Au^{3+}$) | 0.72 |
| 5-amino-8-HQ ($Cu^{2+}$) | 0.85 |
| 5-amino-8-HQ ($Fe^{3+}$) | 0.87 |
| 5-amino-8-HQ ($Pt^{2+}$) | 0.73 |
| 5-amino-1,10-phen ($Au^{3+}$) | 0.91 |
| 5-amino-1,10-phen ($Cu^{2+}$) | 0.86 |
| 5-amino-1,10-phen ($Fe^{3+}$) | 0.87 |
| 5-amino-1,10-phen ($Pt^{2+}$) | 0.73 |
| terpy ($Au^{3+}$) | 2.27 |
| terpy ($Cu^{2+}$) | 1.0 |
| terpy ($Fe^{3+}$) | 10 < |
| terpy ($Pt^{2+}$) | 1.0 |

In general the increased free-metal concentration had no observable effect on the activities of the ligands. Only a very slight increase in activity was ever observed but none was significant enough to suggest that any of the tested metals are involved in the mode of action of 5-amino-8-HQ, 5-amino-1,10-phen or terpy.

These results have three immediate implications. Firstly, it suggests that the mode of action of these ligands does not involve the formation of a toxic metal complex *in situ*. If this were the mode of action, one would expect increased activity with an increase in background metal concentration (as observed in antibacterial studies[96]). Second, the results suggest that none of the ligands act by sequestration of metals (this result is limited to those tested here, iron, copper, gold and platinum) away from the parasite, since the toxic effect is not reversed by the addition of these free metals. Third, this lack of reversal also suggests that the mode of action is not *via* coordination to a metalloenzyme.[118] The free metals should be as easily complexed as enzyme bound metals thus decreasing the amount of free ligand available to block parasite-enzyme metal sites.

An exception to this case is that of terpy with free iron. The activity of terpy with free iron in solution (10 μM < $IC_{50}$) is far worse than that observed for terpy alone (1.0 μM, a more than ten-fold reduction in activity) and suggests that free iron is antagonistic towards the activity of terpy. This is likely due to complexation of free iron by terpy which either prevents its mode of action or alters uptake of the ligand or its transportation into the parasite. Since the other metals do not inhibit the activity to such an extent it seems logical to suggest that the complex formed *in situ* is in fact preventing the mode of action by preventing its transport into the parasite. This is possibly due to the relatively high effective charge density associated with the terpy-iron (III) complex. The ligand also shows reduced activity in the presence of free gold (III), $IC_{50}$ 2.3 μM, although it is not as pronounced as found in the case of iron (III). This could be due to the larger size of the gold (III) ion, leading to a lower effective charge density. Free copper and platinum, both having charges of 2+, showed no observable effect on the activity of terpy.

## Activity of Ligand-Metal Complexes

With the metals, the ligands, and the ligands with metal enhanced growth medium tested, the pre-made complexes remained to be examined. To achieve this, complexes of the ligands with each of the metals were made (see Chapter 4). For 8-HQ and 1,10-phen the complexes made were insoluble and their activities therefore could not be assessed (results not shown). However the complexes of terpy were successfully synthesised, soluble and tested. The antimalarial activities of the terpy complexes are reported in Table 5.2.2.

**Table 5.2.2 Antimalarial activities of terpy complexes (against strain 3D7).**

| Complex | $IC_{50}$/μM |
|---|---|
| [Pt(terpy)Cl]Cl (**30**) | 0.41 |
| [Au(terpy)Cl]$Cl_2$ (**33**) | 0.30 |
| [Cu(terpy)Cl]Cl (**61**) | 0.52 |
| Fe(terpy)$Cl_3$ (**62**) | 0.63 |
| [Pd(terpy)Cl]Cl (**63**) | 0.71 |

Each of the metal complexes has slightly better activity than terpy as a free ligand ($IC_{50}$ = 0.85 μM). The gold-terpy demonstrates the highest activity, approximately three fold that seen for the ligand on its own. If one looks at these results from the perspective of the activity of terpy, it appears as if the metals do little to enhance the activity of the ligand. Furthermore it suggests that metal complexation is not essential for terpy to exhibit its antimalarial cytotoxic effect. In cancer studies it has predominantly been suggested that metals are required for terpy to exhibit activity, [128, 136-140] although a few papers have shown that terpy and some derivatives have similar or even higher anticancer activity than their complexes.[131, 141, 142] It is often the case that the ligands in these studies are not tested for any intrinsic activity.

However these results can also be seen from the perspective of the metals. For each of the metals the activity has been increased significantly. A comparison of the acitivities of the metal salts and the terpy-metal complexes is shown in Table 5.2.3.

**Table 5.2.3 Comparison of the activities of metals and their complexes with terpy.**

| Metal Salt | $IC_{50}/\mu M$ | Complex | $IC_{50}/\mu M$ |
|---|---|---|---|
| $HAuCl_4$ | 23.5 | $[Au(terpy)Cl]Cl_2$ | 0.30 |
| $CuCl_2$ | 35.2 | [Cu(terpy)Cl]Cl | 0.52 |
| $FeCl_3$ | $20 \times 10^3$ | $Fe(terpy)Cl_3$ | 0.63 |
| $K_2PtCl_4$ | 2.41 | [Pt(terpy)Cl]Cl | 0.41 |
| $PdCl_2$ | ND | [Pd(terpy)Cl]Cl | 0.71 |

All the metals show enhanced activity when in the complexed form. The increase is most pronounced for iron (approximately 30 000 fold), followed by gold (approximately 78 fold), copper (approximately 68 fold) and platinum (approximately 6 fold). This increase in activity for the metals is quite possibly due to their enhanced transportation into the parasite when in the complexed form. No direct measurement of the transportation (or uptake) of the metals was performed. It has however been demonstrated before that ligands are capable of facilitating the transport of metal ions into the parasite.[85] Thus it could well be that the ligand allows more of the metal ions to reach their active site(s) in the parasite.

It is interesting to note that the results are open to interpretation from either the ligands' perspective (which suggests only a marginal increase in activity) or from the metals' perspective (which then suggests a significant increase in activity). If the exact mode of action of the ligand, the metals and the complexes were all known one could perhaps determine which of these points holds more scientific weight. It is of course quite possible as well that they all act *via* totally different mechanisms. In light of this it became essential to test whether any of the compounds demonstrated an ability to inhibit β-haematin formation, according to the assay of Egan *et al.*.[28]

## Antimalarial Activities of Derivatized Ligands

The goal of this part of the study was to determine if the activity of the various ligands could be improved by covalently linking them to nutrients for which iRBCs have a higher or exclusive affinity (described in Chapter 3). Hypothetically these new compounds (synthetic procedures described in Chapter 4 and 6) should benefit from enhanced transport into the parasite, hence a higer local concentration, resulting in better acivity. The antimalarial testing results for the derivitized ligands, **78**, **79**, **82**, **83**, **90** and **91**, (as well as the intermediates, **77**, **81** and **89**) are reported in Table 5.2.4.

**Table 5.2.4 Antimalarial activities of intermediates and derivatized ligands (against strain D10).**[i]

| Ligand | $IC_{50}/\mu M$ |
|---|---|
| Chloroquine | 0.016 |
| 8-HQ-gly (**77**) | 29 |
| 8-HQ-Pant (**78**) | 100< |
| 8-HQ-Ade (**79**) | 100< |
| 1,10-Phen-Gly (**81**) | 29 |
| 1,10-Phen-Pant (**82**) | 135 |
| 1,10-Phen-Ade (**83**) | 2.4 |
| Ethylamino-Terpy (**89**) | ND[ii] |
| Terpy-Pant (**90**) | 5.1 |
| Terpy-Ade (**91**) | ND[ii] |

[i] Due to circumstances the later compounds were tested against the D10 strain of malaria and not the 3D7 strain as was used for earlier samples. Both strains are chloroquine susceptible and chloroqine was used as a control in both cases. The chloroquine results are similar in each case (0.022 μM, 3D7, and 0.016 μM, D10).

[ii] Results could not be determined due to interfering absorbance by the compounds. The assay makes use of a colourimetric detection method.

The activities found for the derivatives are all lower than those found for the parent ligands. However there are no clear trends for either of the pantothenic acid derivatives or the adenosine derivatives. In the case of 8-HQ, the introduction of the targeting agents has severely reduced the activity of the ligand, dropping from 2.4 μM to > 100 μM. For 1,10-phen we observe that the adenosine derivative, **83**, has activity in the same region as 1,10-phen, while the activity of the pantothenic acid derivative, **82,** is reduced ten fold. In contrast however, the activity of the terpy pantothenic acid derivative, **90**, is similar to that of terpy, while unfortunately the activity of the adenosine derivative, **91**, could not be determined.

It is apparent from the results that the targeting agents do not confer any ability to enhance the activity of the parent ligands. It is particularly interesting that the activity of 8-HQ is so much lower once derivatized. This suggests that either the toxicity is reduced by derivitization, or the molecule is no longer transported to its active site. However, since one of the 1,10-phen derivatives and one of the terpy derivatives each show activity similar to the parent ligands, a lack of transportation seems unlikely, as 8-HQ is the smallest of the ligands (although the exact location at which the drugs exhibit their toxic effects is unknown and may be different).

In general it is clear that the derivitization of these ligands, 8-HQ, 1,10-phen and terpy, have seen no benefit from being covalently linked to the targeting agents. There are several possible explanations for this lack of activity:

1) the targeting agents might no longer be recognized by the parasite's transport systems;
2) the derivitized ligands may be too big to be transported effectively;
3) derivitization may affect the toxicity or mode of action of the ligands or
4) the ligands may no longer locate in the correct part of the parasite to exert their effect.

Any one or combination of these possibilities would be sufficient to render the derivitization ineffective.

# 5.3 β-Haematin Inhibition Results

As part of this study has involved discussion around the modes of action of the various ligands and complexes, an investigation into their ability to inhibit β-haematin formation was undertaken. It has been suggested that chloroquine exhibits its antimalarial effect by this mode of action by preventing the spontaneous (lipid catalyzed) formation of haemozoin.[29] A simple assay has been developed by Egan *et al.*[28] and it is this method which has been used here to determine whether the compounds from this study do in fact inhibit β-haematin formation. An example of an infrared spectrum positive for β-haematin formation and one negative for β-haematin formation are shown in Figure 5.3.1 and Figure 5.3.2 respectively. The results of these investigations are found in Table 5.3.1.

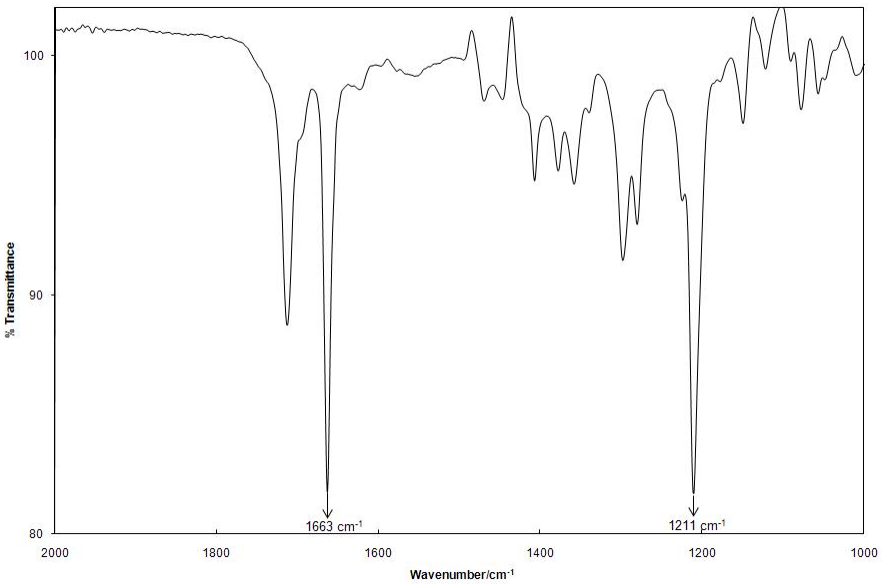



**Figure 5.3.1 An infrared spectrum positive for β-haematin formation.**

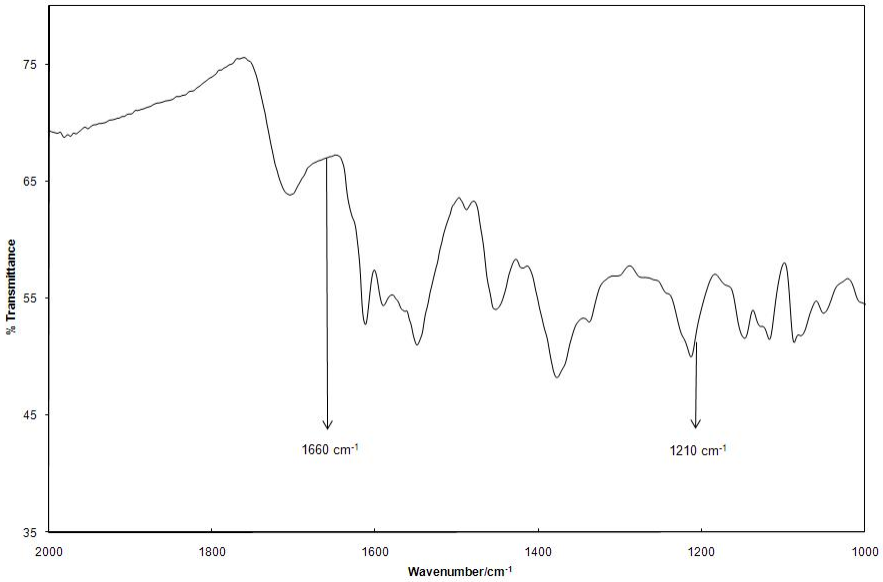



**Figure 5.3.2 An infrared spectrum negative for β-haematin formation (chloroquine).**

Initially the ligands and complexes were tested. It was suspected that these compounds might demonstrate some ability to inhibit β-haematin formation due to their planar structures. However it has been previously reported that several 1,10-phen derivatives, including 5-nitro-1,10-phen did not inhibit β-haematin formation.[116] This is in agreement with the results found here for the various 1,10-phen derivatives. It has also been reported that 8-HQ does not inhibit β-haematin formation,[28] again in agreement with the results observed in this study. In general the results here suggest that the ligands do not act by inhibition of haemazoin formation in the parasite. Similarly the derivatives of each of the ligands (as well as their precursors, **59**, **66**, **77**, **81** and **89**) showed no ability to inhibit β-haematin formation

In contrast however platinum complexes, exemplified by that seen in Figure 5.3.3, have been reported to inhibit β-haematin formation.[116] In this report by Egan *et al.*[116] the ability of these complexes to inhibit β-haematin formation, was attributed to their self-aggregating behaviour a property likely due to the planar nature of the ligand-metal complexes.[116] Thus the complexes (of terpy) in this study were also investigated for

their ability to inhibit β-haematin formation. All the terpy complexes, except that of copper, were found to prevent the formation of β-haematin.

**Table 5.3.1 Results of β haematin inhibition assays (ligands and complexes).**

| Ligand or Complex | β-Haematin Formation |
|---|---|
| None | + |
| Chloroquine | - |
| Pantothenic Acid (**59**) | + |
| Acid-Ade (**66**) | + |
| 8-HQ (**1**) | + |
| 8-HQ-Gly (**77**) | + |
| 8-HQ-Pant (**78**) | + |
| 8-HQ-Ade (**79**) | + |
| 1,10-Phen (**16**) | + |
| 1,10-Phen-Gly (**81**) | + |
| 1,10-Phen-Pant (**82**) | + |
| 1,10-Phen-Ade (**83**) | + |
| Terpy (**29**) | + |
| Ethyl-amino-terpy (**89**) | + |
| Terpy-Pant (**90**) | + |
| Terpy-Ade (**91**) | + |
| [Pt(terpy)Cl]Cl (**30**) | - |
| [Au(terpy)Cl]$Cl_2$ (**33**) | - |
| [Cu(terpy)Cl]Cl (**61**) | + |
| Fe(terpy)$Cl_3$ (**62**) | - |
| [Pd(terpy)Cl]Cl (**63**) | - |

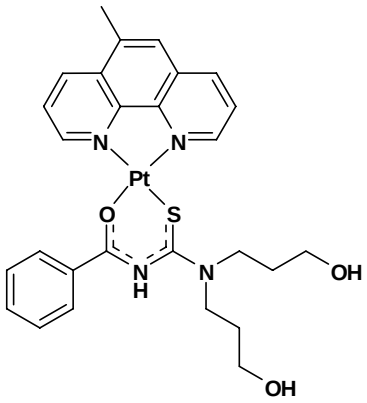



**Figure 5.3.3 A platinum complex designed to inhibit β-haematin formation.[116]**

Having found that several of the terpy complexes inhibited β-haematin formation, it was decided to test the chloride salts of the metals (see Table 5.3.2). This would resolve whether the ability to inhibit β-haematin formation was derived from the metals or if it was a new property resulting from the combination of ligand and metal. The results demonstrated that the metal salts of iron, gold, platinum and palladium were capable of inhibiting β-haematin formation, while that of copper did not. The prevention of β-haematin formation is likely via the coordination of the metal centre to a propionate group, preventing the dimerization of haem as reported.[23] While there is no direct evidence to support this claim it does seem the most likely means by which the metals would exert their action[23] Thus it is suggested that the ability to inhibit β-haematin formation is inherent to the metals, and not a new property of the ligand-metal complexes. This conclusion is supported by the fact that the only complexes which inhibited β-haematin formation, were those containing metals which on their own exhibited this property. Furthermore copper metal does not exhibit any ability to inhibit β-haematin formation, and its complex with terpy, likewise does not. The result implies that the β-haematin inhibiting properties of any complexes of iron, gold, platinum or palladium, may be due to the metals' inherent ability to exhibit this action, and not in fact due to the combination of ligand(s) and metal. However this statement can only be applied with certainty to this study, and may not be true for other cases, such as the those (of platinum) reported by Egan *et al.*.[116]

**Table 5.3.2 Results of β-haematin inhibition assays (metal salts).**

| Metal Salt | β-Haematin Formation |
|---|---|
| $K_2PtCl_4$ | - |
| $HAuCl_4$ | - |
| $CuCl_2$ | + |
| $FeCl_3$ | - |
| $PdCl_2$ | - |

When considered as a whole the results here show that ligands which do not inhibit β-haematin formation can have this property conferred on them by metals such as iron, gold, platinum and palladium. It is also suggestive that in general ligands may act *via* different modes of action from their corresponding complexes, and furthermore that the ligands in the complex form may only be responsible for increasing the transport (and uptake) of these toxic metals into the parasite, and that the activity observed for the complexes is a direct result of the properties of the metal centre. It has been interesting to discover, however, that copper does not demonstrate any ability to inhibit β-haematin formation, neither as the free metal nor in complex form. Interestingly, the terpy-copper complex still shows higher activity than the free metal. While this activity suggests that the complex is toxic, no hypothesis can be put forward as to why copper and its associated complex do not inhibit β-haematin formation.

# CHAPTER 6

# Conclusion

In general the aim of this work was to investigate the relationship between the ligands 8-HQ, 1,10-phen and terpy and the metal ions of gold, copper, iron, platinum and palladium. The secondary aim was to identify two nutrients specific to *Plasmodium falciparum* iRBCs, to covalently link these to the ligands and determine whether this structural alteration enhances the antimalarial activity in any way. The final aim was to use the assay developed by Egan *et al*.[28] to investigate if the studied compounds are capable of β-haematin inhibition, the mode of action attributed to chloroquine.

In order to study the relationship between the ligands and metals several experiments were performed. The background activities of each of the ligands and each of the metals was measured. This appears to be the first report of the activities of the metals, gold, copper, iron, platinum and palladium. Their toxicities ranged from 2.4 μM (platinum) to 20 mM (iron).

With these background values established, the relationship between the activities of the ligands and the free metal concentrations in solution was investigated. It was found that with the exception of iron the free metal concentration had neither an antagonistic nor synergistic effect on the antimalarial activity of the ligands. High concentrations of free iron were antagonistic towards the activity of terpy (reducing the $IC_{50}$ ten fold to approximately 10 μM), but had no effect on the activity of the other two ligands.

Following this work it was decided to investigate whether premade ligand-metal complexes would behave differently from ligands administered in medium with higher concentrations of free metals. For 8-HQ and 1,10-phen, the complexes of iron and copper which were synthesized were found to be too insoluble to permit testing. However in the case of terpy, complexes of each of the metals were successfully

synthesized and sufficiently soluble to allow testing. This is the first apparent report of the antimalarial acitivity of both terpy and its complexes with the metal ions of gold, copper, iron, platinum and palladium. Each of the complexes showed activity similar to that of the ligand alone. All activities were in the sub-micromolar range (0.1-1 μM).

The main synthetic part of this work was to covalently link the ligands (identified in Chapter 2) to the targeting agents (identified in Chapter 3). This work resulted in the synthesis of six novel compounds **78**, **79**, **82**, **83**, **90** and **91** which were then tested for their antimalrial activities. Unfortunately none of these compounds demonstrated any increased activity as a result of this structural modification (covalently linking the ligand to a malaria specific nutrient). While some of the compounds showed much lower activities (**78**, **79** and **82**), others showed activity of the same order of magnitude as the parent ligands (**83** and **91**). Only **90** could not be determined. Clearly the two nutrients selected have no benefit to confer onto the parent ligands. This lack of improvement could be the result of one of several circumstances: the derivitization has in fact removed the toxic effect of the ligand; the targeting agents are no longer recognized by the parasite's transport pathways; the ligand no longer locates where it exerts its effect or the actual mechanism by which the ligand acts is hindered by the structural alteration.

As a secondary study the ability of each of the compounds to inhibit β-haematin formation was investigated. While none of the ligands (basic or derivitized) showed any ability to inhibit β-haematin formation, many of the complexes did. It was found that all of the complexes of terpy, with the exception of copper, inhibited β-haematin formation. Investigations of the chloride salts of each of the metals revealed that β-haematin inhibition was intrinsic to the metals, iron, gold, platinum and palladium but not copper. Thus it is apparent that the complexes of terpy and the ligand itself, might well exert their toxic effects by different mechanisms. It also became apparent that the ligand might serve as a transportation agent, to deliver the metals into the parasite *i.e.* that the ligand serves to enhance the activity of the metals, an idea opposite to the conventional perspective that metals increase the activities of ligands.

This work has established several results. The ambiguity with respect to the activity of 8-HQ has been resolved to be of the micromolar order. The $IC_{50}$ of 1,10-phen was also confirmed (0.99 μM) and the activity of terpy (0.85 μM) reported for the first time. Similarly the activities of the complexes of terpy with gold, copper, iron, platinum and palladium were also reported. The novel compounds **78**, **79**, **82**, **83**, **90** and **91** were synthesized and tested and led to the conclusion that neither adenosine nor pantothenic acid conferred any enhanced activity on the ligands. The toxicities of the metal ions themselves are also apparently reported here for the first time. Furthermore it is evident that the ligands 8-HQ, 1,10-phen and terpy do not require any free metal ions in solution to exert their toxic effects. Some interesting mechanistic discoveries were also made. It was found that the metals exhibit an inherent ability to inhibit β-haematin formation (except copper) and that this property is conferred to complexes of the metals as well (at least in the case of terpy complexes). This may well provide a path for future workers to develop compounds capable of β-haematin inhibition as they strive to develop new drugs in the fight against malaria.

# CHAPTER 7

# Experimental

## 7.1 Instrumentation

All solvents and reagents were obtained from Acros, Aldrich, Fluka, Merck or Alfa-Aesar. Unless otherwise stated, these were used without further purification. Solvents ($CH_3CN$ and DMF) were dried according to the methods in Vogel.[174] All other solvents were used without drying. Thin-layer chromatography (TLC) was conducted on aluminium-backed, precoated silica gel plates (Merck, silica gel 60, 20 cm x 20 cm). Column chromatography was performed with silica gel 60 (Merck, particle size 0.040-0.063 mm). Proton NMR spectra were recorded at 298 K at 300 MHz on a Varian Unity Inova spectrometer; carbon NMR spectra were recorded at 75 MHz with the same instrument under the same conditions. Instances where proton and carbon NMR spectra were recorded at 400 MHz, a Varian Oxford spectrometer or a Bruker Advance spectrometer were used, at an operating temperature of 298 K. NMR spectra recorded at 600 MHz were performed using a Bruker Ultrashield spectrometer. Chemical shifts are reported in parts per million (ppm) and refer to the respective deuterated solvents as reference, $CDCl_3$ ($^1H$ 7.24 ppm, $^{13}C$ 77.0 ppm), $d_6$-DMSO ($^1H$ 2.49 ppm, $^{13}C$ 39.5 ppm) and $D_2O$ ($^1H$ 4.79 ppm, $^{13}C$ ppm referenced to $CH_3CN$). Infrared spectra were collected at 293 K by attenuated total reflection (ATR) using a Perkin Elmer Universal ATR Sampling Accessory attached to a Spectrum 100 FT-IR Spectrometer. Wavenumbers are reported in units of $cm^{-1}$. All melting points are uncorrected. Mass spectra were recorded using a Bruker micrOTOF-Q II Electron Spray Ionization (ESI) Mass Spectrometer (MS).

Liquid Chromatography Mass Spectrometry (LC-MS) analysis was done using an Agilent 1100 Series LC with an Agilent 1100 Series LC/MS Trap by flow injection analysis. Separation techniques were developed using an ACE 5 C18 A55055 column

(150 X 4.6 mm ACE-121-1546). Separations for each compound were developed using gradient elution of water and acetonitrile (both containing 0.1% v/v formic acid). Separation techniques were then scaled up for use on semi-prep HPLC. Purification by semi-prep HPLC was done using a Younglin ACME 9000 UV/VIS Detector and Gradient Pumps. In all cases an ACE 5 C18 A54892 column (150 X 21.2 mm ACE-121-1250) was used.

## 7.2 Synthesized Compounds

### $Fe(8\text{-}HQ)_3$

$FeCl_3.6H_2O$ (0.48 g, 1.79 mmol) was dissolved in $H_2O$ (135 ml). Sodium acetate (4.00 g, 72.7 mmol) was added followed by glacial acetic acid (15 ml). This solution was then heated and 2 % (m/v) 8-hydrozyquinoline in ethanol was added (45 ml). The solution was refluxed for 30 min and the black precipitate collected by filtration (0.851 g, 98%). I.R. ($\upsilon_{max}/cm^{-1}$) 3024, 1574, 1494, 1464, 1375, 1104, 737, 521. Elemental analysis for $C_{27}H_{18}N_3O_3Fe$: calculated %C 66.41, %H 3.72, %N 8.61; found: %C 65.09, %H 3.81, %N 8.06.

### $Cu(8\text{-}HQ)_2$

$CuCl_2.2H_2O$ (0.333 g, 1.99 mmol) was dissolved in $H_2O$ (135 ml). Sodium acetate (4.00g, 72.7 mmol) was added, followed by glacial acetic acid (15 ml) and 2% (m/v) 8-hydroxyquinoline in ethanol (34 ml). The solution was refluxed till a green granular precipitate formed. The green precipitate was filterd and washed with hot $H_2O$ (0.61 g, 87%). I.R. ($\upsilon_{max}/cm^{-1}$) 3043, 1574, 1496, 1461, 1373, 1109, 740, 516. Elemental analysis for $C_{18}H_{12}N_2O_2Cu$: calculated %C 61.45, %H 3.44, %N 7.96; found: %C 61.86, %H 3.51, %N 8.19.

### $Fe(1,10\text{-}phen)_3(ClO_4)_3$

1,10-phenanthroline hydrate was dissolved in $H_2O$ (50 ml) and heated. $Fe(NH_4)_2SO_4.6H_2O$ (0.39 g, 1.32 mmol) was added. Sodium prechlorate (0.30 g, 2.14 mmol) was dissolved in $H_2O$ (10 ml) and added. A dark red precipitate formed and was filtered and washed with hexane (0.70g, 0.74%). I.R. ($\upsilon_{max}/cm^{-1}$) 3436, 1518, 1427,

1086, 853, 722, 626. Elemental analysis for $C_{24}H_{16}C_{l3}N_4O_{12}Fe$: calculated %C 40.34, %H 2.26, %N 7.84; found: %C 39.99, %H 2.21, %N 7.62.

## Cu(1,10-phen)$(ClO_4)_2$

$CuSO_4.5H_2O$ (0.660 g, 2.64 mmol) was dissolved in $H_2O$ (20 ml) and $NaClO_4$ (0.76 g, 6.21 mmol) 1,10-phenantholine hydrate was dissolved in ethanol (20 ml). The two solutions were mixed and precipitate formed. The blue preciptitate that formed was collected (1.33 g, 81%). I.R. ($\upsilon_{max}/cm^{-1}$) 3071, 1527, 1440, 1082, 857, 731, 632. Elemental analysis for $C_{24}H_{16}C_{l2}N_4O_8Cu$: calculated %C 46.28, %H 2.59, %N 9.00; found: %C 45.91, %H 2.61, %N 8.89.

## [Pt(terpy)Cl)]Cl.2$H_2O$[154] (30)

Potassium chloroplatinate(II) (0.100 g, 0.241 mmol) was dissolved in $H_2O$ (5 ml). Solid 2,2':6',2''-terpyridine (0.056 g, 0.24 mmol) was then added to this solution followed by further $H_2O$ (2 ml). The orange solution was then stirred under reflux overnight. The reaction mixture was then cooled, any particulate matter filtered and the solvent removed under reduced pressure. The resulting solid was then redissolved in a minimum of $H_2O$ and the product precipitated out by the addition of HCl (conc. approx. 10 drops). The bright orange product was filtered off and dried in a vacuum desiccator (0.042 g, 33%). Mp >300 °C. $\delta_H$ (400 MHz, DMSO-$d_6$) 8.89 (dd, *J*=1.5 Hz, *J*=5.5 Hz, 2H), 8.64 (m, 5H), 8.51 (td, *J*=8.0 Hz, *J*=1.5 Hz, 2H), 7.95 (m, 2H). $\delta_C$ (100 MHz, DMSO-$d_6$) 158.3, 154.4, 151.3, 142.7, 142.1, 129.2, 125.9, 124.5. I.R. ($\upsilon_{max}/cm^{-1}$) 3297, 3031, 1604, 1476, 1451, 1401, 1314, 1249, 1091, 1030, 779, 721, 593, 546, 521, 461. Calculated for $PtC_{15}H_{11}N_3Cl^+$ *m/z* = 463.0289. Found: *m/z* = 463.0282 [M].

## [Au(terpy)Cl]$Cl_2.3H_2O$[155] (33)

2,2':6',2''-Terpyridine (0.067 g, 0.29 mmol) was added to a solution of $HAuCl_4.H_2O$ (0.100 g, 0.294 mmol) in $H_2O$ (7.5 ml). This was followed by the addition of HCl (1 M, 375 μl, 0.375 mmol). The solution formed a yellow precipitate. The pH was then adjusted to between 3 and 5 by adding NaOH (1 M, 240 μl, 0.240 mmol) giving an orange-red solution with a yellow precipitate. The reaction mixture was refluxed overnight dissolving most of the precipitate. After filtering (hot), the solvent was removed and the product recrystallized from $H_2O$ (0.090 g, 53%). Mp 245-250 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 8.77 (dd, *J*=1.5 Hz, *J*=5.0 Hz, 2H), 8.71 (d, *J*=8.0 Hz, 2H), 8.49 (d, *J*=8.0 Hz, 2H), 8.15 (m, 3H), 7.60 (m, 2H). $\delta_C$ (100 MHz, DMSO-$d_6$) 151.8, 151.5, 146.8, 141.5, 139.7, 126.0, 122.8, 122.7. I.R. ($\upsilon_{max}/cm^{-1}$) 3306, 3010, 1602, 1476, 1449, 1401, 1322, 1249, 1089, 1028, 772, 761, 656, 542, 514, 441. Calculated for $AuC_{15}H_{11}N_3Cl_2^+$ *m/z* = 499.9996. Found: *m/z* = 499.9978 [M].

## [Cu(terpy)Cl]Cl[156] (61)

2,2': 6',2''-Terpyridine (0.118 g, 0.506 mmol) was dissolved in methanol (20 ml) and added to a solution of $CuCl_2.2H_2O$ (0.084 g, 0.493 mmol) in methanol (10 ml). The terpyridine solution was added to the copper(II) chloride solution resulting in a colour change from light to dark green, followed by the formation of a green precipitate. The solution was stirred for ten minutes and then left in a freezer for three days. The product, a dark green powder, was filtered off and recrystallized from water to afford dark green crystals (0.139 g, 75%). Mp >300 °C. I.R. ($\upsilon_{max}/cm^{-1}$) 3338, 3215, 1631, 1595, 1562, 1489, 1428, 1407, 1302, 776, 737, 648, 625, 437, 408. Calculated for $CuC_{15}H_{11}N_3Cl^+$ *m/z* = 330.9938. Found: *m/z* = 330.9936 [M].

## $Fe(terpy)Cl_3$[157] (62)

2,2':6',2''-Terpyridine (0.100 g, 0.430 mmol) was dissolved in methanol (5 ml) and added to a solution of $FeCl_3.6H_2O$ (0.111 g, 0.411 mmol) in methanol (15 ml). The terpyridine solution was added to the iron(III) chloride solution, resulting in a colour change from yellow to red, followed by the formation of a yellow precipitate. The solution was stirred for one hour at room temperature and filtered. The product was recrystallized from water to afford amber crystals (0.122 g, 75%). Mp >300 °C. I.R. ($\upsilon_{max}/cm^{-1}$) 3075, 1596, 1574, 1475, 1444, 1317, 1244, 1155, 1096, 1021, 780, 733, 650, 512, 415. Calculated for $FeC_{15}H_{11}N_3Cl_2^+$ *m/z* = 358.9679. Found: *m/z* = 358.9678 [M].

## $[Pd(terpy)Cl]Cl.3H_2O$ from $PdCl_2$[158] (63)

Palladium(II) chloride was dissolved in $H_2O$ (10 ml) and HCl (32%, 3.00 ml, 31.5 mmol) and heated to reflux. The reaction mixture became clear red and was left for a further 45 minutes. The warm solution was filtered and a solution of 2,2':6',2''-terpyridine (0.120 g, 0.514 mmol) in methanol (10 ml) was added dropwise to the filtrate. The newly formed peach-coloured precipitate was filtered off and the pH of the filtrate adjusted to between 4.5 and 5 using NaOH (2 M). The solution was again filtered and the filtrate left until yellow-orange crystals formed (0.045 g, 19%). Mp >300 °C. $\delta_H$ (400 MHz, DMSO-$d_6$) 8.72 (dd, *J*=1.5 Hz, *J*=5.5 Hz, 2H), 8.64 (m, 5H), 8.46 (td, *J*=8.0 Hz, *J*=1.5 Hz, 2H), 7.88 (m, 2H). $\delta_C$ (100 MHz, DMSO-$d_6$) 158.0, 154.6, 152.0, 142.8, 142.6, 128.9, 125.4, 124.5. I.R. ($\upsilon_{max}/cm^{-1}$) 3307, 3016, 1601, 1448, 1400, 1320, 1247, 1088, 1027, 774, 719, 657, 566, 514, 440. Calculated for $Pd(C_{15}H_{11}N_3)Cl^+$ *m/z* = 373.9676. Found: *m/z* = 373.9675 [M].

## [Pd(terpy)Cl]Cl.3$H_2O$ from $PdCl_2(DMSO)_2$[158] (63)

2,2':6',2''-Terpyridine (0.070 g, 0.30 mmol) was dissolved in methanol (10 ml) and added to a solution of $PdCl_2(DMSO)_2$ (0.100 g, 0.300 mmol) in methanol (20 ml). A yellow precipitate was instantly formed but dissolved during refluxing (one hour). The resulting clear yellow solution was filtered and concentrated under reduced pressure. The concentrated reaction mixture was left to crystallize, resulting in pale yellow needles (0.047 g, 34%). Characterization identical to that obtained in synthesis from $PdCl_2$.

## $PdCl_2(DMSO)_2$[175]

Dimethyl sulfoxide (5.50 g, 70.4 mmol, 5 ml) was added to $PdCl_2$ and the solution refluxed overnight. Diethyl ether (20 ml) was then added to the solution to precipitate out a yellow-orange solid. The mixture was stirred for ten minutes and filtered (0.233 g, 78%). Mp 203-205 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 2.46 (s). $\delta_C$ (100 MHz, DMSO-$d_6$) 40.4. Calculated for $PdC_4H_{13}O_2S_2Cl_2$ *m/z* = 332.8769. Found: *m/z* = 332.9422 [M+H].

## 3-(2,4-Dihydroxy-3,3-dimethylbutanamido)propanoic acid (59)

Pantothenic acid was prepared from the hemi-calcium salt, using Amberlite IR-120 $H^+$ ion exchange resin. The wet resin (20 ml) was loaded into a 50 ml burette. Pantothenic acid hemi-calcium salt (0.238 g, 1 mmol) was dissolved in deionised water (10 ml) and loaded onto the column. The column was eluted with deionised water at a flow rate of 2-4 ml/min. The acidic fractions were collected (approximately 40 ml) and dried by lyophilization to yield a clear to slightly yellow viscous oil (0.183 g, 84%). Drying by any means involving heat resulted in discolouration and the generation of impurities (seen in NMR spectra). $\delta_H$ (400 MHz, DMSO-$d_6$) 3.87 (s, 1H), 3.39 (m, 3H), 3.28 (d, *J*=6.5 Hz, 2H) 0.81 (s, 3H), 0.77 (s, 3H). $\delta_C$ (100 MHz, DMSO-$d_6$) 180.4, 174.8, 75.8, 68.3, 38.5, 36.6, 36.0, 20.5, 19.2.

## (2R,3S,4R,5S)-2-(6-Amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol[176] (64)

Adenosine (2.00 g, 7.5 mmol) was added to $CH_3CN$ (25 ml) and the suspension cooled to -10 °C in an ice bath. Freshly distilled thionyl chloride (2.68 g, 22.5 mmol, 1.64 ml) was then added, followed by dry pyridine (1.20 g, 14.9 mmol, 1.22 ml). Upon the addition of pyridine the solution immediately turned yellow. The reaction mixture was stirred for three hours at -5 °C and then allowed to reach room temperature and stirred overnight. The solution was again cooled (0 °C) and $H_2O$ (60 ml) added. A white precipitate formed on cooling but dissolved when the $H_2O$ was added. This solution was then neutralized (pH 7) using solid $NaHCO_3$, and extracted with ethyl acetate (3 x 100 ml). The organic phase was then washed with $NaHCO_3$ (saturated, 150 ml), $H_2O$ (100 ml), dried over $Na_2SO_4$, filtered and evaporated under vacuum. The white solid obtained was stirred in a mixture of $CH_3OH$ (50 ml), $H_2O$ (10 ml) and $NH_4OH$ (conc. 5 ml) for 30 minutes. The solvent was removed and the product recrystallized from $H_2O$ to give white needles (1.786 g, 72%). Mp 188 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 8.34 (s, 1H), 8.15 (s, 1H), 7.31 (s, 2H), 5.93 (d, *J*=5.5 Hz, 1H), 5.61 (d, *J*=4.5 Hz, 1H), 5.48 (d, *J*=4.5 Hz, 1H), 4.75 (d, *J*=5.5 Hz, 1H), 4.22 (d, *J*=4.0 Hz, 1H), 4.09 (d, *J*=4.0 Hz, 1H), 3.93 (m, 1H), 3.83 (m, 1H). $\delta_C$ (100 MHz, DMSO-$d_6$) 156.1, 152.8, 149.5, 139.8, 119.2, 87.5, 83.7, 72.7, 71.3, 44.9. I.R. ($\upsilon_{max}/cm^{-1}$) 3148, 1650, 1600, 1579, 1483, 1422, 1389, 1337, 1298, 1208, 1131, 1097, 1053, 1038. Calculated for $C_{10}H_{13}ClN_5O_3$ *m/z* = 286.0707. Found: *m/z* = 286.0721 [M+H].

## ((3aS,4R,6R,6aS)-6-(6-Amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol (64)

Acetone (150 ml) was added to adenosine (0.34 g, 1.3 mmol). *para*-Toluenesulphonic acid (PTSA, 2.00 g, 11 mmol) was added to the stirring mixture. Upon the addition of PTSA the adenosine dissolved quickly, resulting in a clear colourless solution which was stirred at room temperature overnight. The reaction mixture was then neutralized with $NaHCO_3$ (1.60 g, 19.0 mmol) in water (50 ml). The acetone was removed under

vacuum and the remaining water was extracted with chloroform (3 X 50 ml). The organic layer was dried ($MgSO_4$), filtered and the solvent removed, to afford a white solid (0.313 g, 80%). Mp 210 °C. $\delta_H$ (400 MHz, DMSO-$d_6$) 8.33 (s, 1H), 8.14 (s, 1H), 7.35 (s, 2H), 6.11 (d, *J*=3.0 Hz, 1H), 5.33 (dd, *J*=3.0 Hz, *J*=6.0 Hz, 1H), 5.24 (t, *J*=6.0 Hz, 1H), 4.95 (dd, *J*=3.0 Hz, *J*=6.0 Hz, 1H), 4.20 (m, 1H), 3.54 (m, 2H), 1.52 (s, 3H), 1.31 (s, 3H). $\delta_C$ (100 MHz, DMSO-$d_6$) 152.7, 148.8, 139.7, 119.1, 113.1, 89.6, 86.4, 83.2, 81.4, 61.6, 27.1, 25.2. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3272, 3127, 2986, 2938, 1679, 1610, 1571, 1475, 1426, 1382, 1335, 1299, 1205, 1108, 1072, 850, 794, 721. Calculated for $C_{13}H_{18}N_5O_4$ *m/z* = 308.1359. Found: *m/z* = 308.1367 [M+H].

## (3aR,4S,6R,6aS)-6-(6-Amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carboxylic acid (65)

Potassium hydroxide (0.179 g, 3.48 mmol) dissolved in water (54 ml) was added to the **64** (0.326 g, 1.16 mmol). Potassium permanganate (0.503 g, 3.48 mmol) was dissolved in water (27 ml) and added to the reaction mixture. The solution was stirred overnight at room temperature and quenched with 7.5% $H_2O_2$ (15 ml). The brown precipitate was filtered off and the filtrate concentrated to approximately 50 ml. The product was precipitated out by acidification to *ca.* pH 4 using a 10% HCl solution. The white solid was collected by filtration and dried in a vacuum desiccator (0.221 g, 64%). Mp 258-262 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 8.23 (s, 1H), 8.07 (s, 1H), 7.26 (s, 2H), 6.31 (s, 1H), 5.52 (dd, *J*=2.0 Hz, *J*=6.0 Hz, 1H), 5.45 (d, *J*=6.0 Hz, 1H), 4.67 (d, *J*=2.0 Hz, 1H), 1.51 (s, 3H), 1.34 (s, 3H). $\delta_C$ (100 MHz, DMSO-$d_6$) 171.8, 157.1, 153.5, 150.3, 141.5, 119.9, 113.8, 90.6, 86.5, 84.9, 84.5, 27.6, 26.1. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3383, 2979, 1702, 1614, 1499, 1378, 1278, 1204, 1104, 1054, 861, 768, 640, 519. Calculated for $C_{13}H_{16}N_5O_5$ *m/z* = 322.1151. Found: *m/z* = 322.1159 [M+H].

## (2S,3R,4S,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylic acid (66)

**65** (0.20 g, 0.623 mmol) was stirred in a TFA/$H_2O$ solution (1:4, 15 ml) at room temperature overnight. The solvent was removed *in vacuo* to yield a white product (0.175 g, 100%). Mp 190 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 8.68 (s, 1H), 8.41 (s, 1H), 6.07 (d, *J*=6.0 Hz, 1H), 4.52 (dd, *J*=5 Hz, *J*=6.0 Hz, 1H), 4.46 (d, *J*=2.0 Hz, 1H), 4.32 (dd, *J*=3.0 Hz, *J*=4.0 Hz, 1H). $\delta_C$ (100 MHz, DMSO-$d_6$) 172.4, 152.9, 149.4, 148.5, 141.4, 119.1, 87.8, 83.3, 74.7, 73.8. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3512, 3215, 3147, 2941, 2654, 2538, 1722, 1694, 1642, 1418, 1216, 1188, 1137, 1104, 1052, 855, 645, 592. Calculated for $C_{10}H_{12}N_5O_5$ *m/z* = 282.0838. Found: *m/z* = 282.0848 [M+H].

## 5-Nitro-1,10-phenanthroline[171] (70)

1,10-Phenantholine hydrate (5.20 g, 26.26 mmol) and 98% $H_2SO_4$ (50.00 g, 500 mmol) were added together in a 250 ml round bottomed flask and heated to 120 °C. To this solution 69% $HNO_3$ (27 ml, 210 mmol) was added, resulting in the generation of brown fumes. The temperature was then brought to approximately 170 °C at which point further $HNO_3$ (69%, 50 ml, 390 mmol) was added dropwise maintaining the reaction temperature between 160 °C and 170 °C. $HNO_3$ was added until the generation of brown fumes (upon addition) ceased. The reaction was then stirred for a further 30 minutes without heating, and then poured directly onto crushed ice. The orange solution was neutralized with 30% NaOH (blue to litmus paper) and then acidified again using 4 M nitric acid. The neutralization results in the formation of a yellow precipitate, which was filtered, washed with water and put in a vacuum desiccator to dry (4.60 g, 78%). Mp 196-198 °C. $\delta_H$ (400 MHz, $CDCl_3$) 9.35 (dd, *J* =2.0 Hz, *J*=4.0 Hz, 1H), 9.29 (dd, *J*=2.0 Hz, *J*=4.0 Hz, 1H), 9.02 (dd, *J*=2.0 Hz, *J*=8.0 Hz, 1H) 8.69 (s, 1H), 8.42 (dd, *J*=2.0 Hz, *J*=8.0Hz, 1H), 7.80 (m, 2H). $\delta_C$ (100 MHz, $CDCl_3$) 153.6, 151.5, 147.7, 146.3, 144.3, 137.9, 132.5, 125.5, 125.4, 124.5, 121.1. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3400, 3082, 1516, 1505, 1420, 1355, 806, 733, 621. Calculated for $C_{12}H_8N_3O_2$: *m/z* = 226.0617. Found: *m/z* = 226.0635[M+H].

## 1,10-Phenanthrolin-5-amine[177] (71)

A solution of 5-nitro-1,10-phenanthroline (0.600 g, 2.67 mmol) in ethanol (100 ml) was heated under nitrogen with stirring. A small amount of Pd/C (1%, 0.300 g) was added to the reaction mixture. A solution of hydrazine hydrate (1.03 g, 32.2 mmol, 1.00 ml) in ethanol (20 ml) was then added drop wise to the reaction mixture. The reaction was then left to reflux overnight. The solution was then filtered hot through sintered glass (porosity no. 4). The filtrate was then concentrated *in vacuo* (temperature < 60 °C) to approximately 5 ml. The yellow-green product was precipitated by adding $H_2O$, vacuum filtered and dried in a vacuum desiccator (0.399 g, 76%). Mp 250 °C. $\delta_H$ (400 MHz, $CDCl_3$) 9.18 (dd, *J*=2.0 Hz, *J*=4.0 Hz, 1H), 8.92 (dd, *J*=2.0Hz, *J*=5.0 Hz, 1H), 8.26 (dd, *J*=2.0 Hz, *J*=8.0 Hz, 1H), 7.96 (dd, *J*=2.0 Hz, *J*=8.0 Hz, 1H), 7.63 (dd, *J*=4.0 Hz, *J*=8.0 Hz, 1H), 7.48 (dd, *J*=4.0 Hz, *J*=8.0 Hz, 1H), 6.93 (s, 1H), 4.25 (b, 2H). $\delta_C$ (100 MHz, $CDCl_3$) 150.1, 147.1, 146.7, 142.4, 139.8, 133.5, 129.8, 129.4, 123.3, 122.3, 122.2, 105.6. .R. ($\upsilon_{max}/cm^{-1}$) 3217, 1630, 1612, 1594, 1488, 1455, 1407, 1301, 859, 802, 736, 625. Calculated for $C_{12}H_{10}N_3$: *m/z* = 196.0875. Found: *m/z* = 196.0922[M+H].

## 2-(*tert*-Butoxycarbonylamino)acetic acid[178] (75)

Di-tert-butyl dicarbonate (9.80 g, 44 mmol) was dissolved in diethyl ether (100 ml) and added to a solution of glycine (3.00 g, 40 mmol) dissolved in NaOH (1.6 M, 100 ml). The two solutions were then stirred vigorously overnight at room temperature. Following this the organic layer was separated from the aqueous layer. The latter was kept and acidified to approximately pH 2 using HCl (10%, approx. 150 ml). This solution was then extracted with ethyl acetate (3 x 150 ml), dried over $Na_2SO_4$ and the solvent removed under vacuum to afford a white solid (5.05 g, 72%). Mp 86-87 °C. Peaks attributed to the *E* or *Z* conformation are indicated by *E* or *Z* in brackets after the chemical shift. $\delta_H$ (400 MHz, $CDCl_3$) 6.70 (Z, 0.33H) 5.06 (*E*, 0.67H), 3.93 (*E* and *Z*, m, 2H), 1.43 (s, 9H). $\delta_C$ (100 MHz, $CDCl_3$) 174.6 (*E*), 173.8 (*Z*), 157.1 (*Z*), 155.9 (*E*),

81.6 (*Z*), 80.4 (*E*), 43.4 (*Z*), 42.2 (*E*), 28.3 (*E* and *Z*). I.R. ($\upsilon_{max}$/$cm^{-1}$) 3407, 3343, 2978, 2939, 1738, 1666, 1529, 1408, 1367, 1280, 1195, 1155, 1055, 957, 856, 579, 565. Calculated for $C_7H_{14}NO_4$ *m/z* = 176.0923. Found: *m/z* = 176.0933 [M+H].

## *tert*-Butyl 2-(8-hydroxyquinolin-5-ylamino)-2-oxoethylcarbamate (76)

A solution of dicyclohexylcarbodiimide (1.65 g, 8 mmol) in dry $CH_3CN$ (20 ml) and a solution of 2-(tert-butoxycarbonylamino)acetic acid (2.80 g, 16.0 mmol) in $CH_3CN$ (30 ml) were added together, resulting in the immediate formation of a white precipitate. This solution was then left to stir for one hour, after which the white solid was filtered (sintered glass porosity no. 4) and the filtrate added to a solution of 5-amino-8-hydroxyquinoline dihydrochloride (0.317 g, 1.29 mmol) in dry $CH_3CN$ (50 ml) with dry pyridine (0.572 g, 5.76 mmol, 0.72 ml). The resulting red solution was left to stir at room temperature, under $N_2$, for 48 hours. The solvent was removed and the resulting solid dissolved in dichloromethane and washed with $NaHCO_3$ (5%, 100 ml). The organic layer was dried using $Na_2SO_4$ and preadsorbed onto silica. The product was purified by column chromatography using gradient elution, from 100% hexane to 50% ethyl acetate/hexane. The product was isolated as a pale yellow solid (0.152 g, 37%). Mp 134-135 °C. $\delta_H$ (400 MHz, DMSO-$d_6$) 9.76 (s, 1H), 8.85 (dd, *J*=1.5 Hz, *J*=8.0 Hz, 1H), 8.31 (dd, *J*=6.5 Hz, *J*=8.0 Hz, 1H), 7.56 (m, 1H), 7.39 (d, *J*=8.0 Hz, 1H), 7.12 (m, 1H), 7.05 (d, *J*=8.0 Hz, 1H), 3.81, (d, *J*=6.5 Hz, 2H), 1.40 (s, 9H). $\delta_C$ (100 MHz, DMSO-$d_6$) 169.4, 156.0, 151.8, 148.1, 138.3, 132.2, 124.7, 124.3, 124.1, 121.4, 110.4, 78.1, 43.7, 28.2. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3318, 2977, 1713, 1691, 1652, 1505, 1365, 1272, 1223, 1167, 1053, 942, 780, 563, 495. Calculated for $C_{16}H_{20}N_3O_4$ *m/z* = 318.1454. Found: *m/z* = 318.1476 [M+H].

## 2-Amino-N-(8-hydroxyquinolin-5-yl)acetamide (77)

A mixture of freshly distilled TFA (5 ml, 29.6 mmol) and dry $CH_2Cl_2$ (5 ml) was added to tert-butyl 2-(8-hydroxyquinolin-5-ylamino)-2-oxoethylcarbamate (0.350 g, 1.10

mmol) and the solution stirred for two hours. The solvent was then removed *in vacuo* affording a green-yellow product (0.234 g, 98%) Mp 210-212 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 10.35 (s, 1H), 8.93 (d, *J*=4 Hz, 1H), 8.52 (d, *J*=8.0 Hz, 1H), 8.17 (b, 1H), 7.69 (dd, *J*=2 Hz, *J*=4 Hz, 1H), 7.52 (d, *J*=8.0 Hz, 1H), 7.16 (d, *J*=8.0 Hz, 1H), 3.92 (m, 2H). $\delta_C$ (100 MHz, DMSO-$d_6$) 166.1, 158.5, 151.0, 147.7, 136.6, 134.0, 124.7, 123.4, 121.8, 111.6, 40.8. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3277, 3061, 1721, 1687, 1662, 1601, 1562, 1315, 1185, 1123, 1084, 842, 828, 797, 726, 720. Calculated for $C_{11}H_{12}N_3O_2$ *m/z* = 218.0930. Found: *m/z* = 218.0954 [M+H].

## 8-Hydroxyquinoline-pantothenic acid derivative (78)

Dry pantothenic acid (0.0954 g, 0.436 mmol) was dissolved in dry DMF (5 ml). **77** (0.0945 g, 0.436 mmol) was added to the reaction mixture, which turned green-brown, followed by DPPA (0.132 g, 0.479 mmol). The reaction was then cooled to 0 °C and TEA (0.067 ml, 0.479 mmol) was added. The reaction was stirred at 0 °C for 2 hours and then allowed to warm to room temperature and stirred overnight. The reaction was monitored using LC-MS and the product purified using semi-prep HPLC to afford a viscous yellow oil (0.067 g, 36 %). Gradient elution (water and acetonitrile both containing 0.1% v/v formic acid) for semi-prep HPLC was as follows: using a flow rate of 17 $ml.min^{-1}$ the gradient was held at 90% water to for two minutes and then changed to 87% water over 6 minutes. Product retention time 3.1 min. $\delta_H$ (400 MHz, DMSO-$d_6$) 9.87 (s, 1H), 8.91 (d, *J*=2.0 Hz, 1H), 8.44 (d, *J*=8.0 Hz, 1H), 8.35 (t, *J*=5.0 Hz, 1H), 8.13 (s, 1H), 7.76 (t, *J*=5.0 Hz, 1H), 7.68 (dd, *J*=4.0 Hz, *J*=8.0 Hz, 1H), 7.44 (d, *J*=8.0 Hz, 1H), 7.13 (d, *J*=8.0 Hz, 1H), 3.97 (d, *J*=6.0 Hz, 2H), 3.69 (s, 1H), 3.16- 3.28 (m, 4H, suppressed), 2.41 (m, 2H), 0.79 (s, 3H), 0.78 (s 3H). $\delta_C$ (100 MHz, DMSO-$d_6$) 172.9, 171.4, 169.1, 163.1, 150.7, 147.5, 136.7, 134.2, 125.0, 124.9, 124.3, 121.6, 111.4, 75.1, 68.0, 42.6, 35.1, 34.8, 21.0, 20.4. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3278, 3072, 2964, 1646, 1598, 1524, 1475, 1277, 1197, 1179, 1130, 1078, 1036, 832, 798, 719, 598. Calculated for $C_{20}H_{27}N_4O_6$ *m/z* = 419.1931. Found: *m/z* = 419.1944 [M+H].

## 8-Hydroxyquinoline-adenosine derivative (79)

**77** (0.039 g, 0.178 mmol) and **66** (0.050 g, 0.178 mmol) were dissolved in dry DMF (5 ml). HBTU (0.0674 g, 0.178 mmol) and TEA (0.148 g, 1.068 mmol) were added to the reaction mixture. The resulting solution was stirred at room temperature overnight and monitored by LC-MS. The product was purified using semi-prep HPLC to afford a yellow solid (0.027 g, 32 %). Gradient elution (water and acetonitrile both containing 0.1% v/v formic acid) conditions for semi-prep HPLC were as follows: using a flow rate of 17 ml.min$^{-1}$ the gradient was changed from 96% water to 95% water over 20 minutes. Product retention time 4.5 min. Mp 226-228 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 9.94 (s, 1H), 9.27 (s, 1H), 8.89 (d, *J*=3.0 Hz, 1H), 8.51 (s, 1H), 8.41 (d, *J*=3.0 Hz, 1H), 8.26, (s, 1H), 7.63, (dd, *J*=4 Hz, *J*=8.0 Hz, 1H), 7.45 (d, *J*=8.0 Hz, 1H), 7.09 (d, *J*=8.0 Hz, 1H), 6.03 (d, *J*=7.0 Hz, 1H), 4.67 (dd, *J*=4.0 Hz, *J*=7.0 Hz, 1H), 4.46 (s, 1H), 4.17-4.24 (m, 3H). $\delta_C$ (100 MHz, DMSO-$d_6$) 170.8, 169.0, 156.8, 151.9, 151.5, 149.4, 148.3, 141.3, 138., 125.1, 124.9, 124.6, 122.0, 119.7, 111.3, 87.9, 85.1, 73.8, 72.8, 42.8. I.R. ($\upsilon_{max}$/cm$^{-1}$) 3327, 3192, 3070, 2926, 1643, 1597, 1503, 1475, 1374, 1207, 1123, 1051, 835, 555. Calculated for $C_{21}H_{21}N_8O_6$ *m/z* = 481.1584. Found: *m/z* = 481.1612 [M+H].

## *tert*-Butyl 2-(1,10-phenanthrolin-5-ylamino)-2-oxoethylcarbamate[165] (80)

A solution of dicyclohexylcarbodiimide (1.65 g, 8 mmol) in dry $CH_3CN$ (20 ml) and a solution of 2-(tert-butoxycarbonylamino)acetic acid (2.80 g, 16.0 mmol) in dry $CH_3CN$ (30 ml) were added together, resulting in the immediate formation of a white precipitate. This solution was then left to stir for one hour, after which the white solid was filtered off (sintered glass porosity no. 4) and the filtrate added to a solution of 5-amino-1,10-phenanthroline (0.300 g, 1.54 mmol) in dry $CH_3CN$ (50 ml). The resulting yellow solution was left to stir at room temperature, under $N_2$, overnight. The reaction was monitored using UV TLC plates with a mobile phase of 20% methanol in dichloromethane (product $R_f$ = 0.50). Normal TLC plates showed no distinction

between product and starting material. The solvent was removed and the resulting solid dissolved in dichloromethane and then washed with $NaHCO_3$ (5%, 100 ml). The organic layer was dried using $Na_2SO_4$ and preadsorbed onto silica. The product was purified by column chromatography using gradient elution, from 100% $CH_2Cl_2$ to 6% $CH_3OH/CH_2Cl_2$. The product was isolated as a pale yellow solid (0.295 g, 55%). Mp 140-150 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 10.15 (s, 1H), 9.12 (d, *J*=2.0 Hz, 1H), 9.03 (d, *J*=2.0 Hz, 1H), 8.61 (d, *J*=8.0 Hz, 1H), 8.45 (d, *J*=8.0 Hz, 1H), 8.13 (s, 1H), 7.75 (m, 2H), 7.20 (s, 1H), 3.93 (d, *J*=6.0 Hz, 2H), 1.48 (s, 9H). $\delta_C$ (100 MHz, DMSO-$d_6$) 169.6, 156.1, 149.9, 149.4, 145.8, 143.9, 135.9, 131.8, 131.6, 128.1, 124.8, 123.6, 122.8, 120.3, 78.2, 43.9, 28.2. I.R. ($\upsilon_{max}/cm^{-1}$) 3192, 2979, 1710, 1685, 1644, 1620, 1524, 1481, 1420, 1366, 1282, 1167, 740. Calculated for $C_{19}H_{21}N_4O_3$ *m/z* = 353.1614. Found: *m/z* = 353.1627 [M+H].

## 2-Amino-*N*-(1,10-phenanthrolin-5-yl)acetamide[165] (81)

A mixture of freshly distilled trifluoroacetic acid (TFA, 5 ml, 29.6 mmol) and dry $CH_2Cl_2$ (5 ml) was added to tert-butyl 2-(1,10-phenanthrolin-5-ylamino)-2-oxoethylcarbamate (0.100 g, 0.284 mmol) and the solution stirred for two hours. The solvent was then removed *in vacuo* affording an orange product (0.072 g, 100%) Mp 122 °C. $\delta_H$ (400 MHz, DMSO-$d_6$) 10.91 (s, 1H), 9.30 (d, *J*=4.0 Hz, 1H), 9.19 (d, *J*=4.5 Hz, 1H), 8.98 (t, *J*=8.0 Hz, 2H), 8.44 (s, 1H), 8.29 (s, 1H), 8.11 (m, 1H), 4.08 (d, *J*=4.0 Hz, 2H) . $\delta_C$ (100 MHz, DMSO-$d_6$) 166.5, 158.1, 149.8, 147.5, 142.1, 139.9, 133.7, 131.9, 128.7, 124.9, 124.8, 124.3, 119.4, 41.2. I.R. ($\upsilon_{max}/cm^{-1}$) 3284, 3243, 2920, 1670, 1593, 1540, 1502, 1466, 1425, 1191, 1174, 1131, 866, 835, 810, 797, 722, 621, 474. Calculated for $C_{14}H_{13}N_4O$ *m/z* = 253.1089. Found: *m/z* = 253.1108 [M+H].

## 1,10-Phenanthroline-pantothenic acid derivative (82)

Dry pantothenic acid (0.107 g, 0.488 mmol) was dissolved in dry DMF (5 ml). **81** (0.123 g, 0.489 mmol) was added to the reaction mixture, which turned bright orange,

followed by DPPA (0.148 g, 0.534 mmol). The reaction was then cooled to 0 °C and triethylamine (TEA, 0.074 ml, 0.534 mmol) was added. The reaction was stirred at 0 °C for two hours and then allowed to warm to room temperature and stirred overnight. The reaction was monitored using LC-MS and the product purified using semi-prep HPLC to afford a viscous orange oil (0.042 g, 19 %). Gradient elution (water and acetonitrile both containing 0.1% v/v formic acid) for semi-prep HPLC was as follows: using a flow rate of 17 $ml.min^{-1}$ the gradient was changed from 90% water to 86% water over 8 minutes. Product retention time 7 minutes. $\delta_H$ (400 MHz, DMSO-$d_6$) 10.20 (s, 1H), 9.13 (d, *J*=3.0 Hz, 1H), 9.05 (d, *J*=3.0 Hz, 1H), 8.60 (d, *J*=8 Hz, 1H), 8.46 (d, *J*=8 Hz, 1H), 8.41 (t, *J*=5 Hz, 1H), 7.83 (dd, *J*=5.0 Hz, *J*=8.0 Hz, 1H) 7.76 (m, 2H), 4.08 (d, *J*=5 Hz, 2H), 3.71 (s, 1H), 3.18-3.41 (m, 4H, suppressed), 2.42 (m, 2H), 0.81 (s, 3H), 0.79 (s, 3H). $\delta_C$ (100 MHz, DMSO-$d_6$) 173.4, 171.9, 169.7, 163.6, 150.4, 149.9, 146.3, 144.4, 136.3, 132.3, 132.1, 128.5, 125.4, 124.1, 123.3, 121.1, 75.6, 74.7, 68.5, 43.2, 38.1, 35.6, 35.3, 21.3, 20.8. I.R. ($\upsilon_{max}/cm^{-1}$) 3632, 3277, 1776, 1653, 1599, 1543, 1466, 1119, 1024, 991, 937, 765, 724, 557. Calculated for $C_{23}H_{28}N_5O_5$ *m/z* = 454.2090. Found: *m/z* = 454.2113 [M+H].

## 1,10-Phenanthroline-adenosine derivative (83)

**81** (0.045 g, 0.178 mmol) and **66** (0.050 g, 0.178 mmol) were dissolved in dry DMF (5 ml). HBTU (0.0674 g, 0.178 mmol) and TEA (0.148 g, 1.068 mmol) were added to the reaction mixture. The resulting solution was stirred at room temperature overnight and monitored by LC-MS. The product was purified using semi-prep HPLC to afford an orange solid (0.018 g, 20 %). Gradient elution (water and acetonitrile both containing 0.1% v/v formic acid) for semi-prep HPLC was as follows: using a flow rate of 17 $ml.min^{-1}$ the gradient was changed from 95% water to 91% water over eight minutes. Product retention time 8.7 minutes. Mp 250-254 °C (decomp.). $\delta_H$ (400 MHz, DMSO-$d_6$) 10.30 (s, 1H), 9.52 (t, *J*=5.0 Hz, 1H), 9.13 (d, *J*=3.5 Hz, 1H), 9.04 (d, *J*=3.5 Hz, 1H), 8.66 (d, *J*=8.0 Hz, 1H), 8.46 (d, *J*=8.0 Hz, 1H), 8.44 (s, 1H) 8.16 (s, 1H), 8.13 (s, 1H) 7.83 (dd, *J*=4.0 Hz, *J*=4.0 Hz, 1H), 7.74 (dd, *J*=4.0 Hz, *J*=4.0 Hz, 1H), 7.42 (s, 2H), 6.03 (d, *J*=8.0 Hz, 1H), 4.71 (dd, *J*=5.0 Hz, *J*=3.0 Hz, 1H), 4.46 (s, 1H), 4.28 (m, 3H).

$\delta_C$ (100 MHz, DMSO-$d_6$) 170.5, 168.8, 156.2, 152.5, 149.9, 149.2, 148.9, 145.5, 143.5, 140.5, 136.1, 131.9, 131.6, 128.1, 124.7, 123.7, 122.9, 120.1, 119.5, 57.5, 84.7, 73.3, 72.0, 42.5. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3435, 3171, 3067, 2920, 1643, 1594, 1540, 1421, 1329, 1206, 1129, 1046, 798, 739, 522. Calculated for $C_{24}H_{22}N_9O_5$ *m/z* = 516.1744. Found: *m/z* = 516.1768 [M+H].

## 4-(*N*-Ethylamino)-amino-2,2':6',2''-terpyridine[173] (89)

4-Chloro-2;2':6';2"-terpyridine (0.180 g, 0.672 mmol) was refluxed in ethylenediamine (11.12 g, 189 mmol, 10 ml,). The solution was initially yellow and became dark brown overnight. The solution was then concentrated under vacuum, and the product precipitated out by adding $H_2O$. This golden precipitate was filtered, washed with water and dried in a vacuum desiccator (0.133 g, 68%). Mp 156-159 °C. $\delta_H$ (400 MHz, $CDCl_3$) 8.65 (ddd, *J*=1.5 Hz, *J*=2.0 Hz, *J*=5.5 Hz, 2H), 8.59 (dt, (*J*=8.0 Hz, *J*=1.0 Hz, 2H), 7.80 (ddd, *J*=1.5 Hz, *J*=5.5 Hz, *J*=8.0 Hz, 2H), 7.68 (s, 2H), 7.28 (ddd, *J*=1.0 Hz, *J*=5.5 Hz, *J*=8.0 Hz, 2H), 4.86 (t, *J*=5.0 Hz, 1H), 3.41 (m, 2H), 3.00 (m, 2H). $\delta_C$ (100 MHz, $CDCl_3$) 156.8, 155.9, 155.5, 148.8, 136.7, 123.5, 121.3, 104.8, 45.3, 41.0. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3289, 2878, 1606, 1582, 1563, 1462, 1331, 1225, 1040, 984, 789, 744, 731, 629, 404. Calculated for $C_{17}H_{18}N_5$ *m/z* = 292.1562. Found: *m/z* = 292.1574 [M+H].

## 2,2':6',2'' Terpyridine-pantothenic acid derivative (90)

Dry pantothenic acid (0.183 g, 0.836 mmol) was dissolved in dry DMF (5 ml). **89** (0.243 g, 0.836 mmol) was added to the reaction mixture followed by DPPA (0.200 g, 0.920 mmol). The reaction was then cooled to 0 °C and TEA (0.130 ml, 0.920 mmol) was added. The reaction was stirred at 0 °C for two hours and then allowed to warm to room temperature and stirred overnight. The reaction was monitored using LC-MS and the product purified using semi-prep HPLC to afford a viscous purple oil (0.216 g, 55.3%). Gradient elution (water and acetonitrile both containing 0.1% v/v formic acid) conditions for semi-prep HPLC were as follows: using a flow rate of 17 $ml.min^{-1}$ the

gradient was changed from 82% water to 81% water over eight minutes, then to 65% water over two minutes where it was held constant for four minutes. Product retention time 10 minutes. $\delta_H$ (400 MHz, $D_2O$) 8.60 (m, 2H), 8.38 (s, 1H), 7.99 (m, 4H), 7.59 (t, *J*=5.0 Hz, 2H), 7.33 (t, *J*=6.0 Hz, 2H), 7.15 (m, 2H), 3.89 (s, 1H), 3.27-3.40 (m, 8H), 2.44 (t, *J*=6.0 Hz, 2H) 0.82 (s, 3H), 0.78 (s, 3H). $\delta_C$ (100 MHz, $D_2O$-$d_6$) 175.0, 174.4, 168.9, 149.5, 138.8, 129.7, 127.0, 124.4, 121.7, 120.1, 120.0, 75.6, 68.2, 41.8, 38.5, 37.9, 35.3, 20.4, 19.0. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3242, 3061, 2936, 2872, 1637, 1584, 14878, 1234, 1206, 1089, 1072, 909, 893, 765, 692, 532. Calculated for $C_{26}H_{33}N_6O_4$ *m/z* = 493.2563. Found: *m/z* = 493.2570 [M+H].

## 2,2';6',2'' Terpyridine-adenosine derivative (91)

**89** (0.051 g, 0.178 mmol) and **66** (0.050 g, 0.178 mmol) were dissolved in dry DMF (5 ml). HBTU (0.0674 g, 0.178 mmol) and TEA (0.148 g, 1.068 mmol) were added to the reaction mixture. The resulting solution was stirred at room temperature overnight and monitored by LC-MS. The product was purified using semi-prep HPLC to afford a purple solid (0.023 g, 28%). Gradient elution (water and acetonitrile both containing 0.1% v/v formic acid) conditions for semi-prep HPLC were as follows: using a flow rate of 17 $ml.min^{-1}$ the gradient was changed from 80% water to 60% water over ten minutes. Product retention time 10 minutes. Mp 175 °C. $\delta_H$ (400 MHz, DMSO-$d_6$) 9.29 (s, 1H), 8.73 (m, 2H), 8.49 (m, 2H), 8.37 (s, 1H), 8.23 (s, 1H), 8.00 (m, 2H), 7.68 (s, 2H), 7.51 (m, 2H), 7.41 (s, 2H), 5.95 (d, *J*=8.0 Hz, 1H), 4.63 (d, *J*=4.0 Hz, 1H), 4.35 (s, 1H), 4.20 (s, 1H), 3.50 (t, *J*=6.0 Hz, 2H suppressed), 2.90 (t, *J*=6.0 Hz, 2H suppressed). $\delta_C$ (100 MHz, DMSO-$d_6$) 170.6, 163.6, 156.7, 153.0, 149.7, 149.6, 149.2, 141.2, 138.0, 124.7, 123.8, 121.4, 120.1, 88.3, 85.2, 73.6, 72.4, 42.2, 38.3. I.R. ($\upsilon_{max}$/$cm^{-1}$) 3183, 1647, 1585, 1567, 1472, 1337, 1119, 1052, 790, 744, 693. Calculated for $C_{27}H_{27}N_{10}O_4$ *m/z* = 555.2217. Found: *m/z* = 555.2204 [M+H].

## 7.3 Inhibition of β-Haematin Formation Assays

A simple method for screening a compounds ability to inhibit the formation of β-haematin was developed by Egan, Ross and Adams.[28] Their method was adapted for this study. A sample of haemin (15 mg, 0.023 mmol) was placed in a test tube with NaOH (0.1 M, 3 ml). This mixture was heated to 70 °C. To this solution was added first an acetate buffer solution (12.9 M, pH 5, 1.75 ml) followed by HCl (1 M, 0.30 ml) both pre-incubated at 70 °C. After stirring for two hours in a temperature controlled bath, the test tube was placed on ice for five minutes. In the study by Egan *et al*[28] β-haematin was found to form within 30 minutes at 60 °C, however here it was found that the formation of β-haematin was only complete after two hours at 70 °C. Once cooled the contents of the test tubes were transferred to vials, and centrifuged at 6000 rpm for five minutes. The supernatant was decanted, and the remaining solid was shaken with deionised water (8 ml) and centrifuged again at 6000 rpm for five minutes. The solid was washed in this way three times following which, while still wet, it was analysed using an ATR infrared spectrophotometer (no difference was found whether the sample was left to dry on the spectrophotometer, usually within a few minutes, or if it was dried in a desiccator prior to infrared analysis.) This control experiment was used to confirm β-haematin formation under the reaction conditions. The effect of each compound was determined by adding three equivalents to the test tube prior to the addition of the acetate buffer solution. In each run a negative inhibition control (haemin alone) and a positive inhibition control (a reaction mixture containing three equivalents of chloroquine phosphate) was used. The formation of β-haematin may be determined by the appearance of two peaks on the infrared spectrum of solid residue, one at 1660 $cm^{-1}$ and one at 1207 $cm^{-1}$.[28]

# 7.4 Antimalarial Activity Screening Method*

## Parasite culture

The 3D7 and D10 strains of *P. falciparum* was cultured at 37 °C in RPMI-1640 medium supplemented with 50 mM glucose, 0.65 mM hypoxanthine, 25 mM Hepes, 0.2% (w/v) $NaHCO_3$, 0.048 mg/ml gentamicin, 0.5% (w/v) Albumax II, and 2-4% (v/v) human $O^+$ erythrocytes, under an atmosphere of 3% $CO_2$, 4% $O_2$, balance $N_2$.

## Parasite viability dose-response assays

Culture-derived parasitised erythrocytes were mixed with fresh culture medium and erythrocytes to yield a 2% parasitemia, 2% haematocrit suspension and distributed in microtitre plates at 100 µl/well. Serial dilutions of test drug in culture medium were prepared in quadruplicate wells in a separate plate and transferred to the parasite plate to yield a final volume of 200 µl/well. The plates were incubated at 37$^o$C for 48 hours and parasite viability in each well measured by the colorimetric determination of parasite lactate dehydrogenase activity.[179] The $IC_{50}$-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

* All antimalarial testing was performed either by Dr H Hoppe or Prof. P. Smith at the University of Cape Town. The screening method reported here is reproduced with the permission of Dr H. Hoppe, personal communication.

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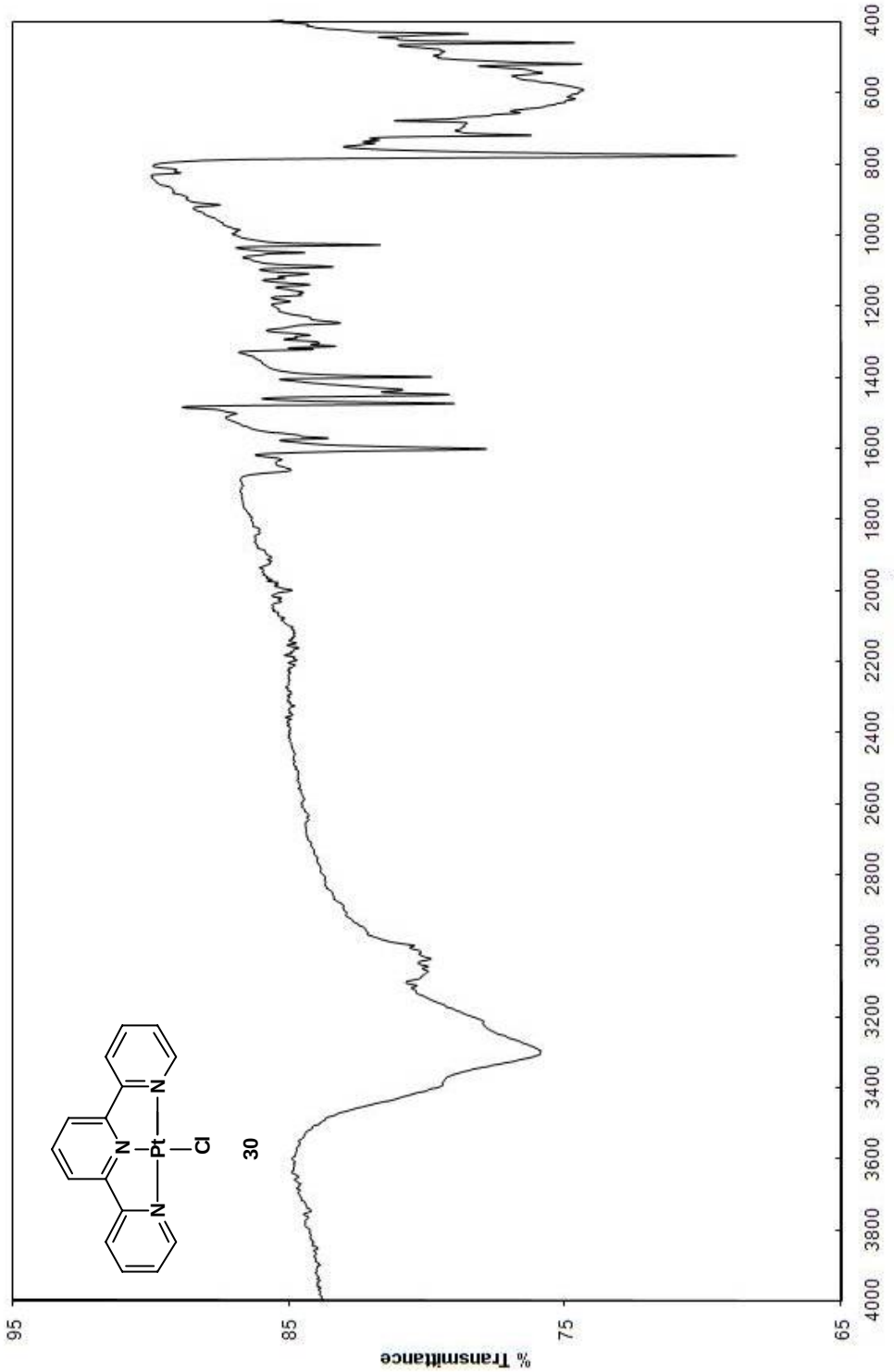
# Appendix 1

# Characterization




**Spectrum 1 IR spectrum of 30.**

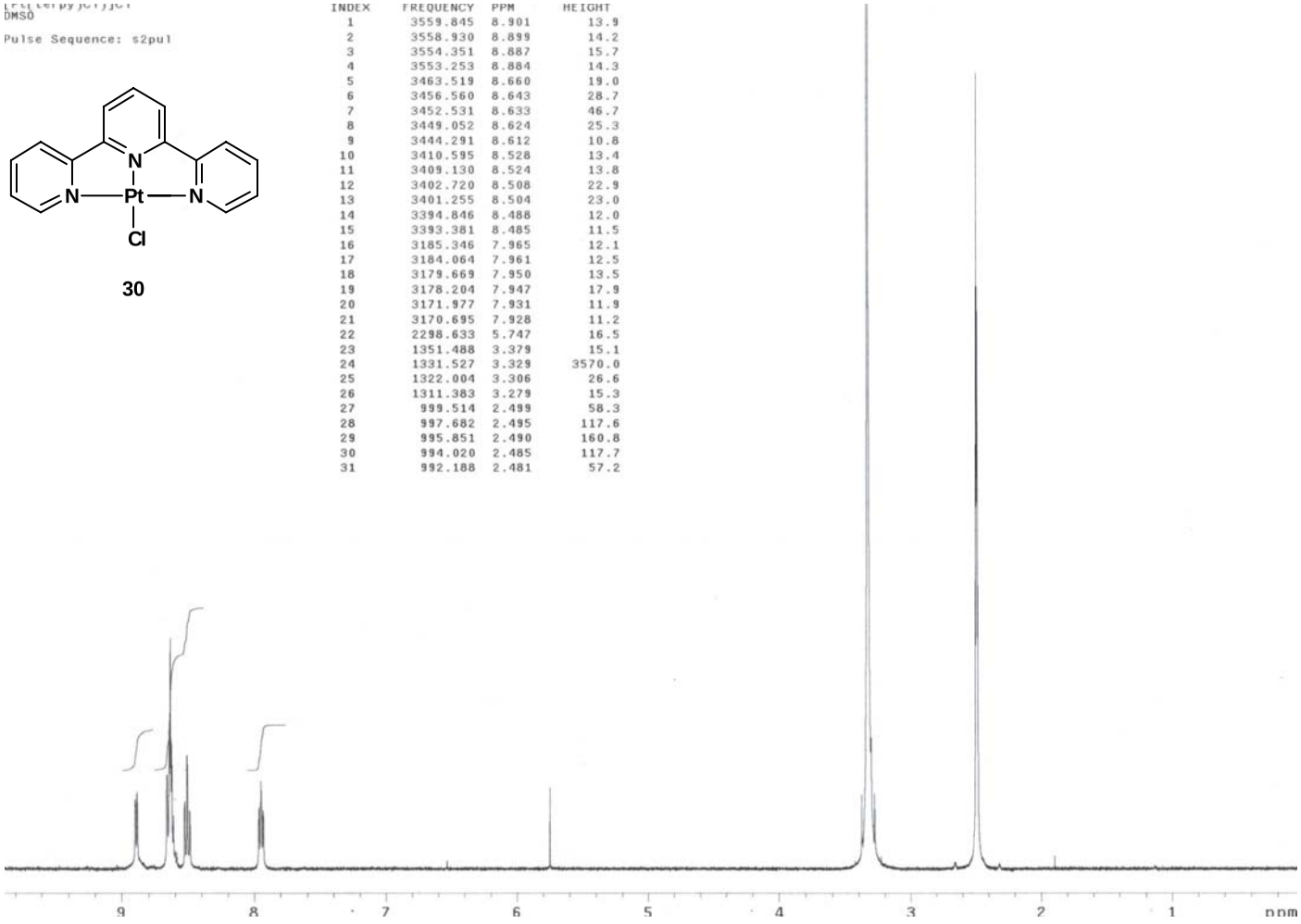
DMSO

Pulse Sequence: s2pul




30

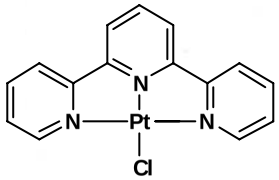
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 3559.845 | 8.901 | 13.9 |
| 2 | 3558.930 | 8.899 | 14.2 |
| 3 | 3554.351 | 8.887 | 15.7 |
| 4 | 3553.253 | 8.884 | 14.3 |
| 5 | 3463.519 | 8.660 | 19.0 |
| 6 | 3456.560 | 8.643 | 28.7 |
| 7 | 3452.531 | 8.633 | 46.7 |
| 8 | 3449.052 | 8.624 | 25.3 |
| 9 | 3444.291 | 8.612 | 10.8 |
| 10 | 3410.595 | 8.528 | 13.4 |
| 11 | 3409.130 | 8.524 | 13.8 |
| 12 | 3402.720 | 8.508 | 22.9 |
| 13 | 3401.255 | 8.504 | 23.0 |
| 14 | 3394.846 | 8.488 | 12.0 |
| 15 | 3393.381 | 8.485 | 11.5 |
| 16 | 3185.346 | 7.965 | 12.1 |
| 17 | 3184.064 | 7.961 | 12.5 |
| 18 | 3179.669 | 7.950 | 13.5 |
| 19 | 3178.204 | 7.947 | 17.9 |
| 20 | 3171.977 | 7.931 | 11.9 |
| 21 | 3170.695 | 7.928 | 11.2 |
| 22 | 2298.633 | 5.747 | 16.5 |
| 23 | 1351.488 | 3.379 | 15.1 |
| 24 | 1331.527 | 3.329 | 3570.0 |
| 25 | 1322.004 | 3.306 | 26.6 |
| 26 | 1311.383 | 3.279 | 15.3 |
| 27 | 999.514 | 2.499 | 58.3 |
| 28 | 997.682 | 2.495 | 117.6 |
| 29 | 995.851 | 2.490 | 160.8 |
| 30 | 994.020 | 2.485 | 117.7 |
| 31 | 992.188 | 2.481 | 57.2 |




**Spectrum 2 $^{1}$H NMR spectrum of 30 in DMSO-$d_6$.**

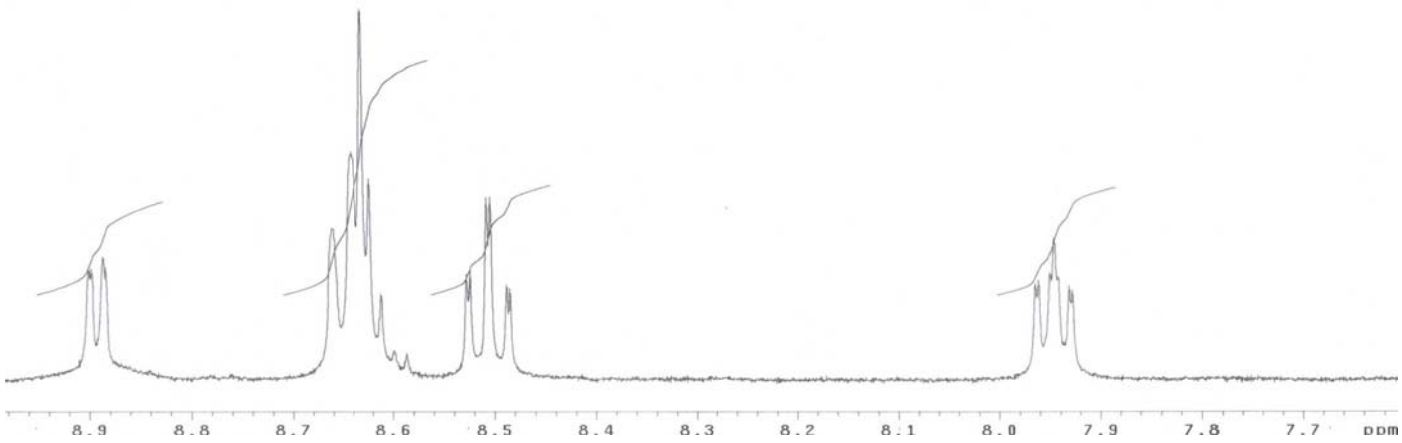
DMSO

Pulse Sequence: s2pul

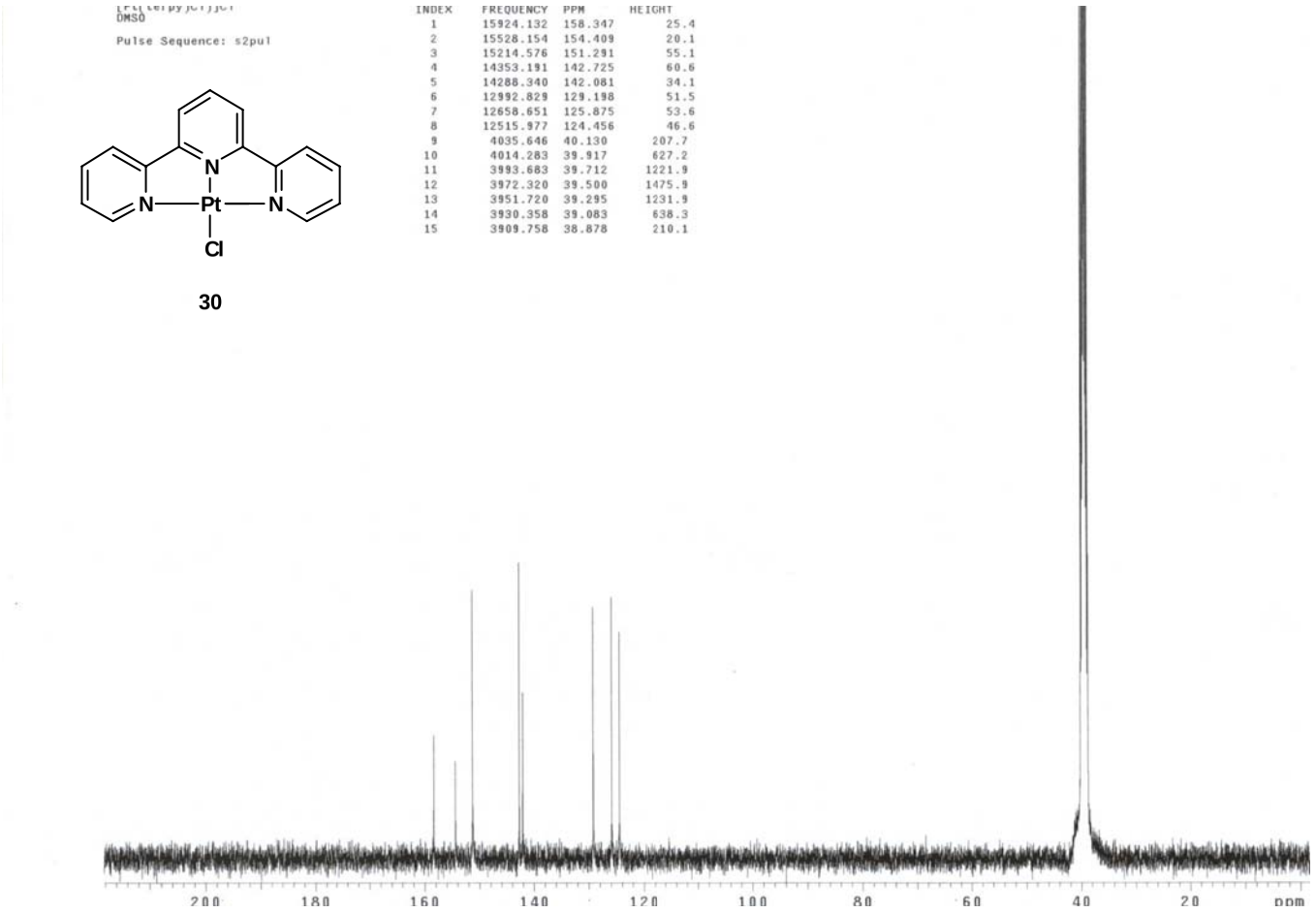



**30**

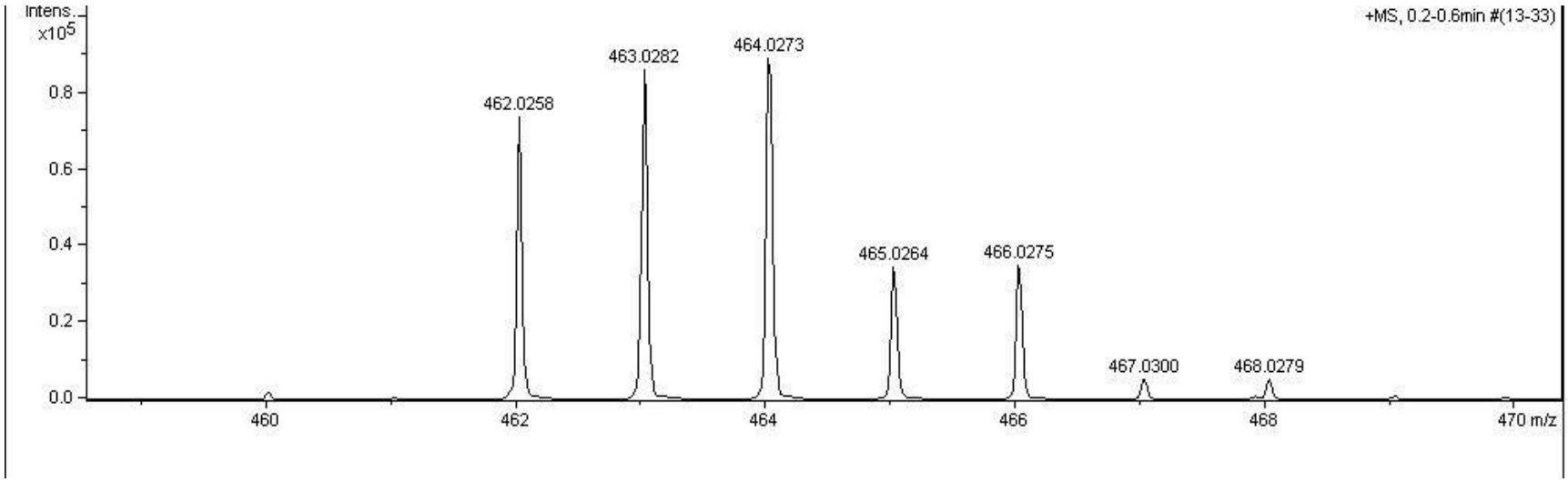
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 3559.845 | 8.901 | 25.2 |
| 2 | 3558.930 | 8.899 | 25.7 |
| 3 | 3554.351 | 8.887 | 28.2 |
| 4 | 3553.253 | 8.884 | 25.8 |
| 5 | 3463.519 | 8.660 | 34.0 |
| 6 | 3456.560 | 8.643 | 50.4 |
| 7 | 3452.531 | 8.633 | 80.7 |
| 8 | 3449.052 | 8.624 | 44.7 |
| 9 | 3444.291 | 8.612 | 20.2 |
| 10 | 3410.595 | 8.528 | 24.5 |
| 11 | 3409.130 | 8.524 | 25.3 |
| 12 | 3402.720 | 8.508 | 40.7 |
| 13 | 3401.255 | 8.504 | 40.8 |
| 14 | 3394.846 | 8.488 | 22.2 |
| 15 | 3393.381 | 8.485 | 21.5 |
| 16 | 3185.346 | 7.965 | 22.4 |
| 17 | 3184.064 | 7.961 | 23.2 |
| 18 | 3179.669 | 7.950 | 24.8 |
| 19 | 3178.204 | 7.947 | 32.2 |
| 20 | 3171.977 | 7.931 | 22.1 |
| 21 | 3170.695 | 7.928 | 21.0 |

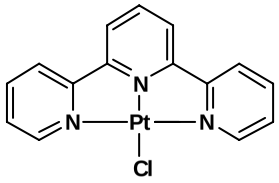



**Spectrum 3 Expanded $^{1}$H NMR spectrum of 30 in DMSO-$d_6$.**



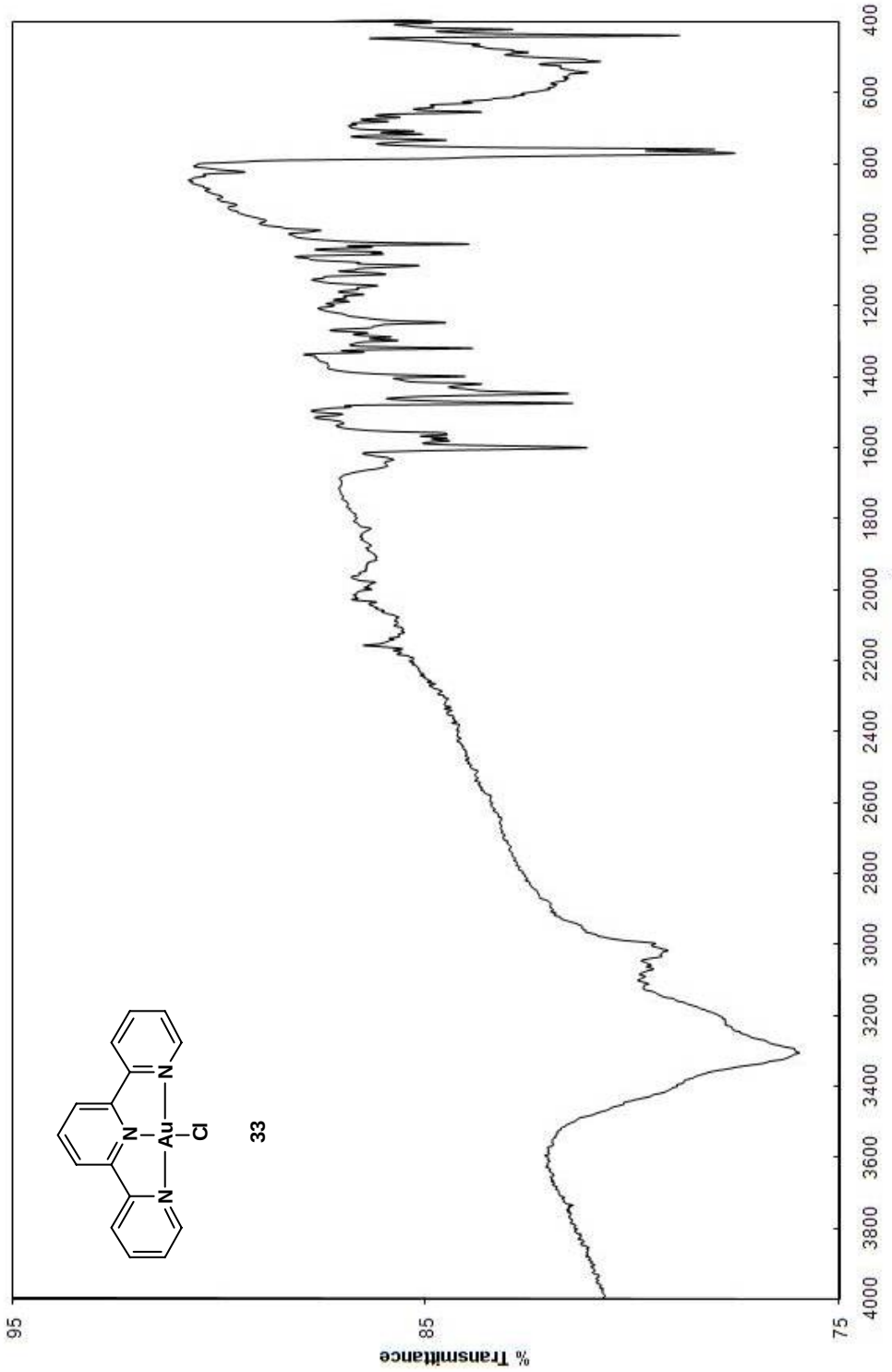

**Spectrum 4 $^{13}$C NMR spectrum of 30 in DMSO-$d_6$.**




**30**

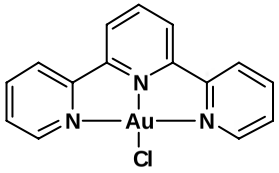
**Spectrum 5 MS isotopic pattern for Pt(terpy)Cl$^{+}$.**




**Spectrum 6 IR spectrum of 33.**

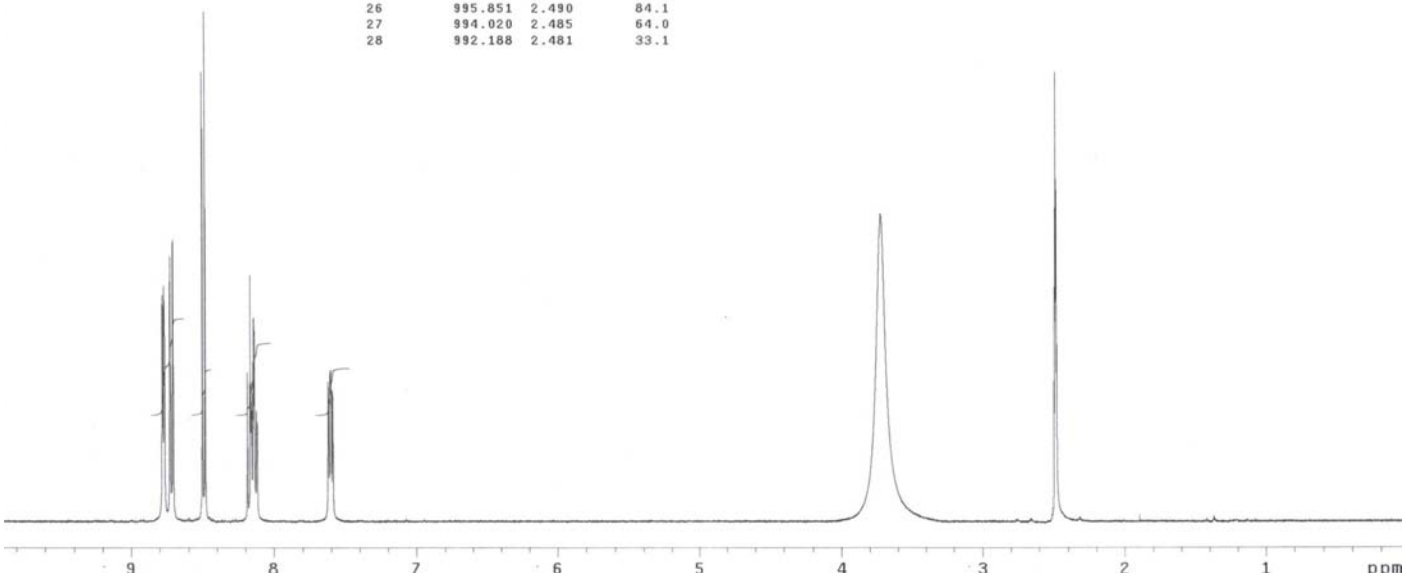
DMSO

Pulse Sequence: s2pul




**33**

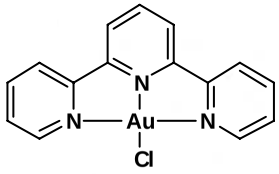
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 3510.950 | 8.779 | 42.1 |
| 2 | 3510.034 | 8.776 | 42.3 |
| 3 | 3506.005 | 8.766 | 44.0 |
| 4 | 3505.273 | 8.764 | 41.1 |
| 5 | 3489.157 | 8.724 | 49.5 |
| 6 | 3481.100 | 8.704 | 52.8 |
| 7 | 3399.973 | 8.501 | 84.0 |
| 8 | 3392.099 | 8.482 | 95.4 |
| 9 | 3272.882 | 8.183 | 27.9 |
| 10 | 3265.007 | 8.164 | 46.0 |
| 11 | 3262.443 | 8.157 | 22.3 |
| 12 | 3260.978 | 8.154 | 20.8 |
| 13 | 3257.132 | 8.144 | 30.0 |
| 14 | 3254.752 | 8.138 | 38.2 |
| 15 | 3253.287 | 8.134 | 35.7 |
| 16 | 3247.060 | 8.119 | 20.7 |
| 17 | 3245.412 | 8.115 | 18.7 |
| 18 | 3047.632 | 7.620 | 26.2 |
| 19 | 3042.688 | 7.608 | 27.6 |
| 20 | 3041.772 | 7.606 | 28.2 |
| 21 | 3040.124 | 7.601 | 28.4 |
| 22 | 3035.180 | 7.589 | 24.6 |
| 23 | 1489.018 | 3.723 | 57.5 |
| 24 | 999.514 | 2.499 | 26.2 |
| 25 | 997.682 | 2.495 | 58.6 |
| 26 | 995.851 | 2.490 | 84.1 |
| 27 | 994.020 | 2.485 | 64.0 |
| 28 | 992.188 | 2.481 | 33.1 |




**Spectrum 7 $^{1}$H NMR spectrum of 33 in DMSO-$d_6$.**

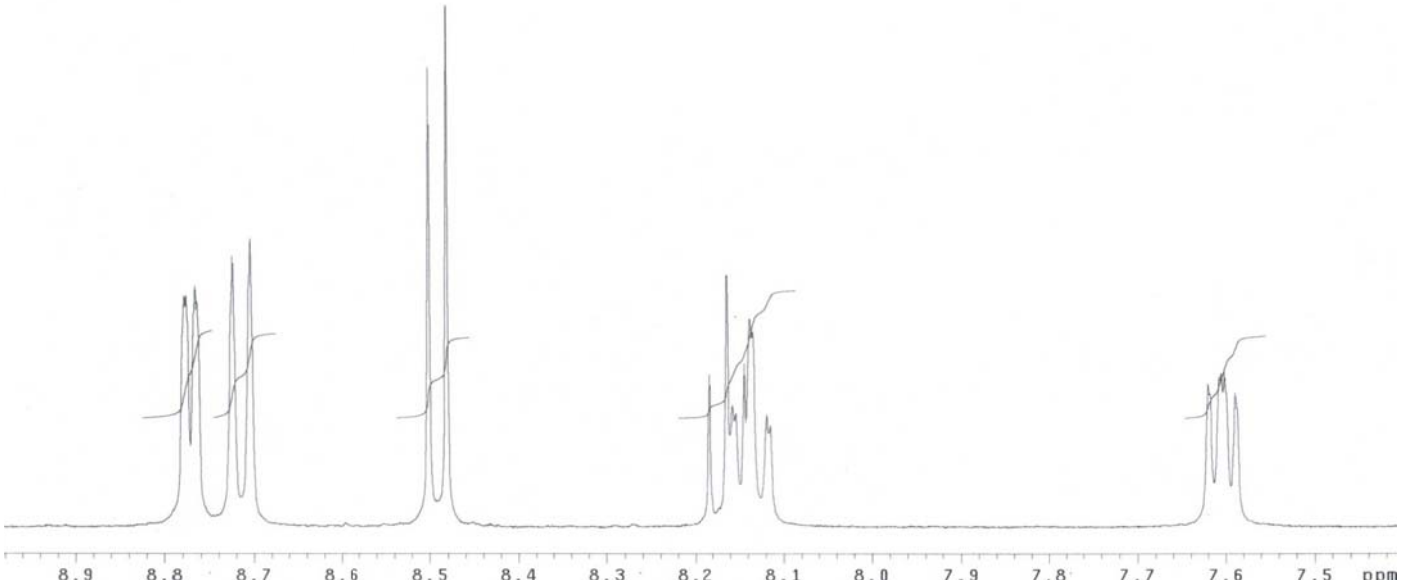
DMSO

Pulse Sequence: s2pul

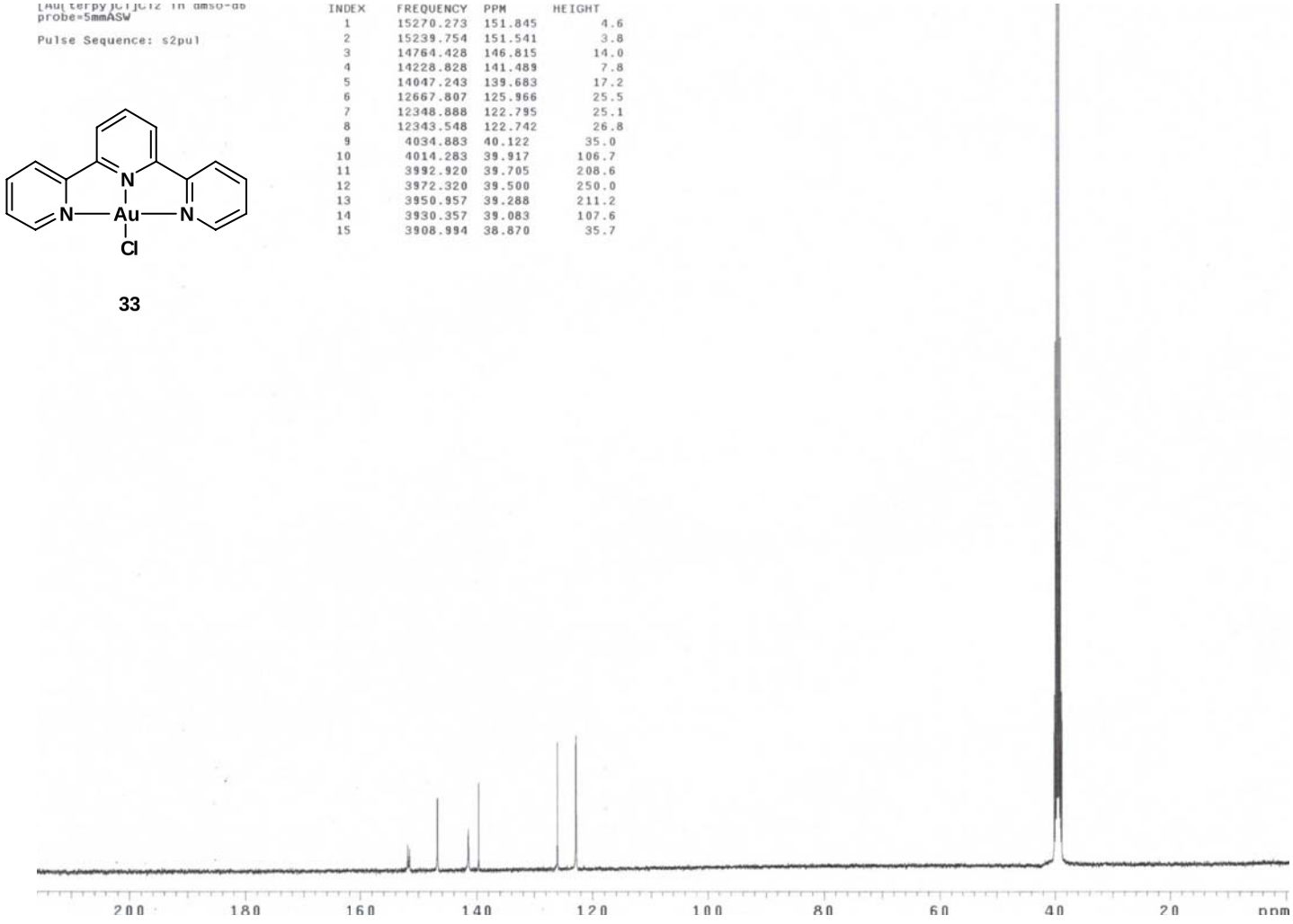



33

| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 3510.950 | 8.779 | 42.1 |
| 2 | 3510.034 | 8.776 | 42.3 |
| 3 | 3506.005 | 8.766 | 44.0 |
| 4 | 3505.273 | 8.764 | 41.1 |
| 5 | 3489.157 | 8.724 | 49.5 |
| 6 | 3481.100 | 8.704 | 52.8 |
| 7 | 3399.973 | 8.501 | 84.0 |
| 8 | 3392.099 | 8.482 | 95.4 |
| 9 | 3272.882 | 8.183 | 27.9 |
| 10 | 3265.007 | 8.164 | 46.0 |
| 11 | 3262.443 | 8.157 | 22.3 |
| 12 | 3260.978 | 8.154 | 20.8 |
| 13 | 3257.132 | 8.144 | 30.0 |
| 14 | 3254.752 | 8.138 | 38.2 |
| 15 | 3253.287 | 8.134 | 35.7 |
| 16 | 3247.060 | 8.119 | 20.7 |
| 17 | 3245.412 | 8.115 | 18.7 |
| 18 | 3047.632 | 7.620 | 26.2 |
| 19 | 3042.688 | 7.608 | 27.6 |
| 20 | 3041.772 | 7.606 | 28.2 |
| 21 | 3040.124 | 7.601 | 28.4 |
| 22 | 3035.180 | 7.589 | 24.6 |

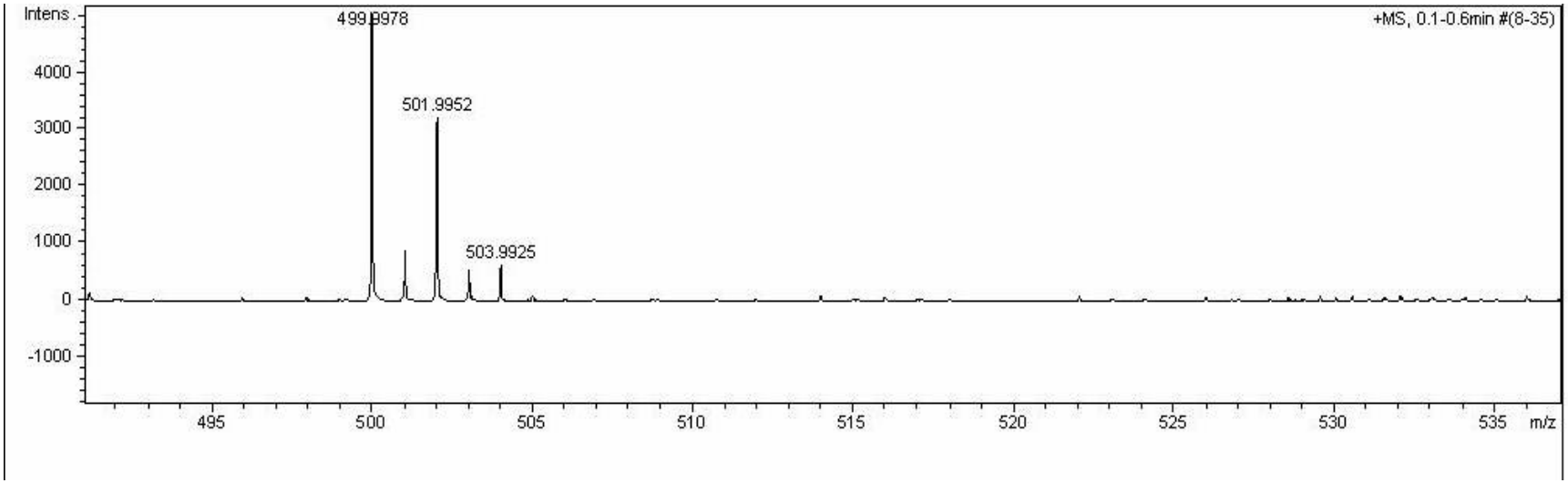


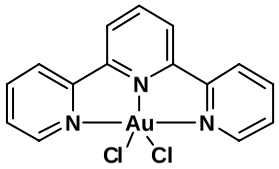

**Spectrum 8 Expanded $^{1}$H NMR spectrum of 33 in DMSO-$d_6$**



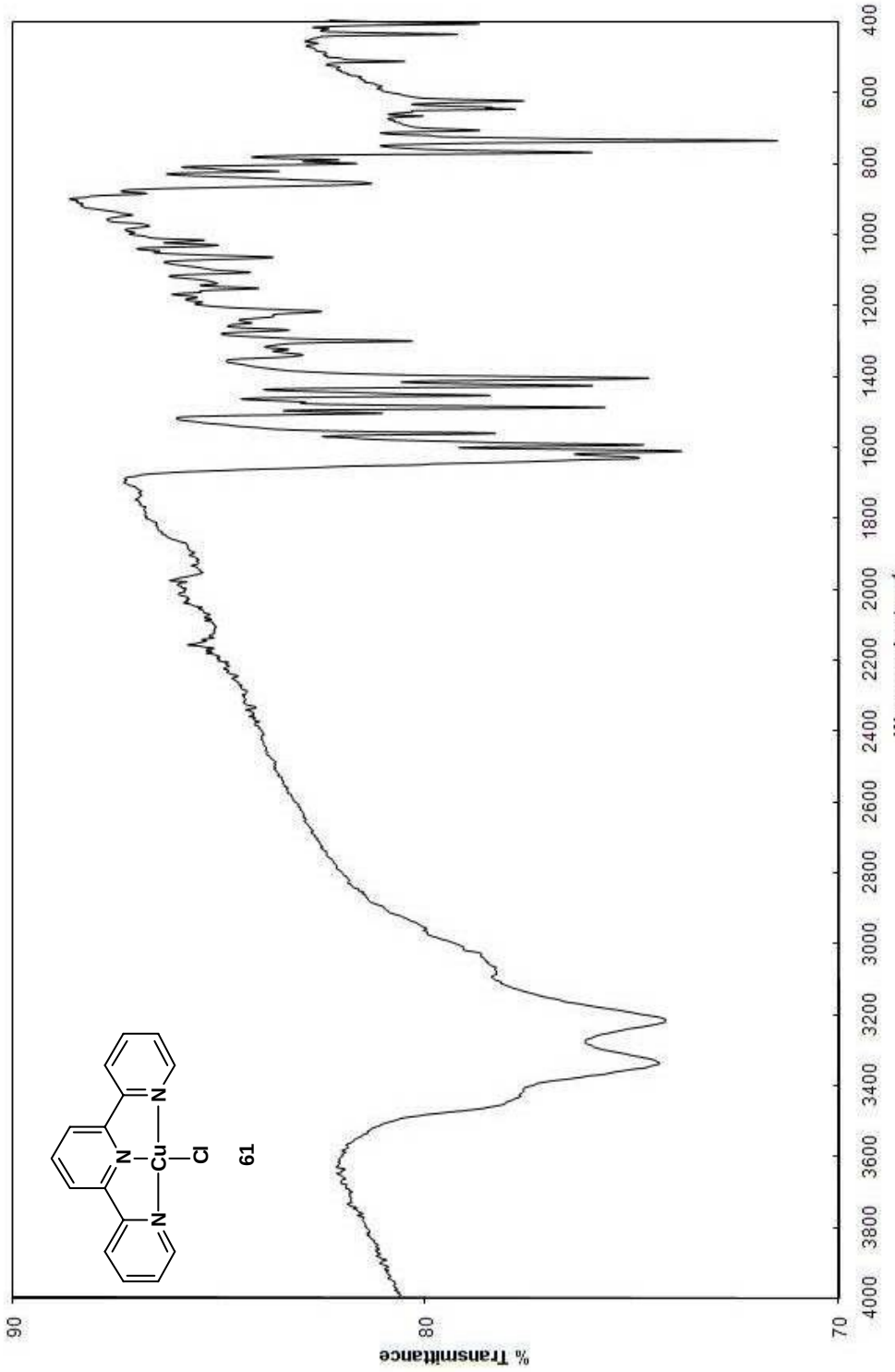

**33**

**Spectrum 9 $^{13}$C NMR spectrum of 33 in DMSO-$d_6$.**

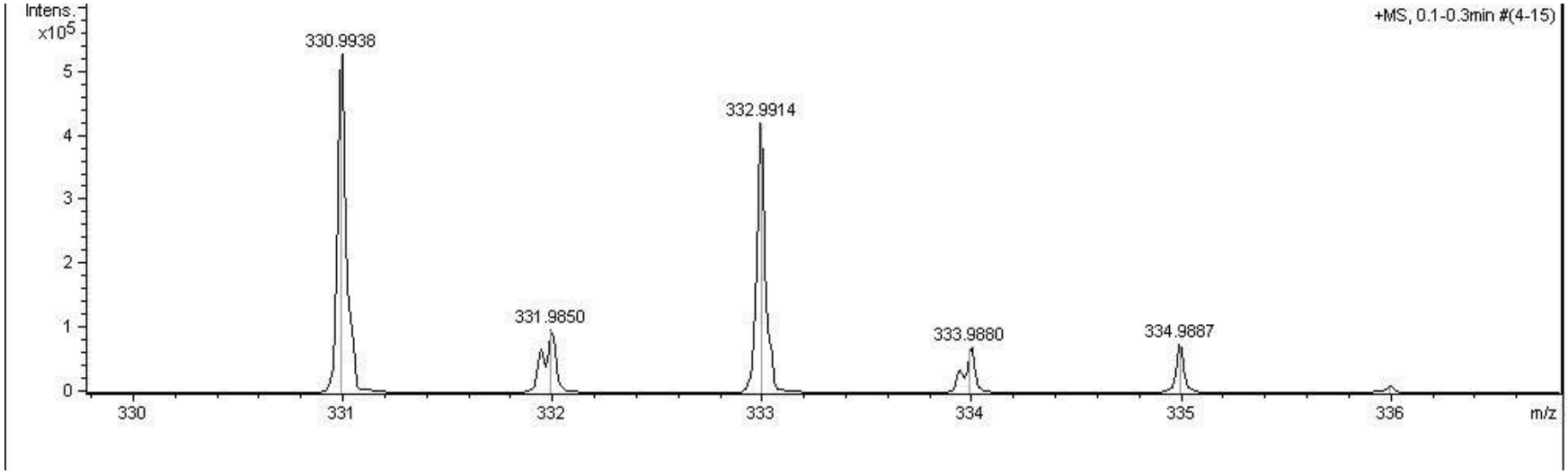


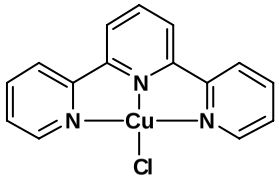




**Spectrum 10 MS isotopic pattern for Au(terpy)$Cl_2^+$.**



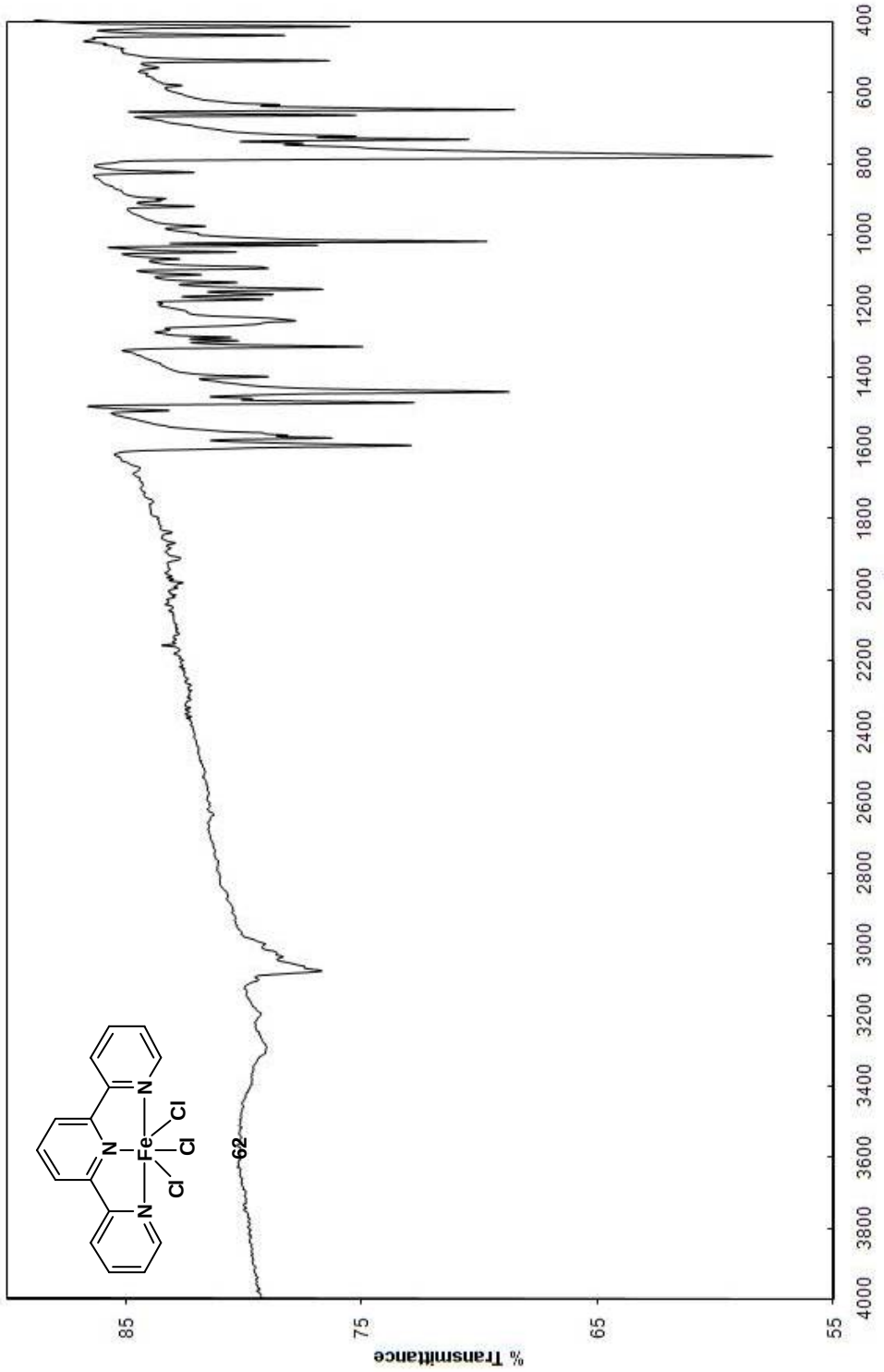

**Spectrum 11 IR spectrum of 61.**



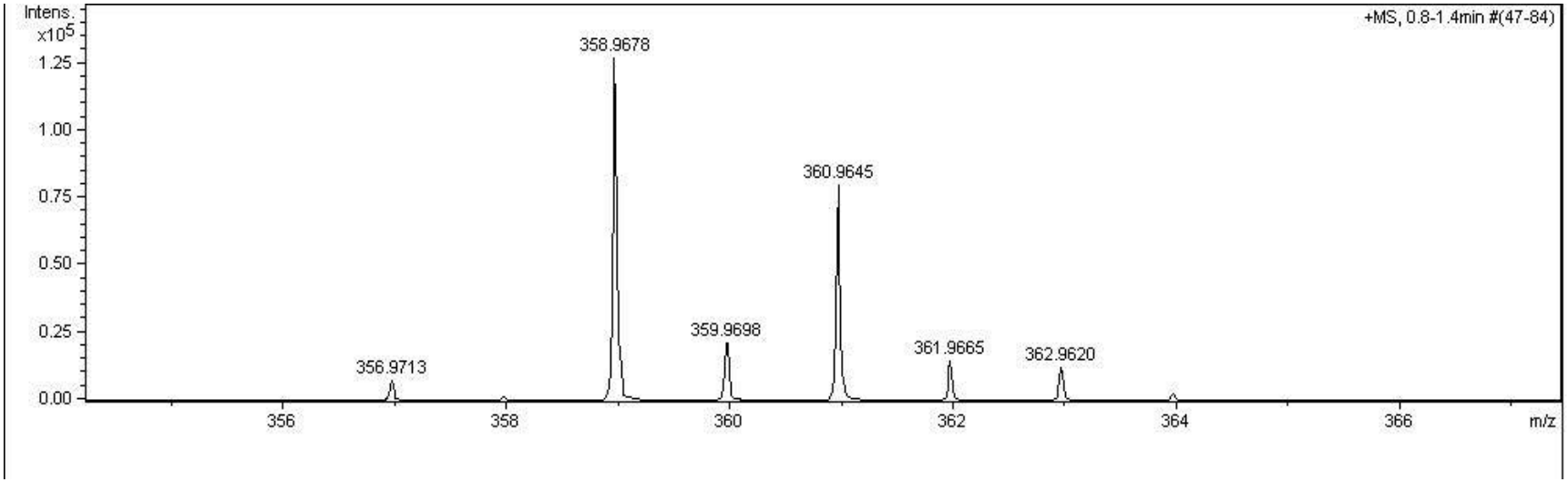



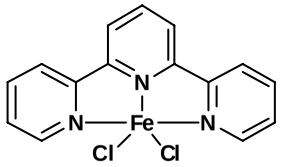

**61**

**Spectrum 12 MS isotopic pattern for Cu(terpy)Cl$^{+}$.**

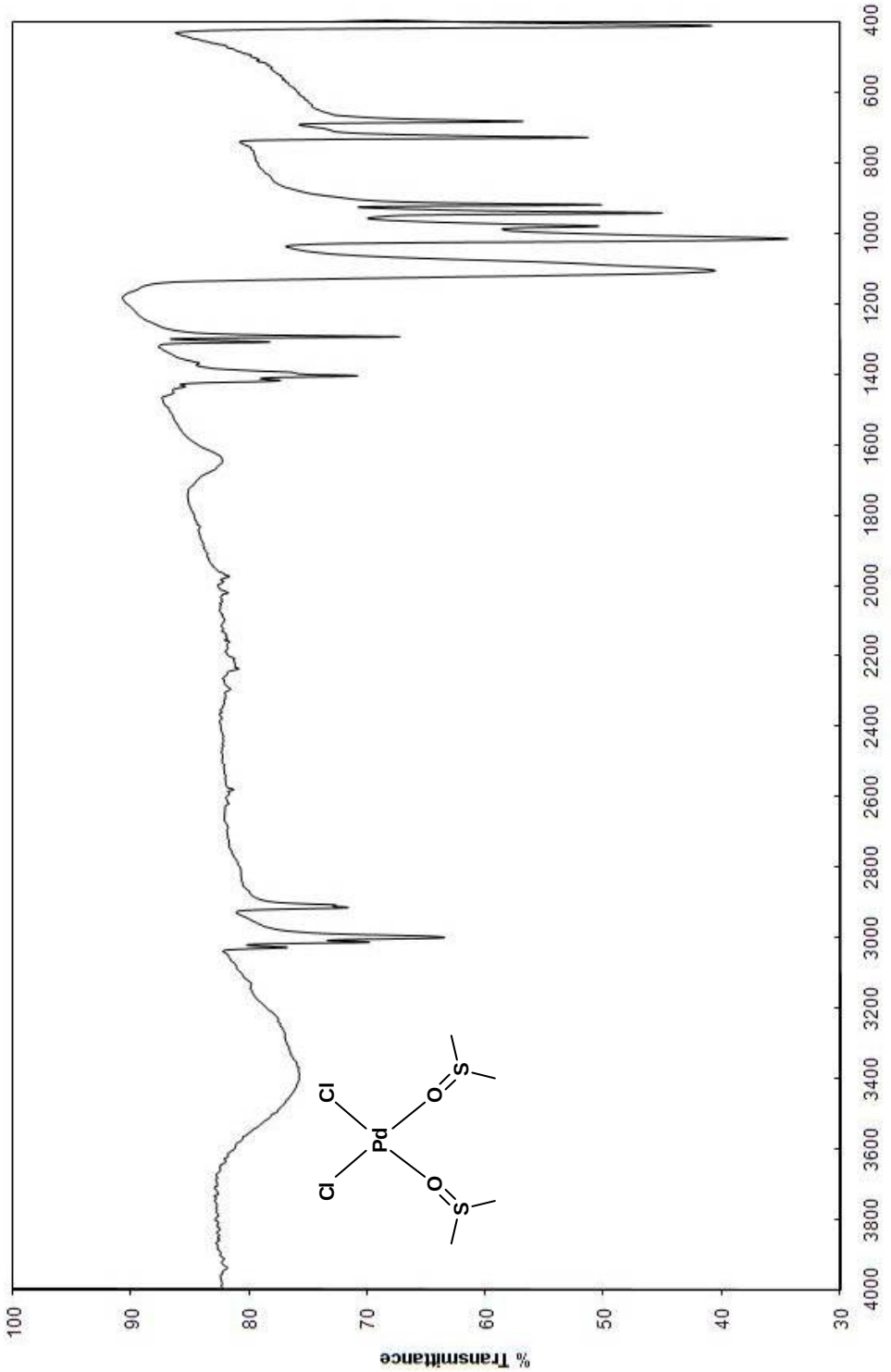



**Spectrum 13 IR spectrum of 62.**

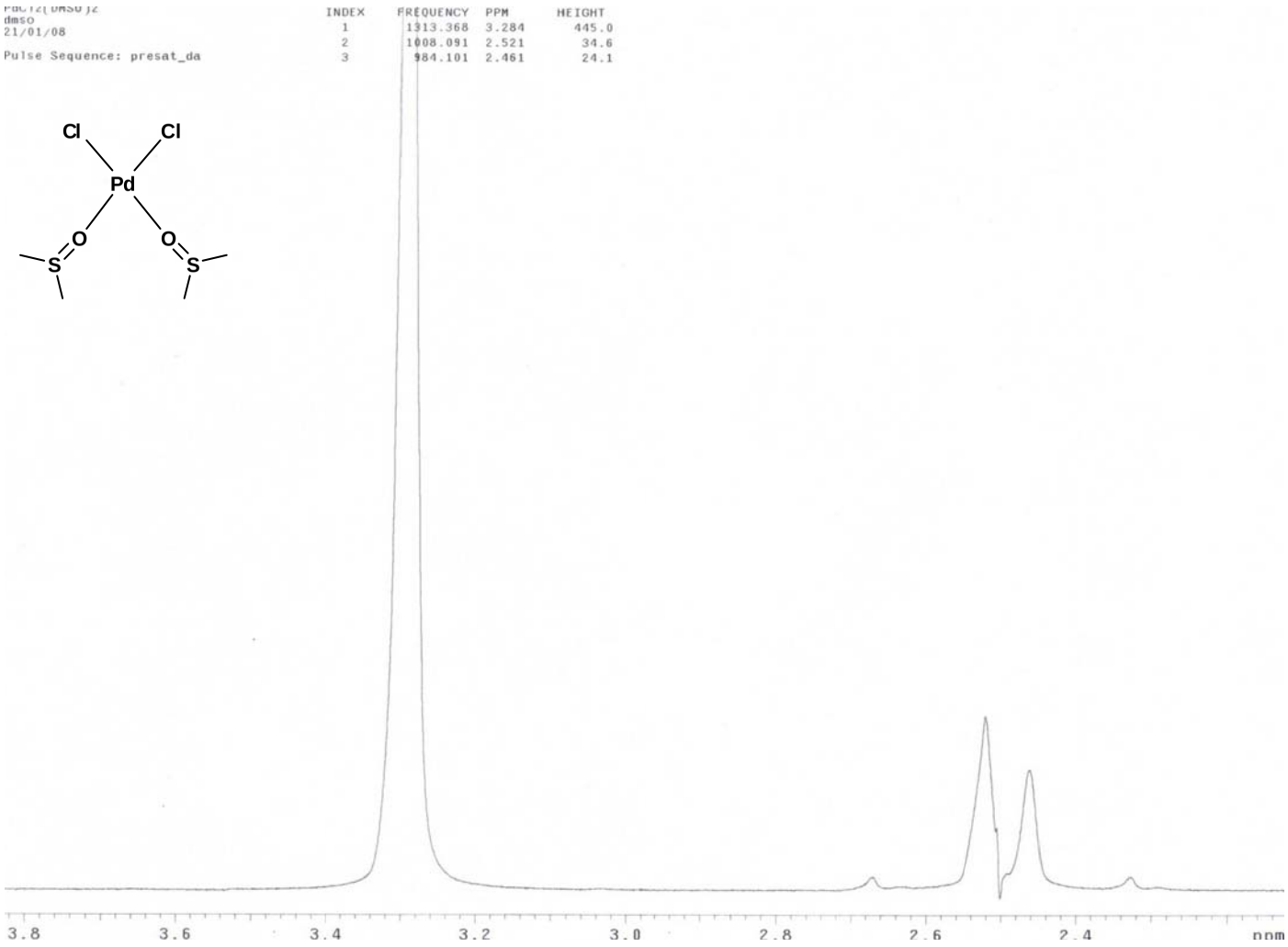


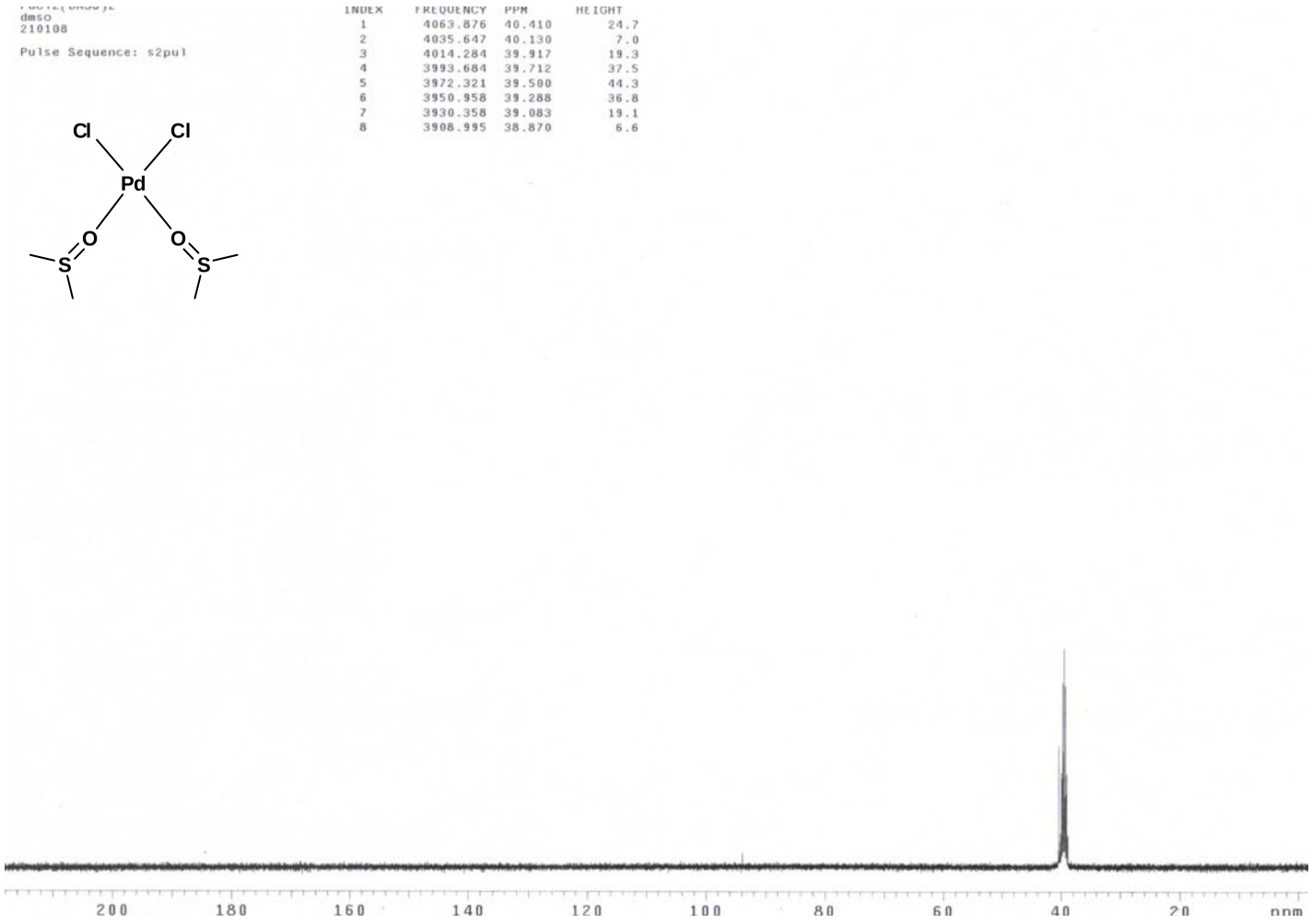

**Spectrum 14 MS isotopic pattern for Fe(terpy)$Cl_2^+$.**



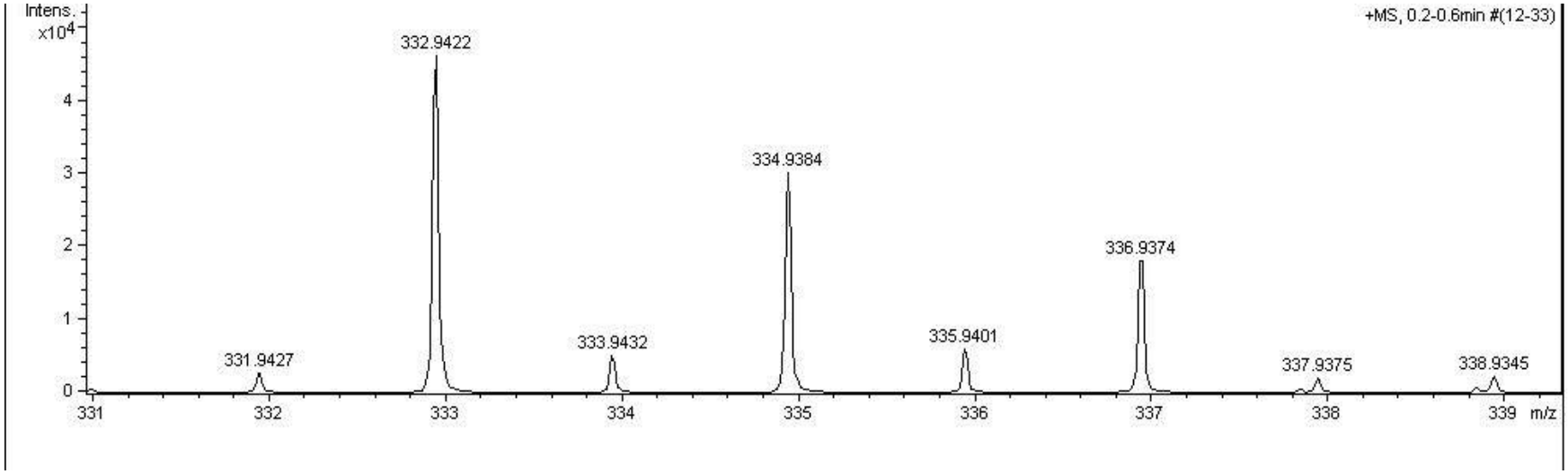

**Spectrum 15 IR spectrum of $PdCl_2(DMSO)_2$.**

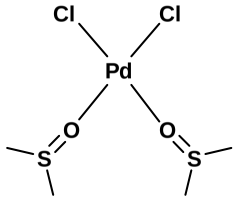



**Spectrum 16 $^{1}$H NMR spectrum of $PdCl_2(DMSO)_2$ in DMSO-$d_6$.**

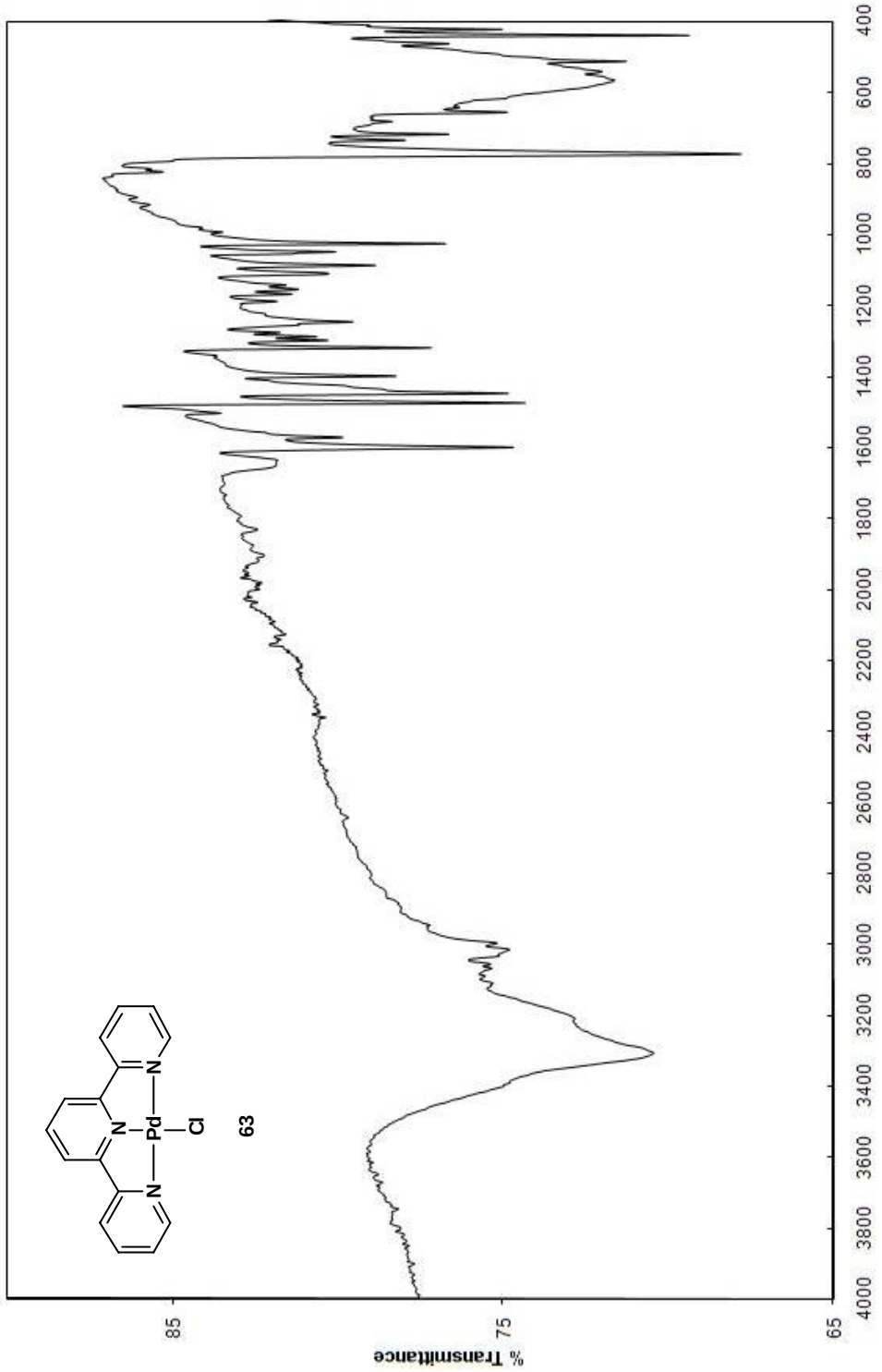



**Spectrum 17 $^{13}$C NMR spectrum of $PdCl_2(DMSO)_2$ in DMSO-$d_6$.**

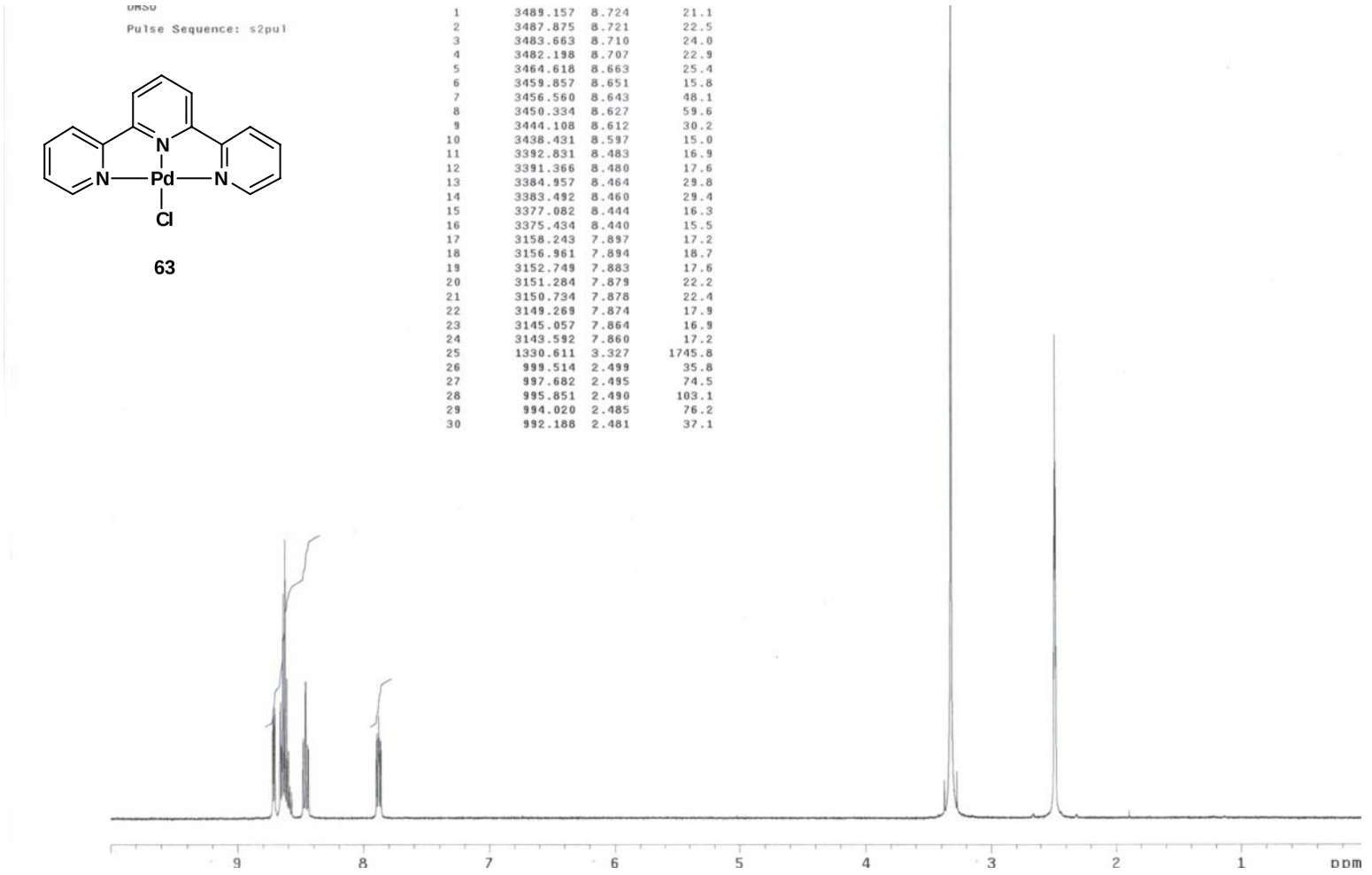



**Spectrum 18 MS isotopic pattern for $PdCl_2(DMSO)_2H^+$.**



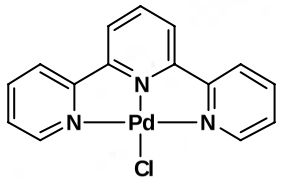

**Spectrum 19 IR spectrum of 63.**




**Spectrum 20 $^{1}$H NMR spectrum of 63 in DMSO-$d_6$.**

DMSO

Pulse Sequence: s2pul

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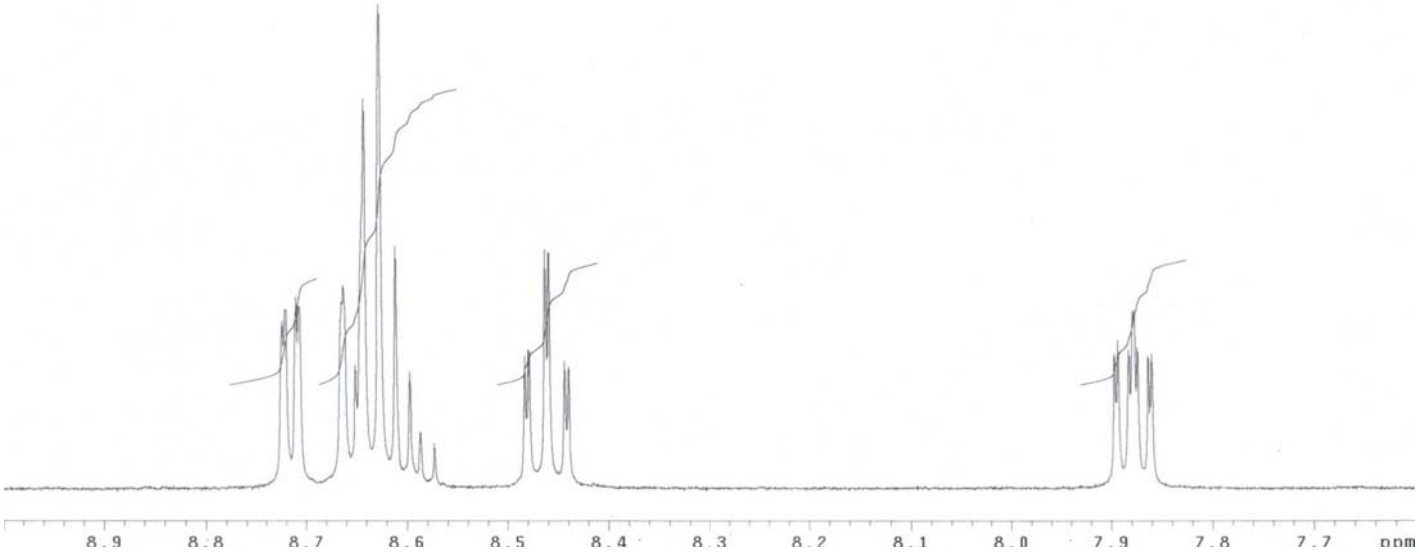
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 3489.157 | 8.724 | 31.7 |
| 2 | 3487.875 | 8.721 | 33.8 |
| 3 | 3483.663 | 8.710 | 36.0 |
| 4 | 3482.198 | 8.707 | 34.3 |
| 5 | 3464.618 | 8.663 | 38.1 |
| 6 | 3459.857 | 8.651 | 23.7 |
| 7 | 3456.560 | 8.643 | 72.1 |
| 8 | 3450.334 | 8.627 | 89.4 |
| 9 | 3444.108 | 8.612 | 45.3 |
| 10 | 3438.431 | 8.597 | 22.4 |
| 11 | 3434.219 | 8.587 | 11.3 |
| 12 | 3428.725 | 8.573 | 9.4 |
| 13 | 3392.831 | 8.483 | 25.3 |
| 14 | 3391.366 | 8.480 | 26.4 |
| 15 | 3384.957 | 8.464 | 44.6 |
| 16 | 3383.492 | 8.460 | 44.1 |
| 17 | 3377.082 | 8.444 | 24.4 |
| 18 | 3375.434 | 8.440 | 23.3 |
| 19 | 3158.243 | 7.897 | 25.7 |
| 20 | 3156.961 | 7.894 | 28.1 |
| 21 | 3152.749 | 7.883 | 26.4 |
| 22 | 3151.284 | 7.879 | 33.3 |
| 23 | 3150.734 | 7.878 | 33.7 |
| 24 | 3149.269 | 7.874 | 26.9 |
| 25 | 3145.057 | 7.864 | 25.4 |
| 26 | 3143.592 | 7.860 | 25.8 |




**Spectrum 21 Expanded $^{1}$H NMR spectrum of 63 in DMSO-d$_6$.**

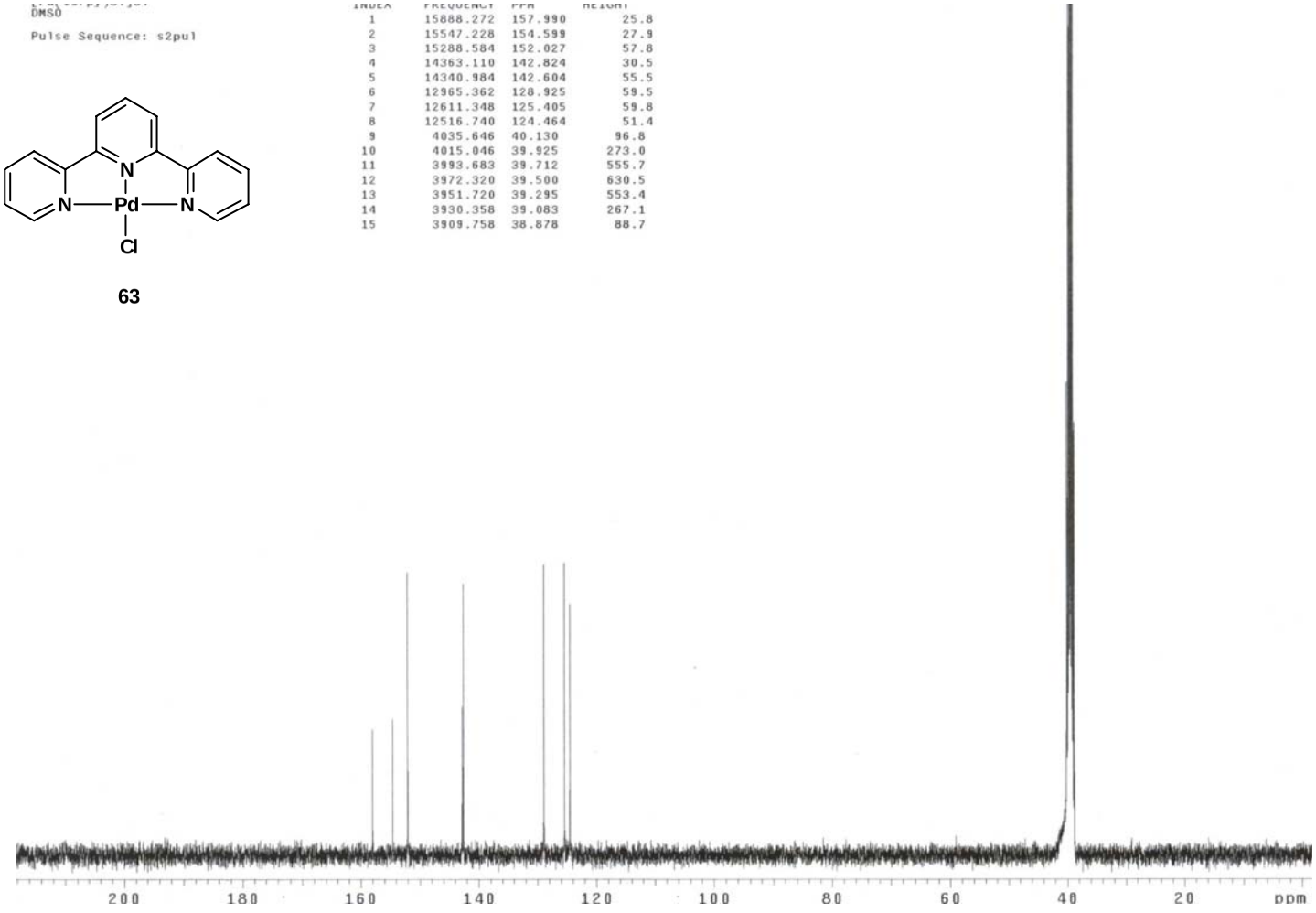
DMSO

Pulse Sequence: s2pul

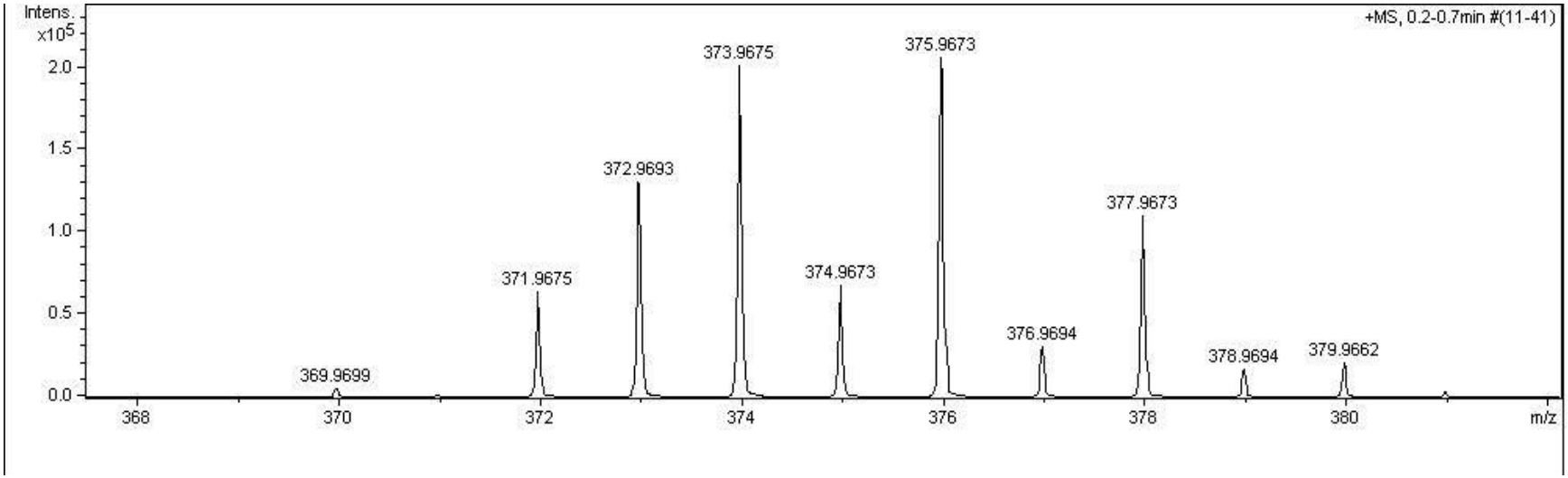


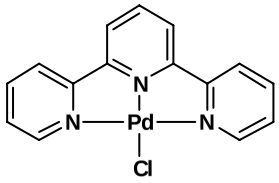

**63**

| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 15888.272 | 157.990 | 25.8 |
| 2 | 15547.228 | 154.599 | 27.9 |
| 3 | 15288.584 | 152.027 | 57.8 |
| 4 | 14363.110 | 142.824 | 30.5 |
| 5 | 14340.984 | 142.604 | 55.5 |
| 6 | 12965.362 | 128.925 | 59.5 |
| 7 | 12611.348 | 125.405 | 59.8 |
| 8 | 12516.740 | 124.464 | 51.4 |
| 9 | 4035.646 | 40.130 | 96.8 |
| 10 | 4015.046 | 39.925 | 273.0 |
| 11 | 3993.683 | 39.712 | 555.7 |
| 12 | 3972.320 | 39.500 | 630.5 |
| 13 | 3951.720 | 39.295 | 553.4 |
| 14 | 3930.358 | 39.083 | 267.1 |
| 15 | 3909.758 | 38.878 | 88.7 |



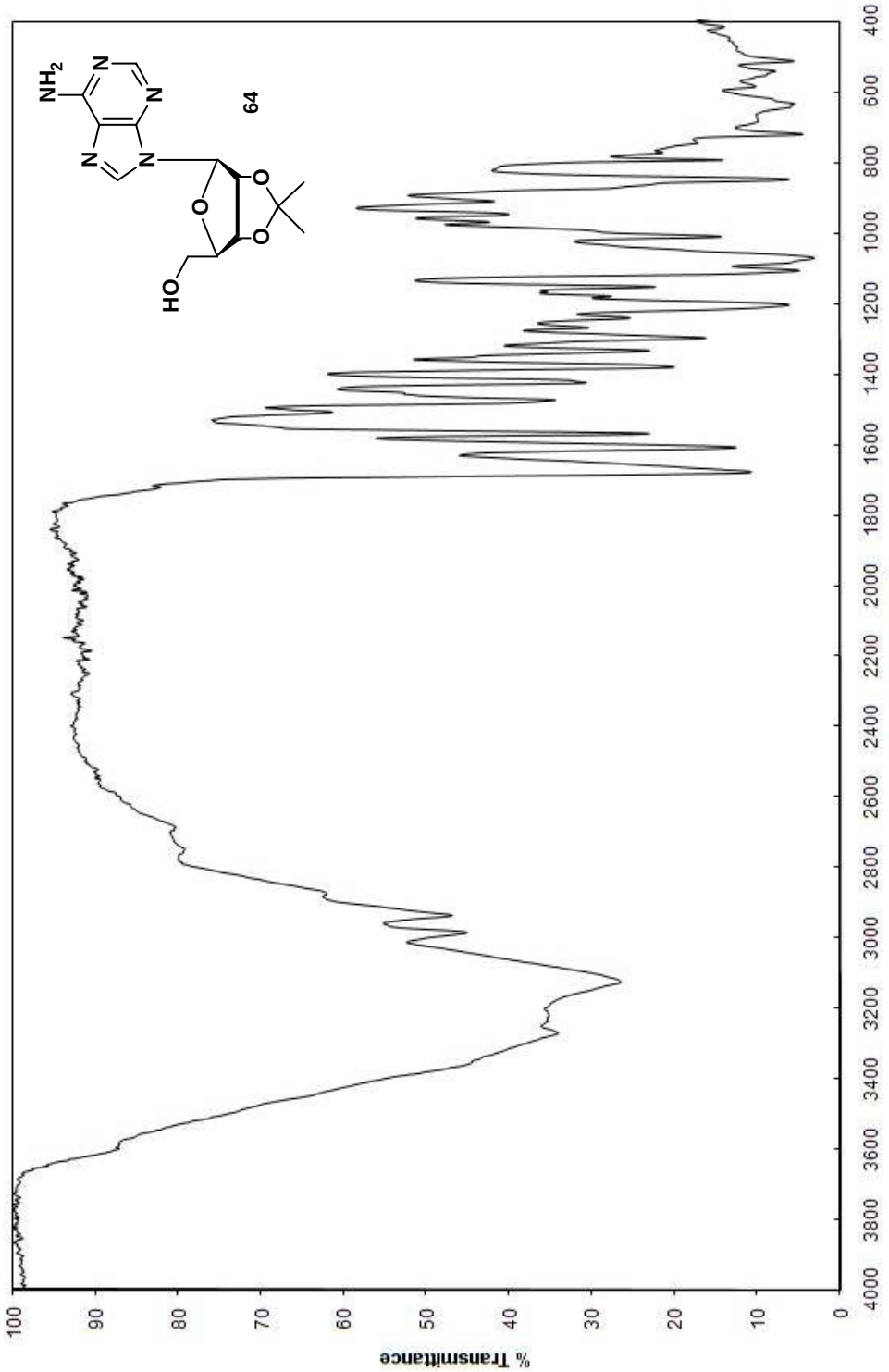

**Spectrum 22 $^{13}$C NMR spectrum of 63 in DMSO-$d_6$.**



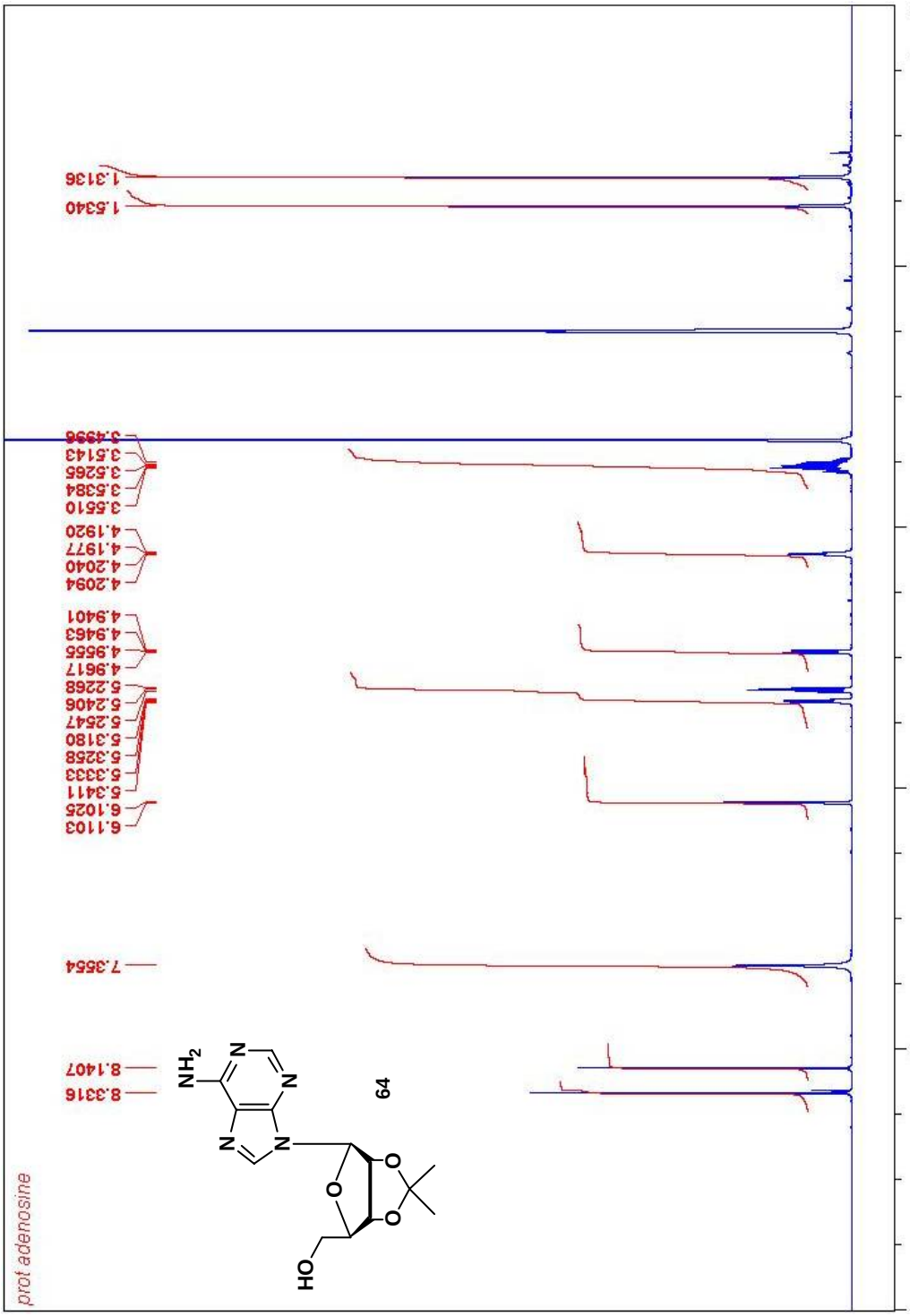




**63**

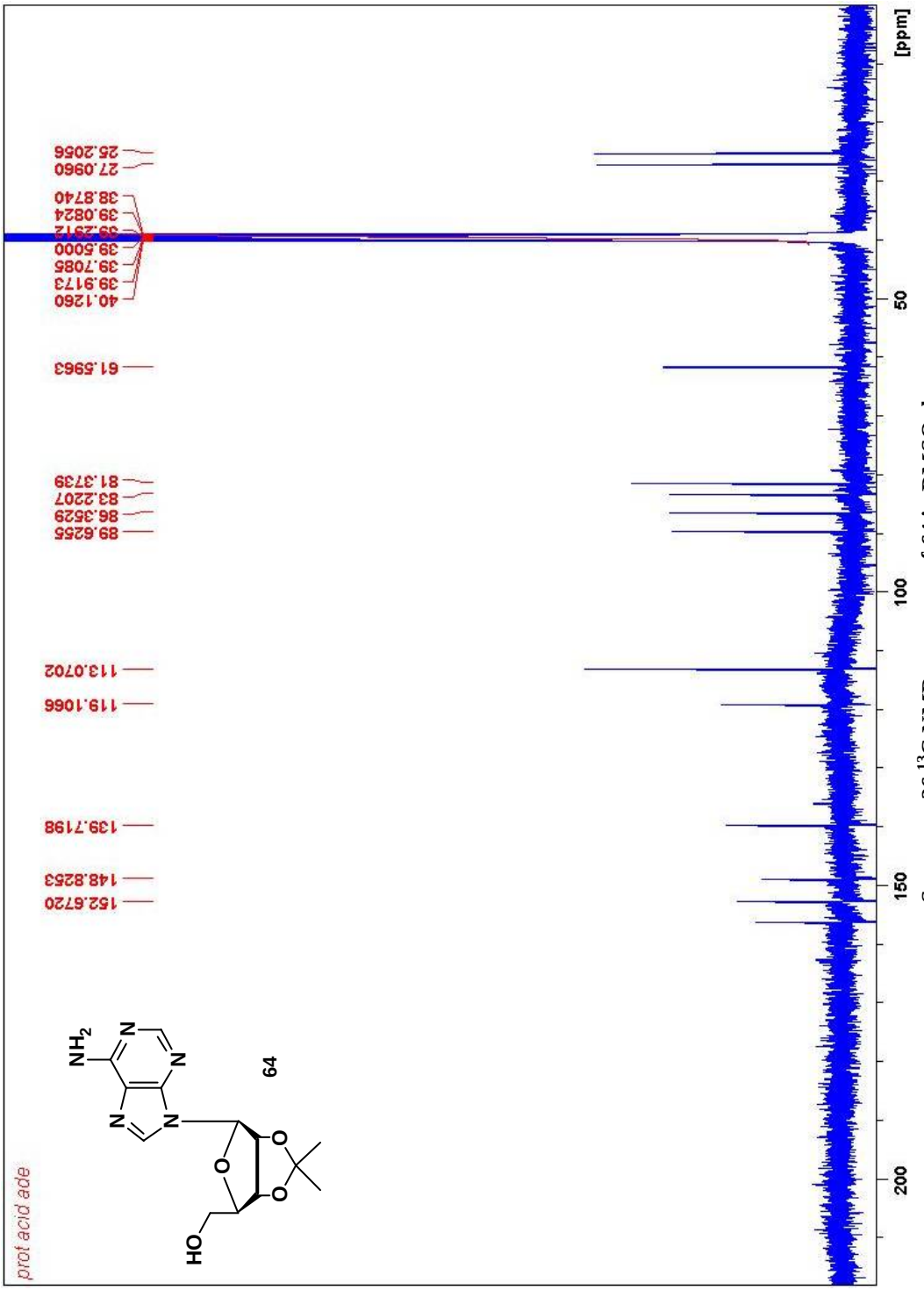
**Spectrum 23 MS isotopic pattern for Pd(terpy)Cl$^{+}$.**



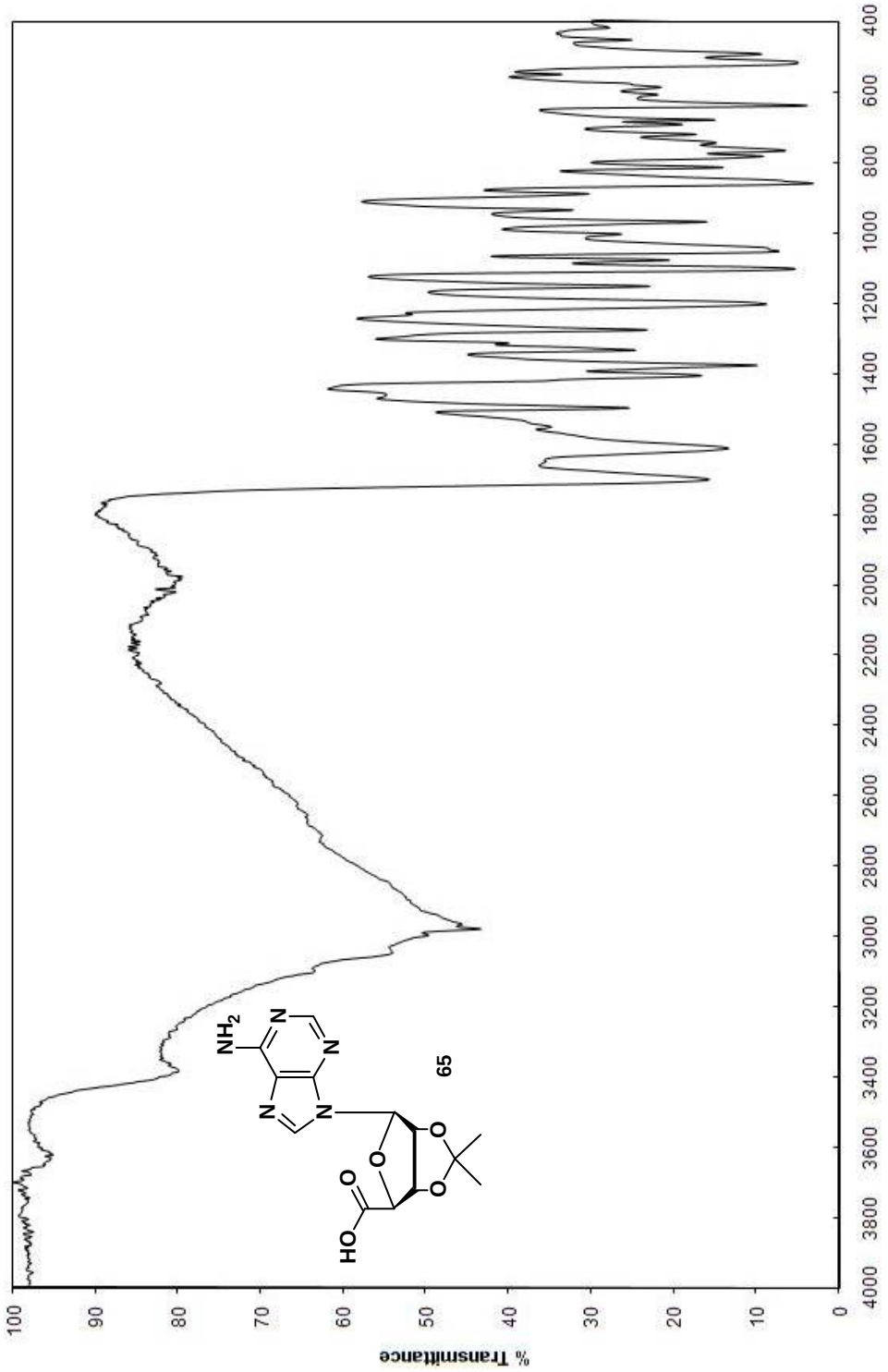

**Spectrum 24 IR spectrum of 64.**



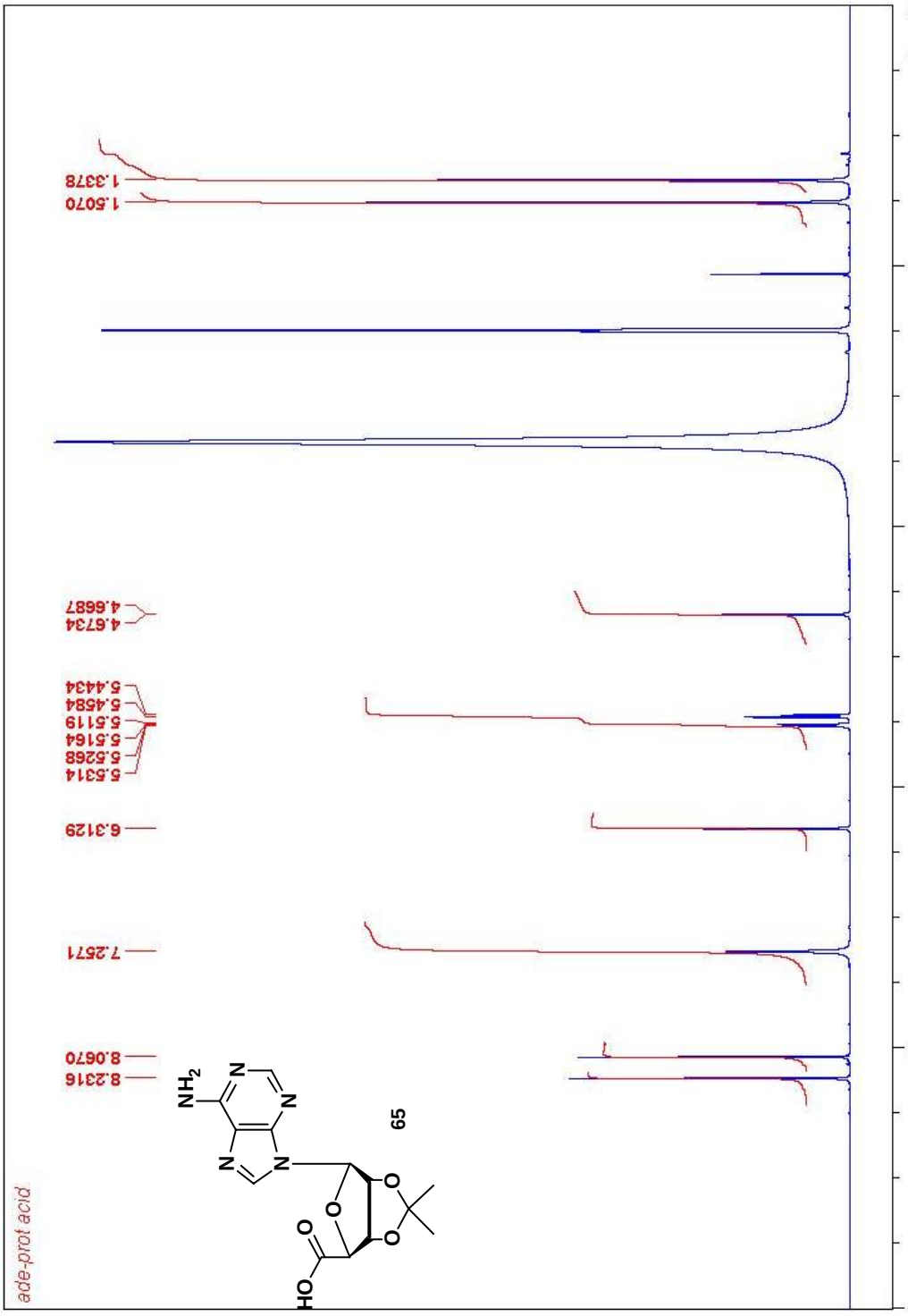

**Spectrum 25 $^{1}$H NMR spectrum of 64 in DMSO-$d_6$.**



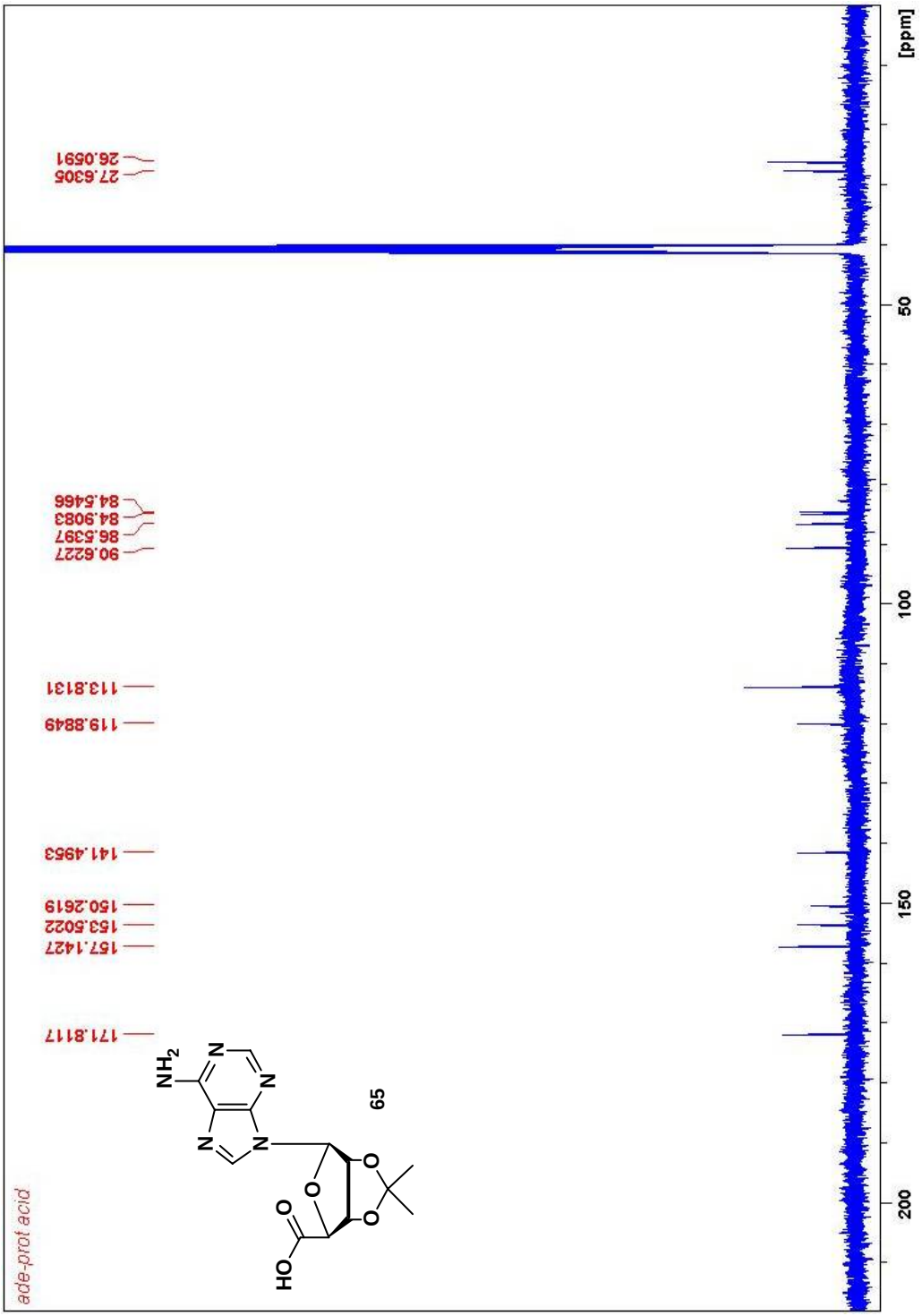

**Spectrum 26 $^{13}$C NMR spectrum of 64 in DMSO-$d_6$.**



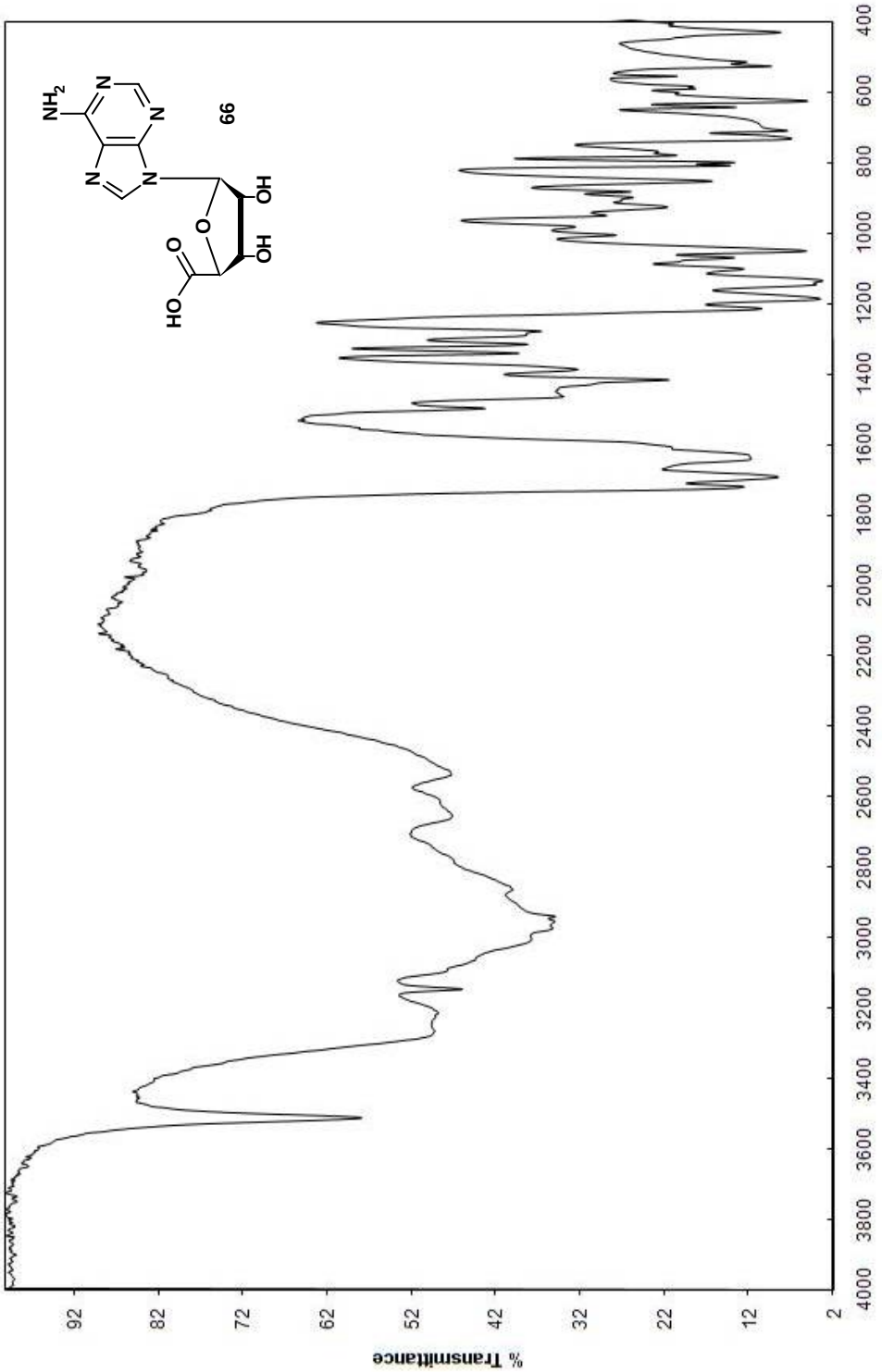

**Spectrum 27 IR spectrum of 65.**



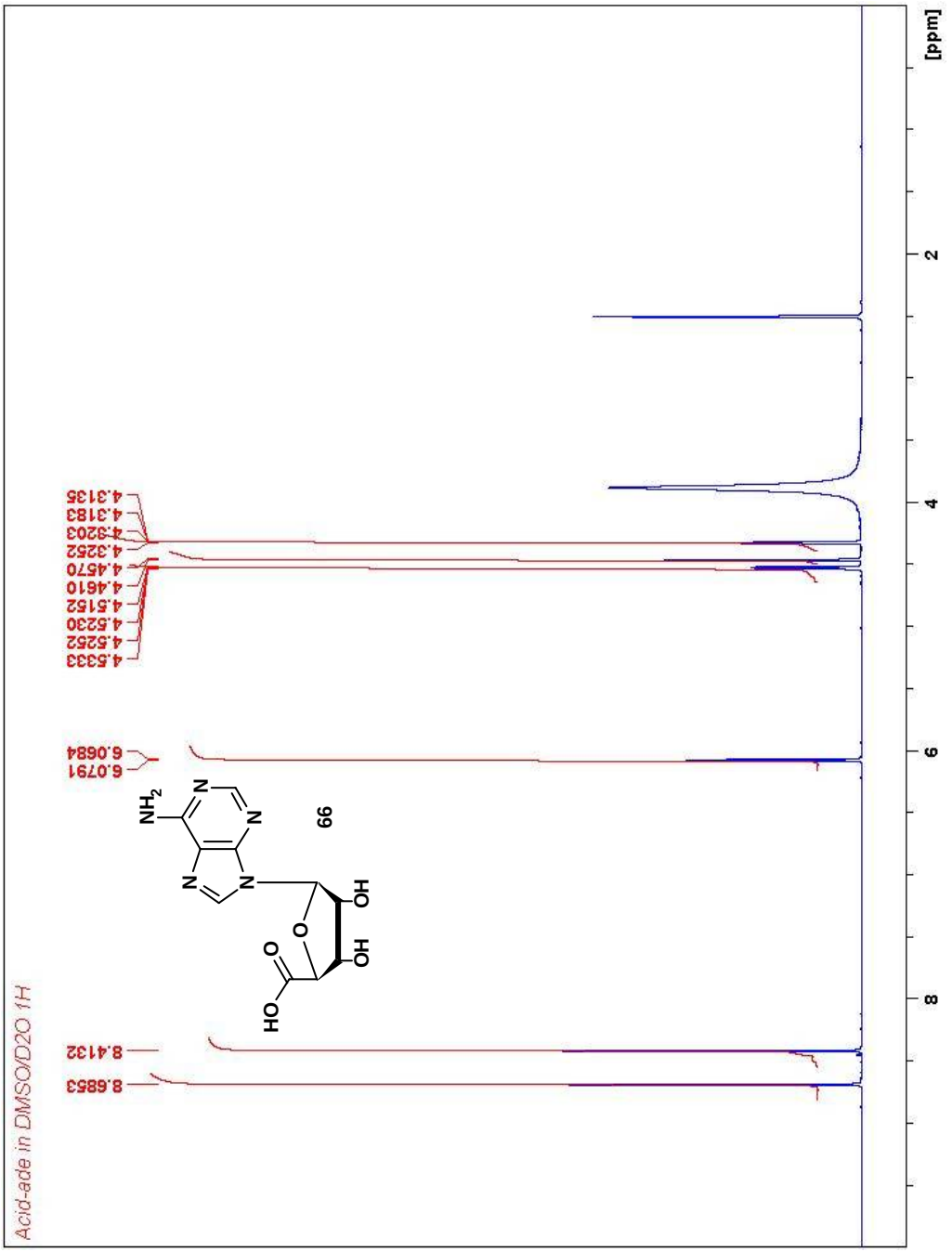

**Spectrum 28 $^{1}$H NMR spectrum of 65 in DMSO-$d_6$.**



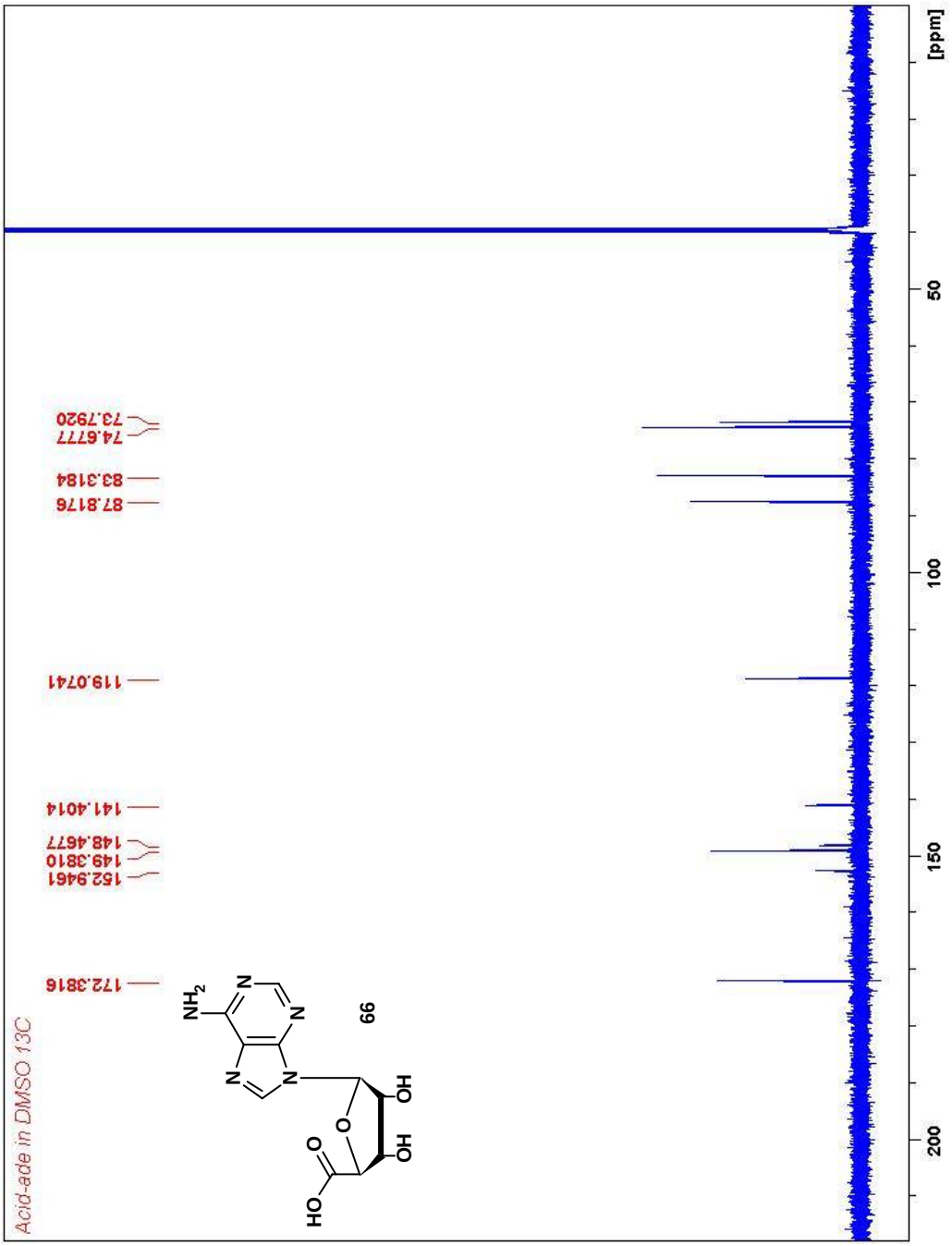

**Spectrum 29 $^{13}$C NMR spectrum of 65 in DMSO-$d_6$.**



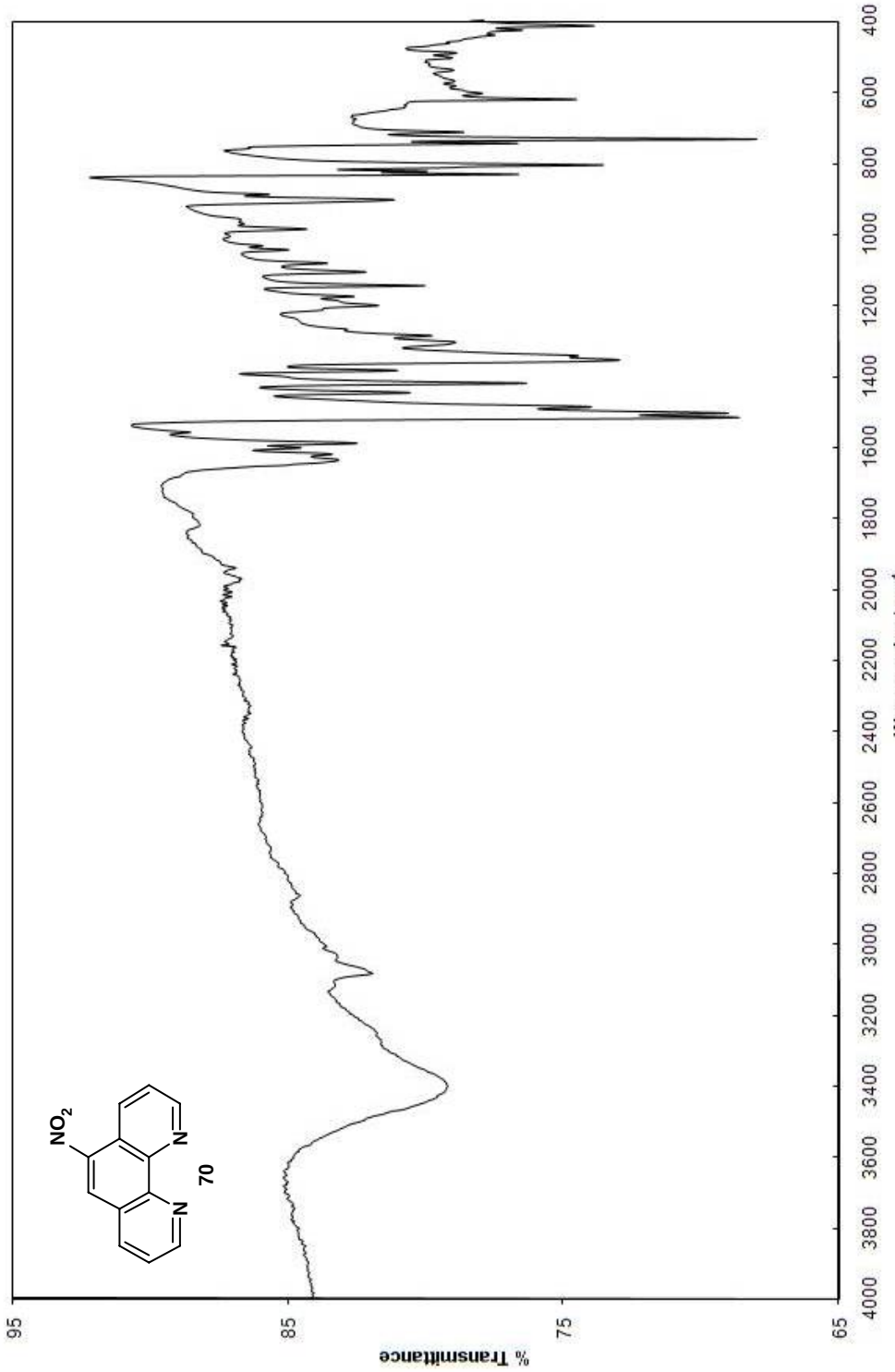

**Spectrum 30 IR spectrum of 66.**



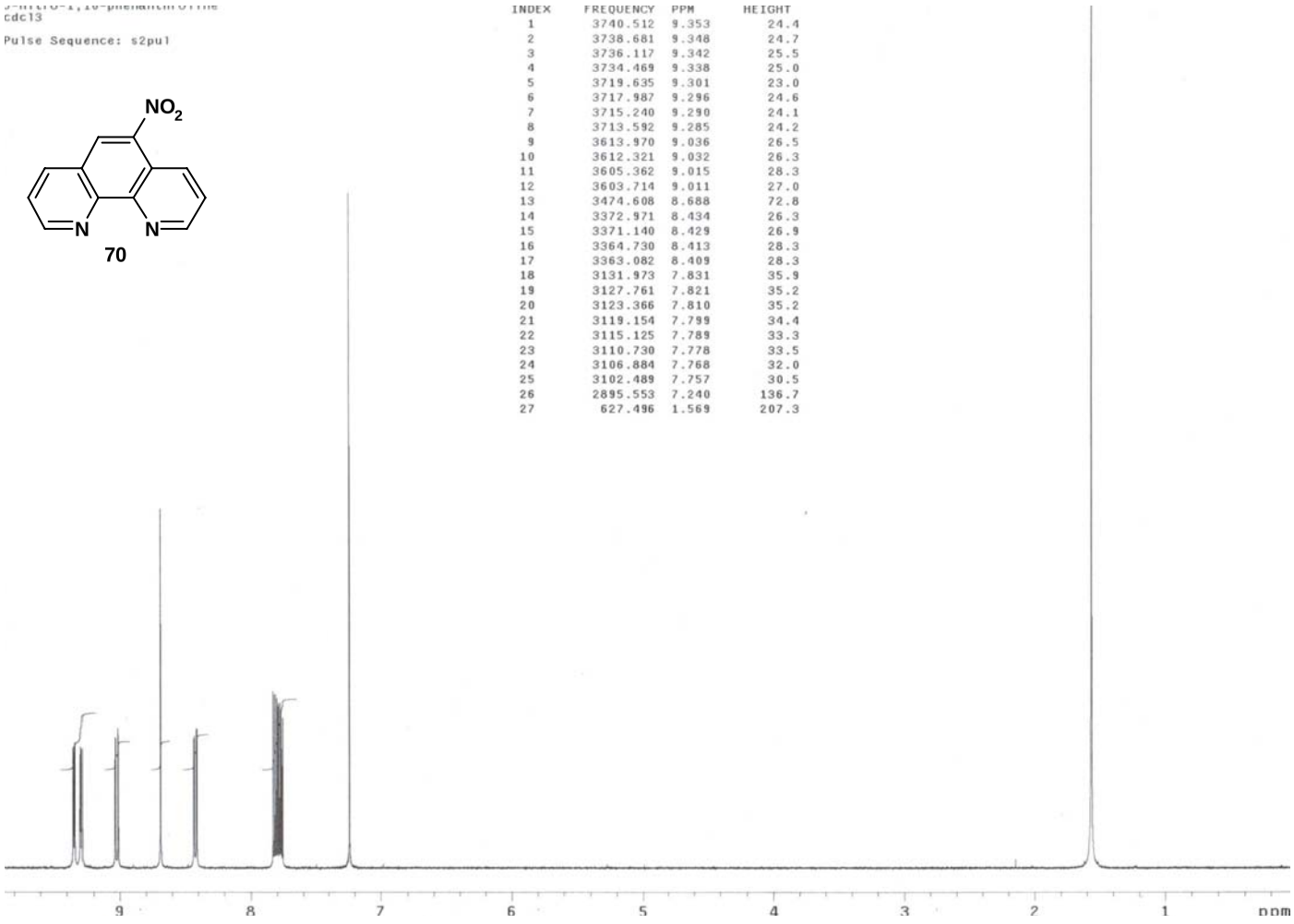

**Spectrum 31 $^{1}$H NMR spectrum of 66 in DMSO-$d_6$ with $D_2O$.**



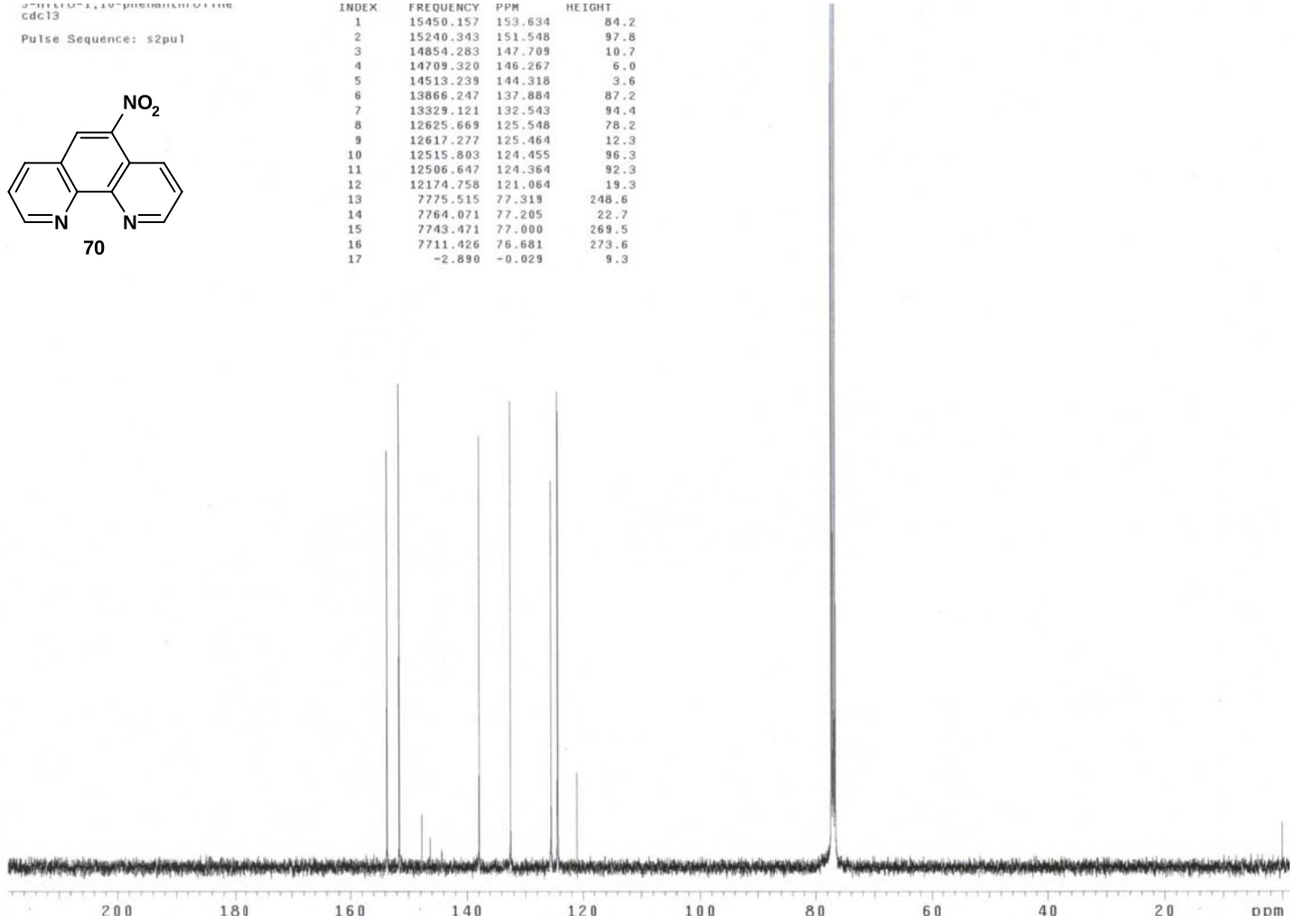

**Spectrum 32 $^{13}$C NMR spectrum of 66 in DMSO-$d_6$.**



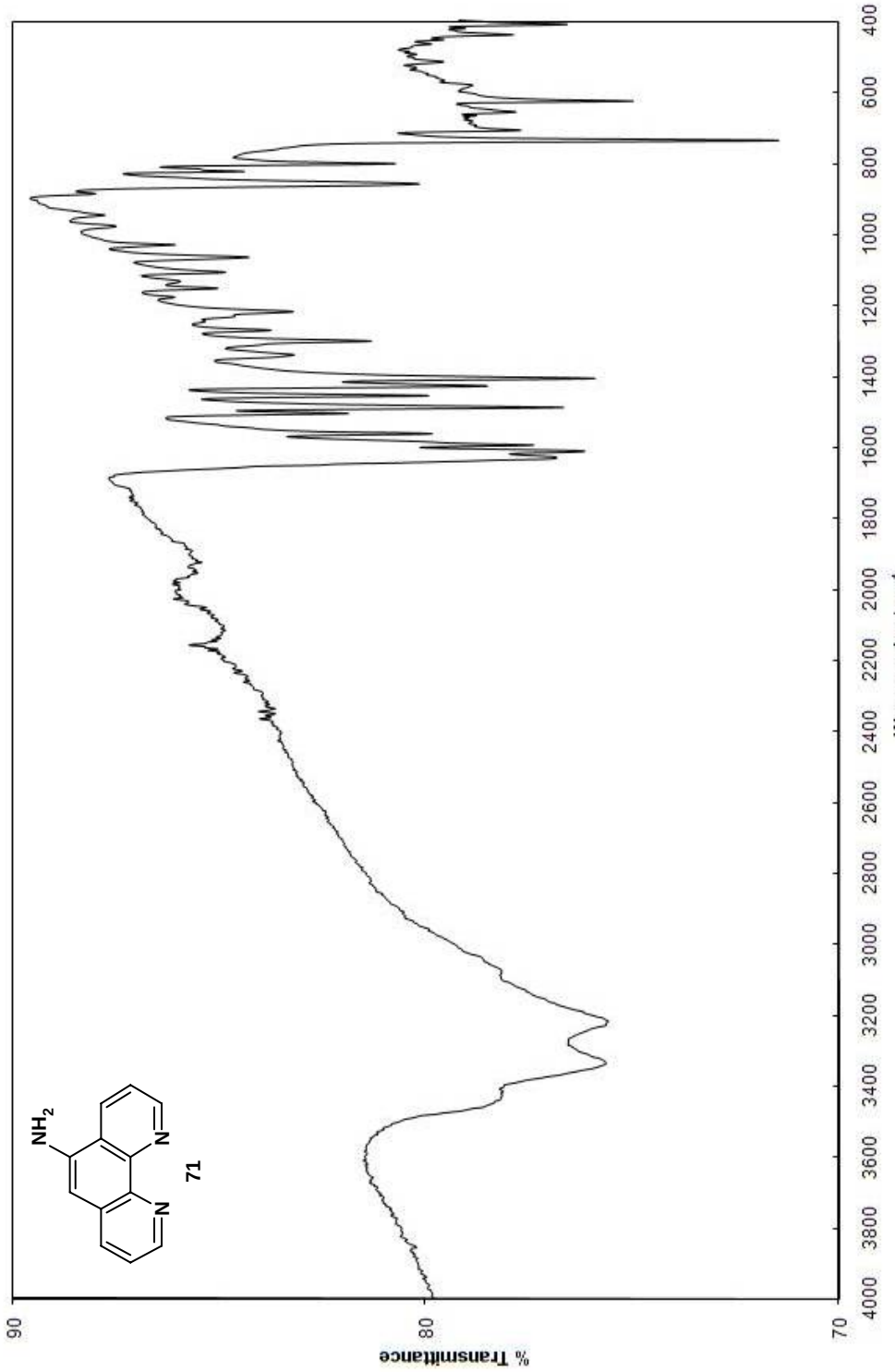

**Spectrum 33 IR spectrum of 70.**



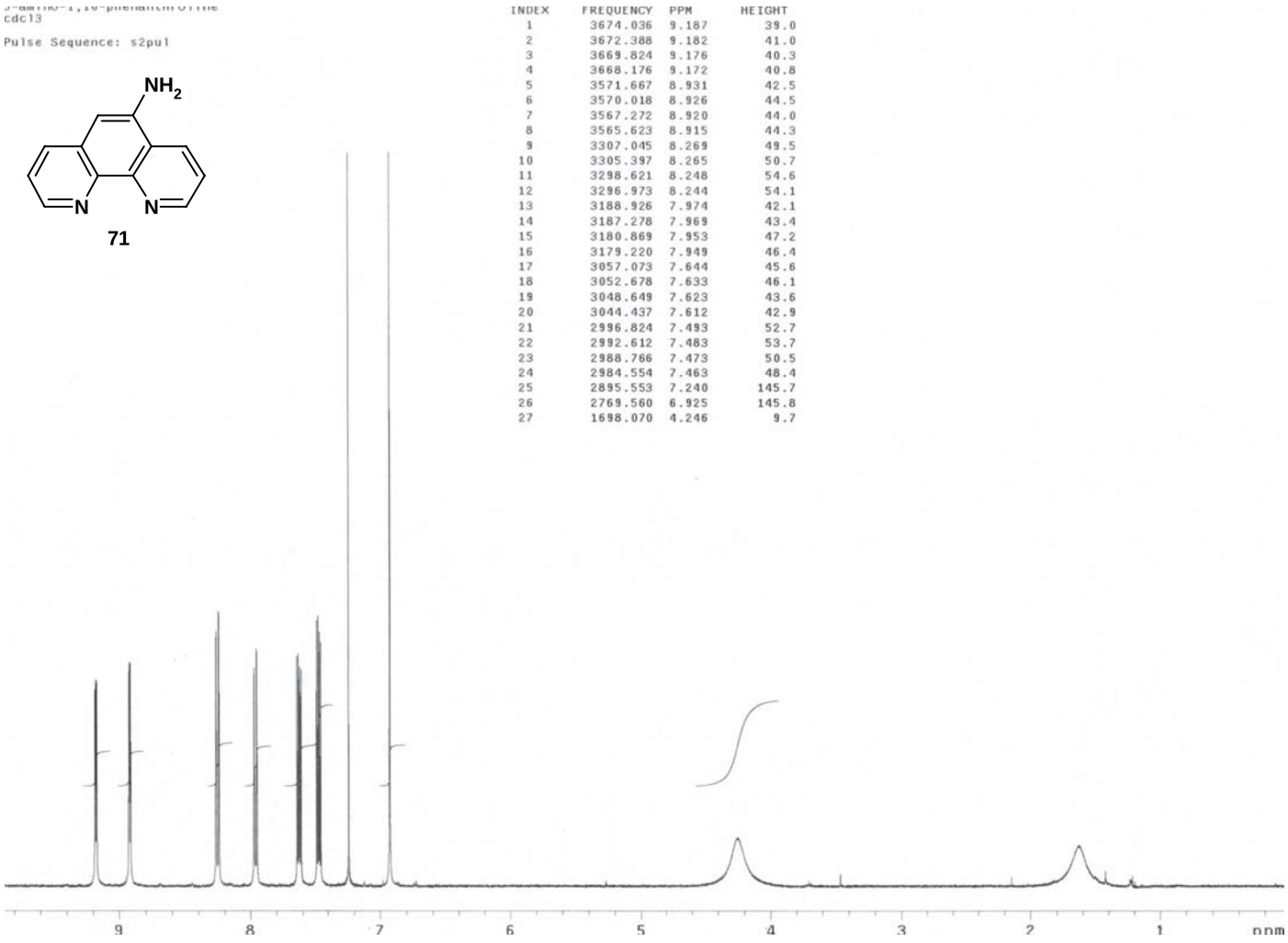

**Spectrum 34 $^{1}$H NMR spectrum of 70 in $CDCl_3$.**




**Spectrum 35 $^{13}C$ NMR spectrum of 70 in $CDCl_3$.**



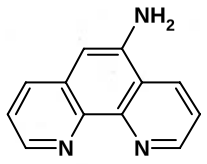

**Spectrum 36 IR spectrum of 71.**




**Spectrum 37 $^{1}$H NMR spectrum of 71 in $CDCl_3$.**

5-amino-1,10-phenanthroline
cdcl3

Pulse Sequence: s2pul

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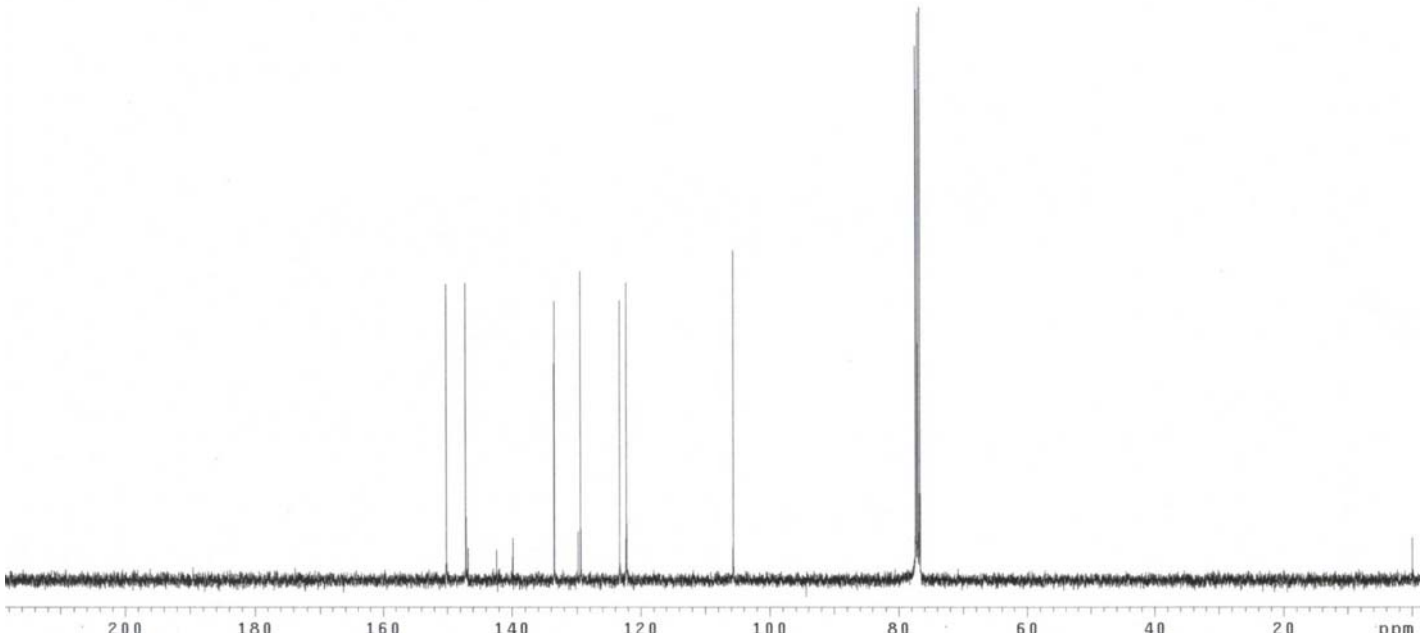
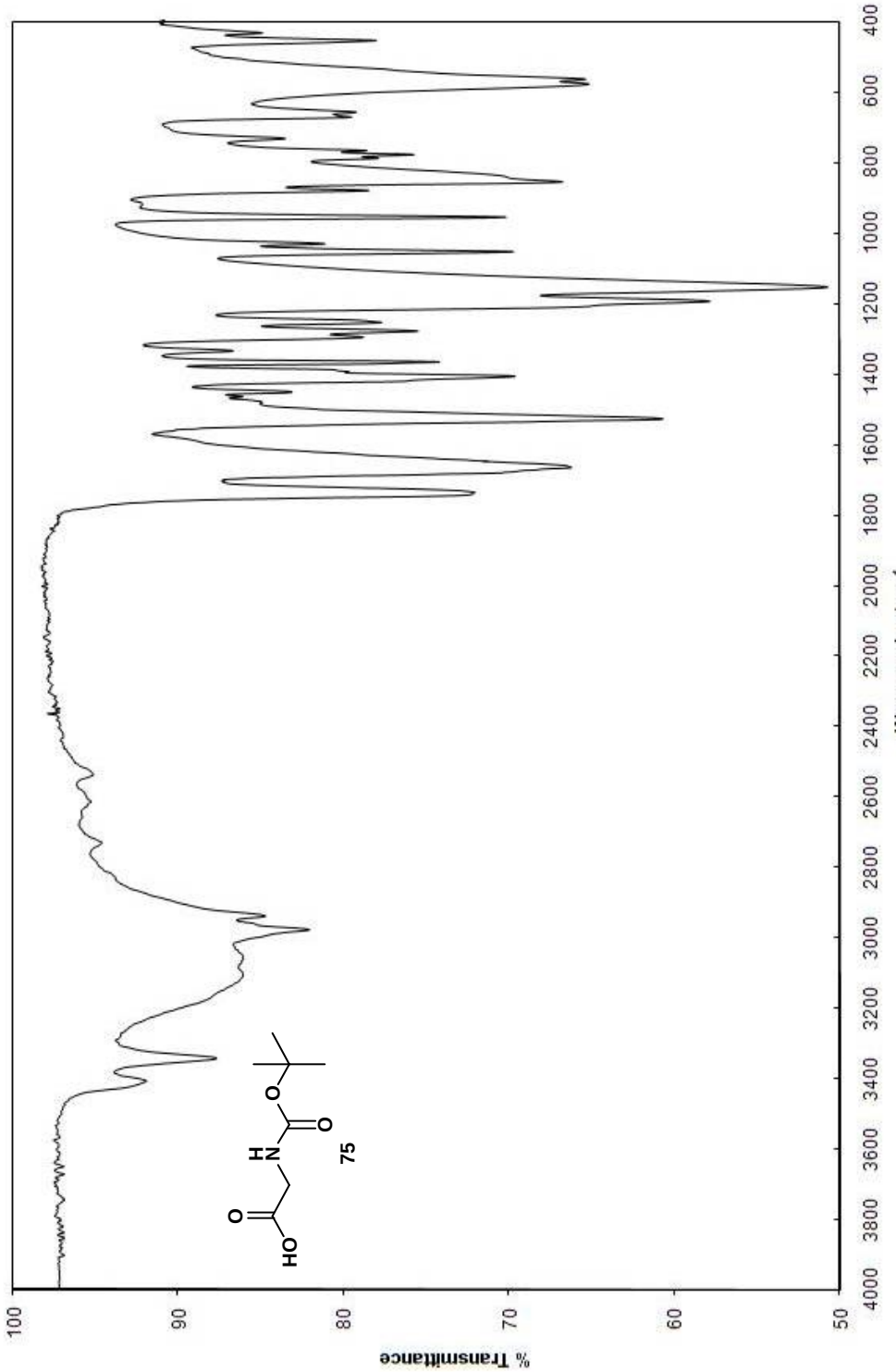
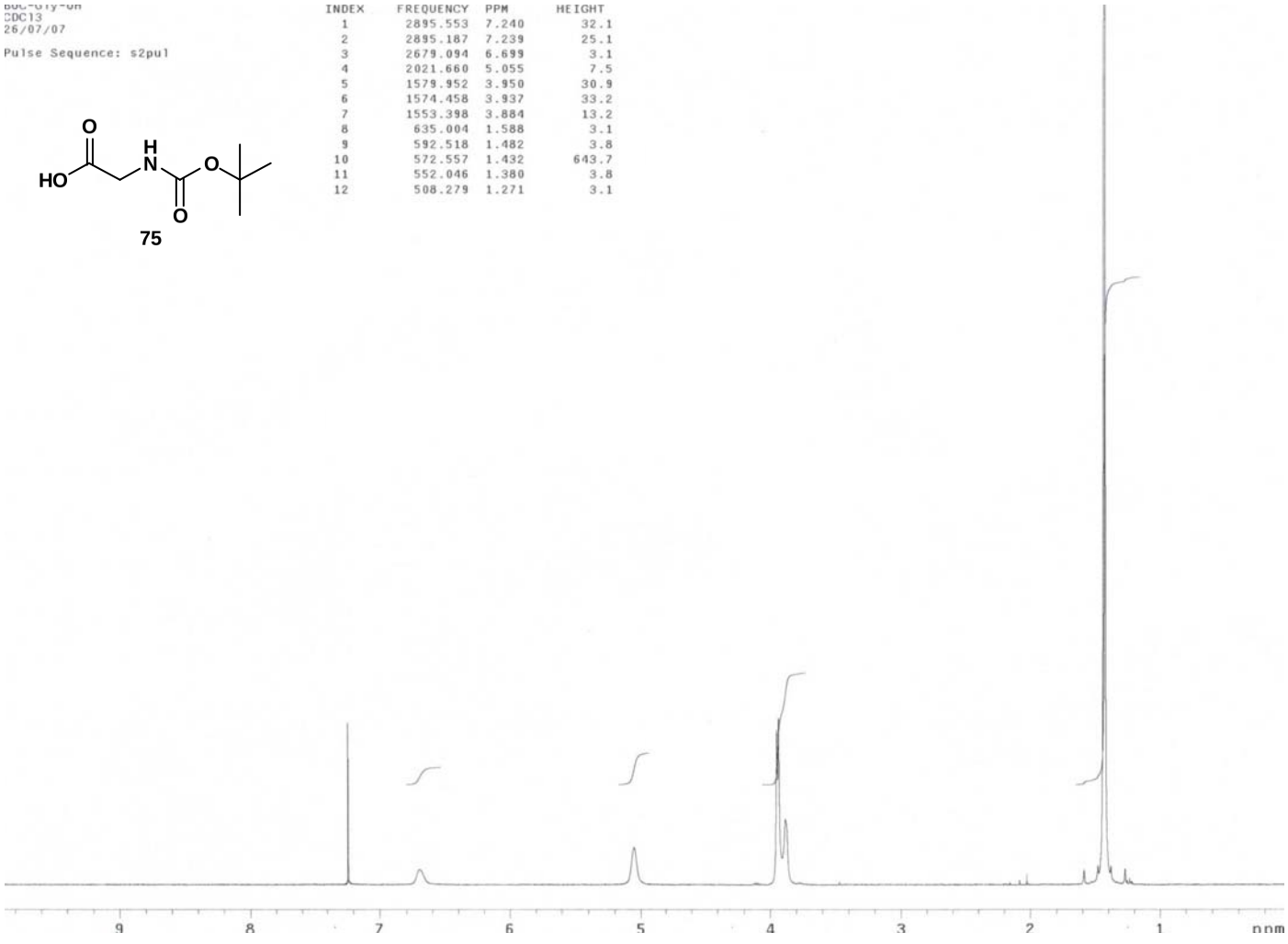
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 15093.091 | 150.084 | 54.1 |
| 2 | 14790.958 | 147.079 | 54.3 |
| 3 | 14758.150 | 146.753 | 5.9 |
| 4 | 14315.632 | 142.353 | 5.6 |
| 5 | 14060.802 | 139.819 | 7.8 |
| 6 | 13423.728 | 133.484 | 50.9 |
| 7 | 13055.217 | 129.819 | 8.9 |
| 8 | 13012.491 | 129.394 | 56.5 |
| 9 | 12399.832 | 123.302 | 51.1 |
| 10 | 12296.832 | 122.278 | 54.4 |
| 11 | 12284.625 | 122.157 | 10.3 |
| 12 | 10623.655 | 105.640 | 60.3 |
| 13 | 7775.515 | 77.319 | 97.7 |
| 14 | 7743.471 | 77.000 | 103.9 |
| 15 | 7711.426 | 76.681 | 104.9 |
| 16 | -3.653 | -0.036 | 7.9 |



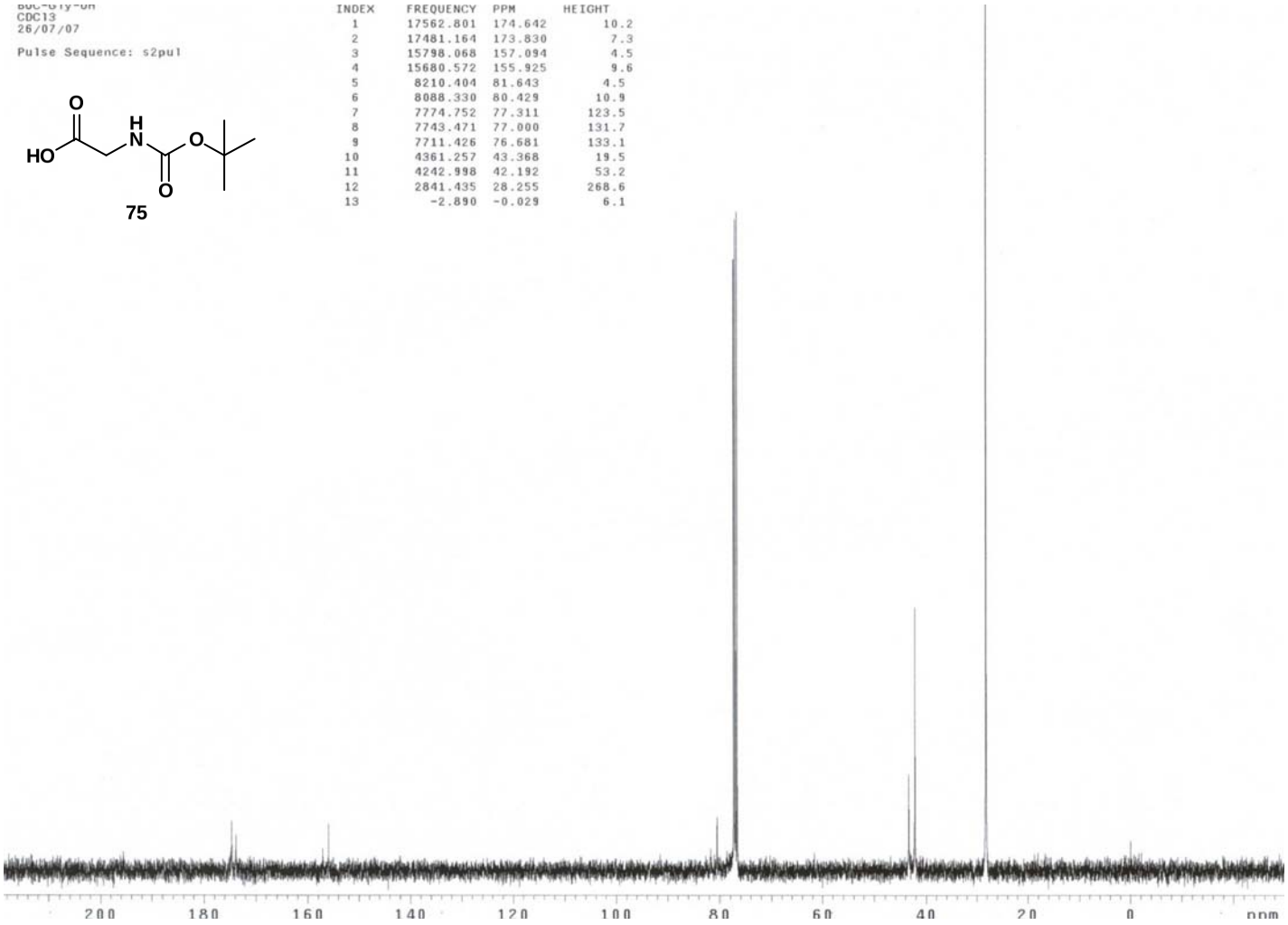

**Spectrum 38 $^{13}$C NMR spectrum of 71 in $CDCl_3$.**



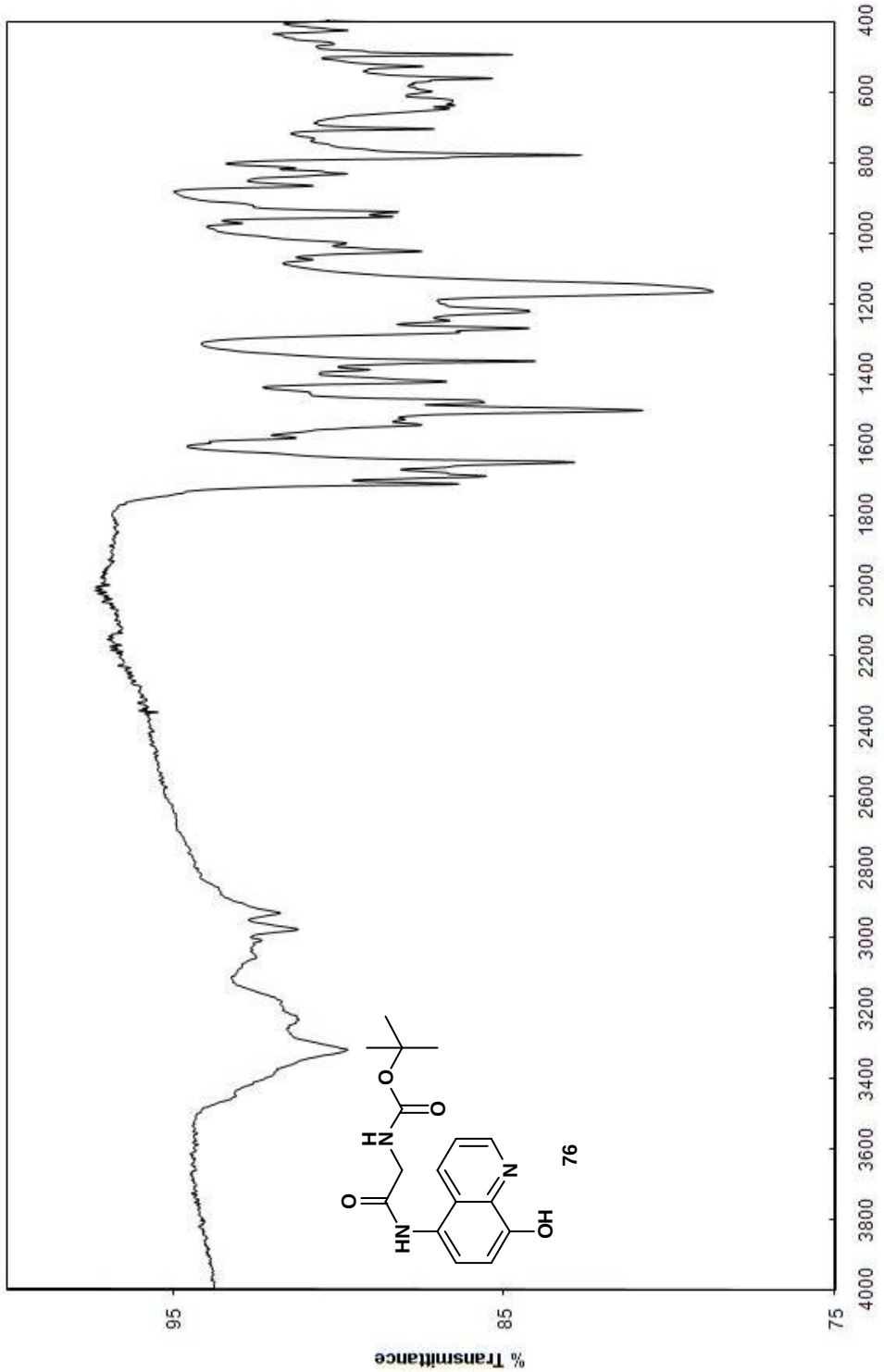

**Spectrum 39 IR spectrum of 75.**



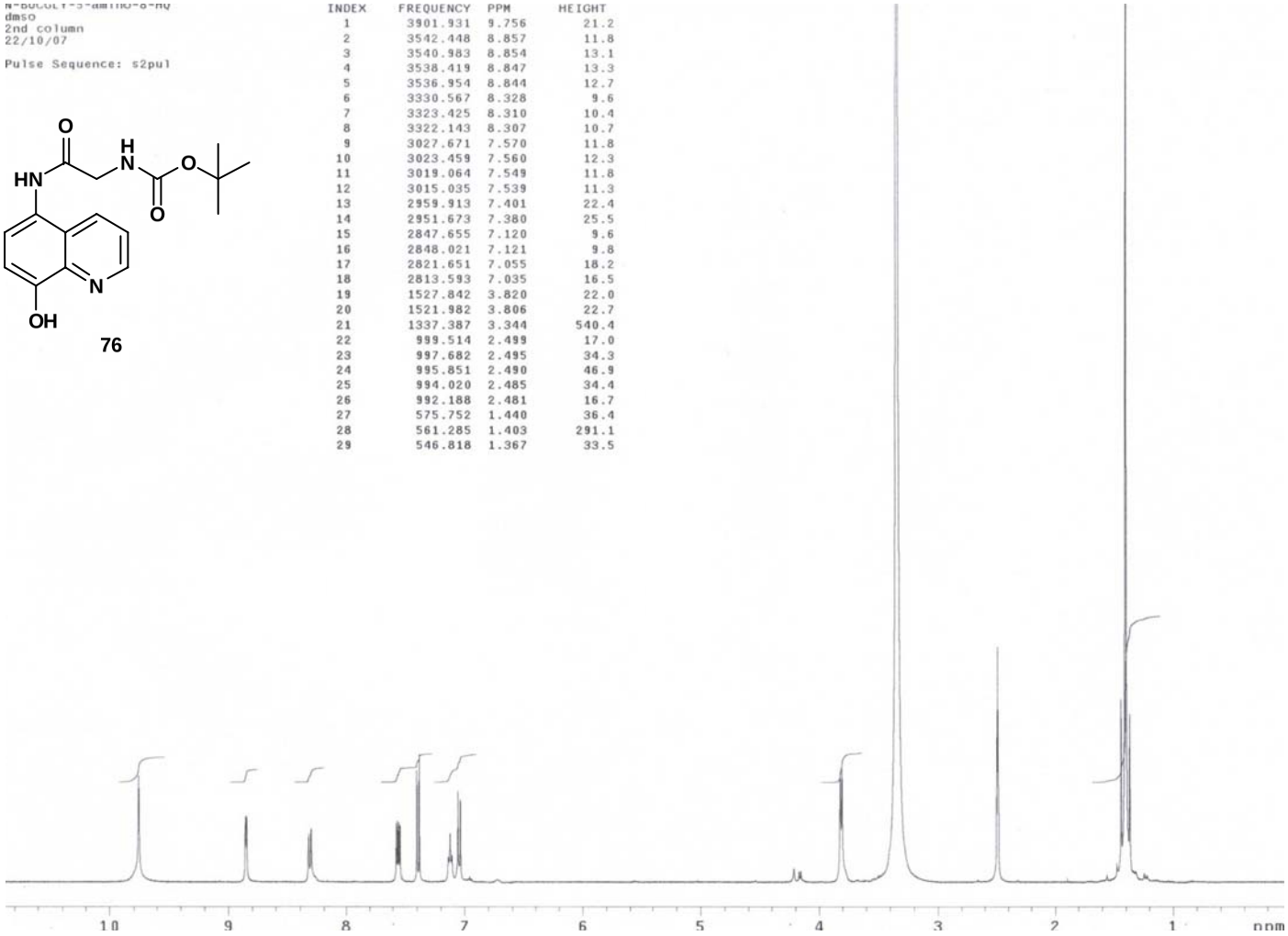

**Spectrum 40 $^{1}$H NMR spectrum of 75 in $CDCl_3$.**



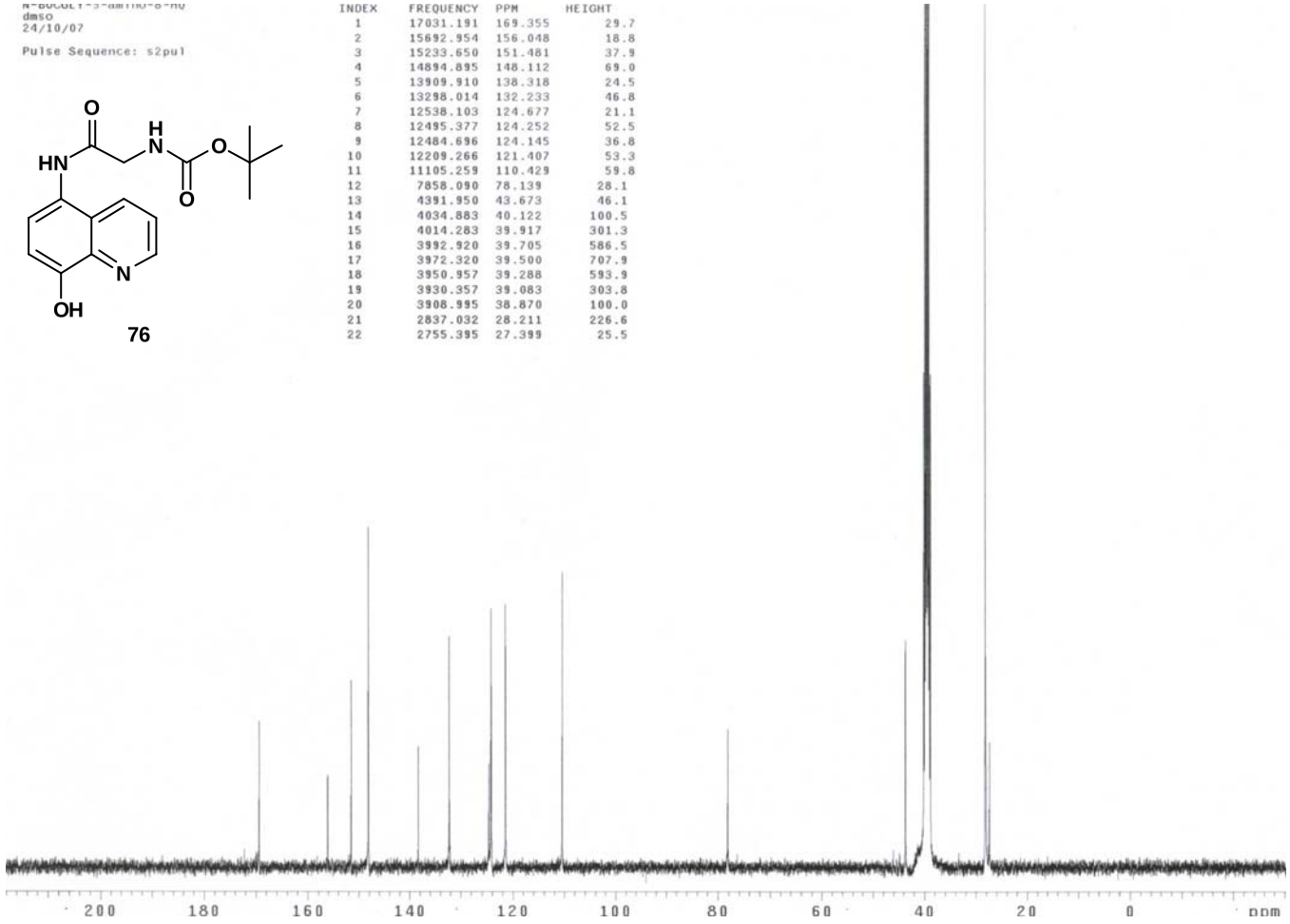

**Spectrum 41 $^{13}C$ NMR spectrum of 75 in $CDCl_3$.**



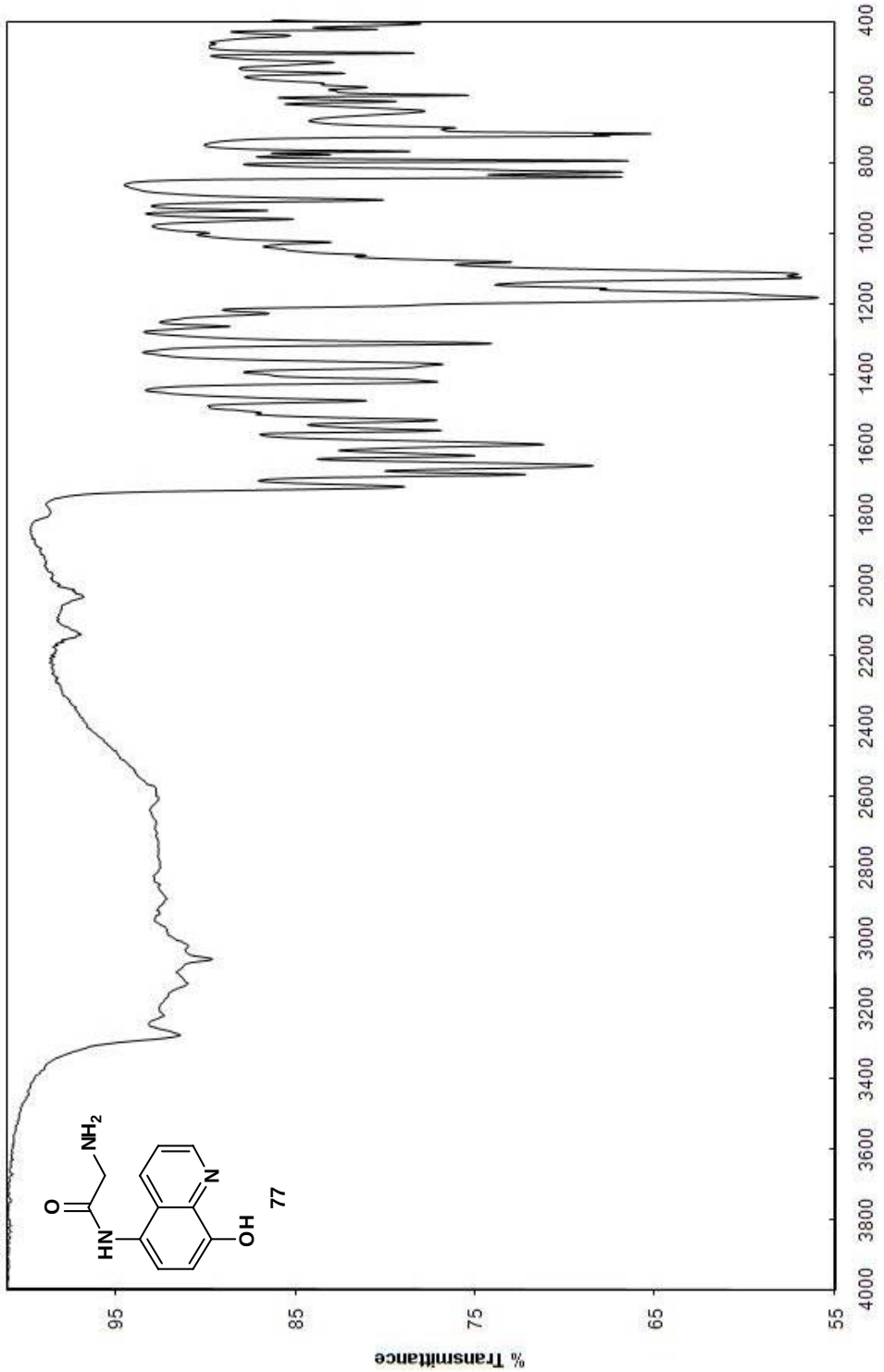

**Spectrum 42 IR spectrum of 76.**




**Spectrum 43 $^{1}$H NMR spectrum of 76 in DMSO-$d_6$.**



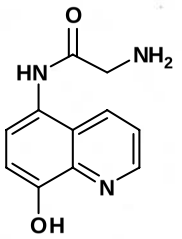

**Spectrum 44 $^{13}$C NMR spectrum of 76 in DMSO-$d_6$.**




**Spectrum 45 IR spectrum of 77.**

N-Gly-5-amino-8HQ
dmso
12/11/07

Pulse Sequence: presat_da

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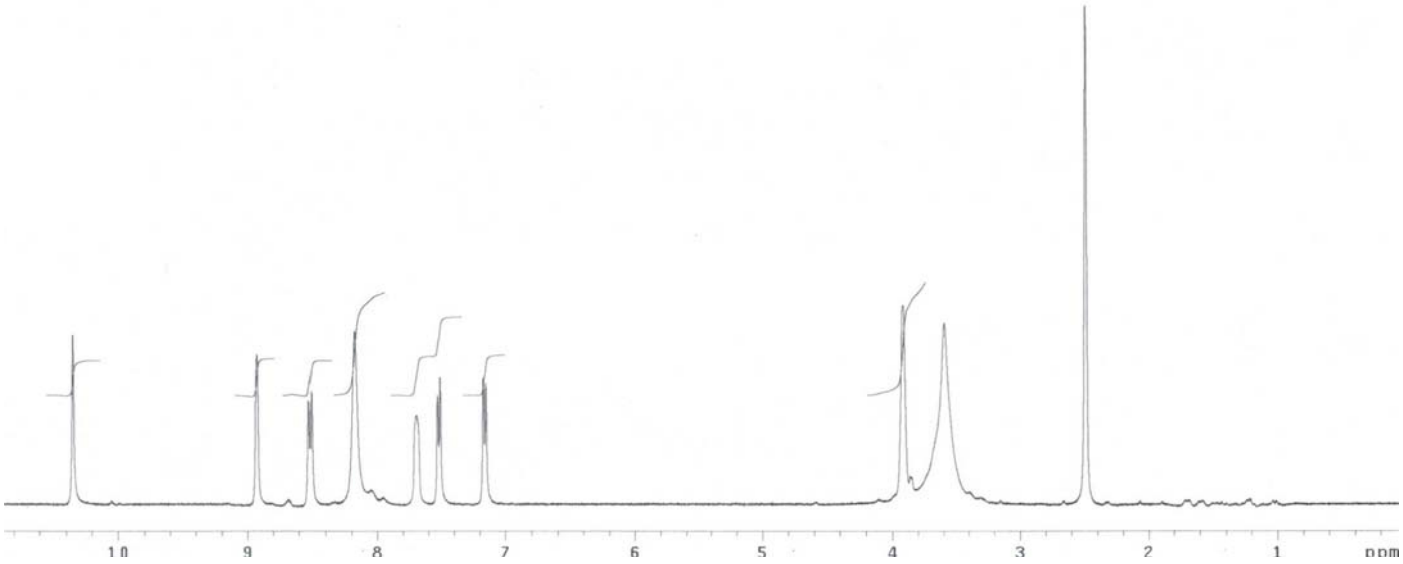
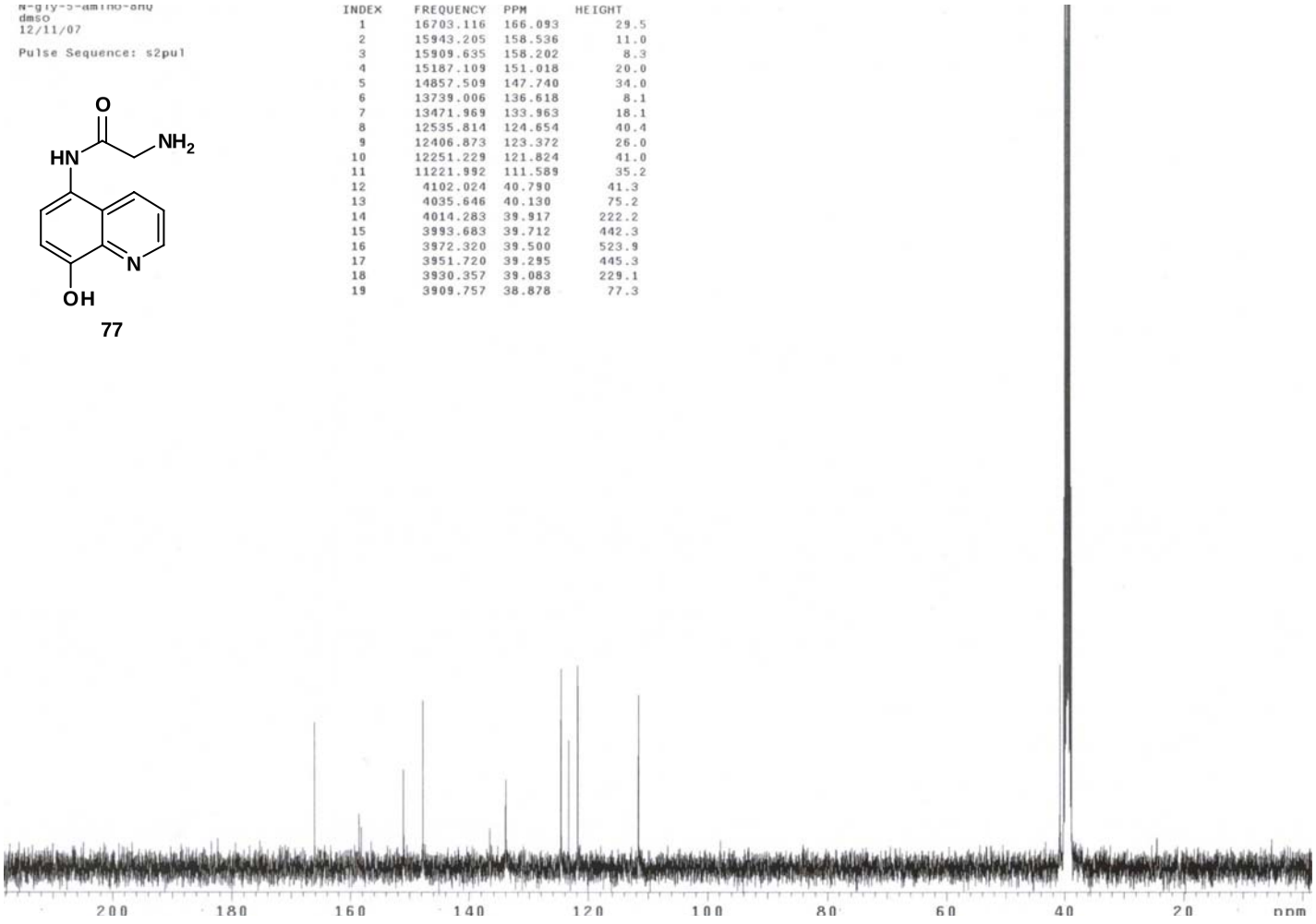
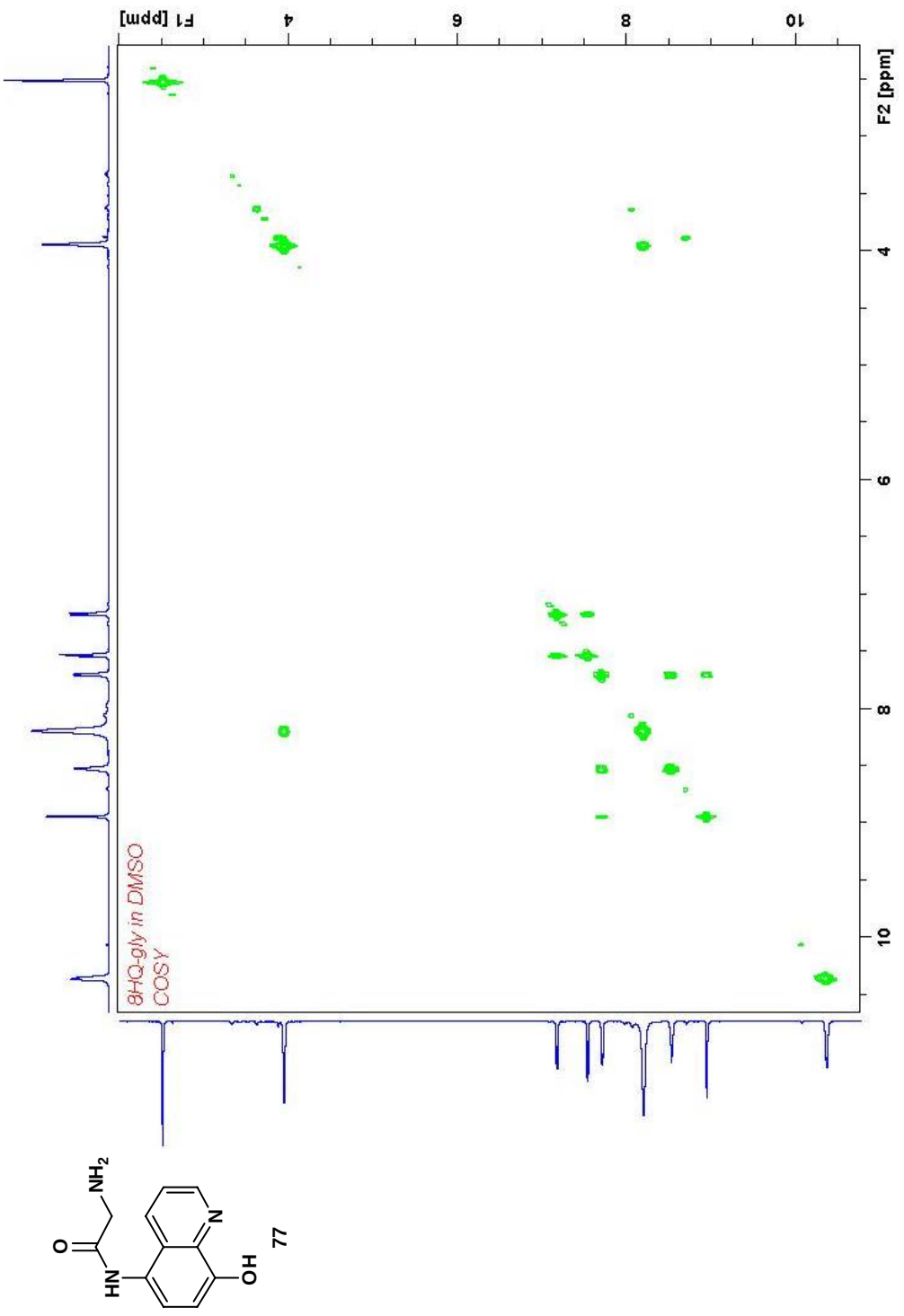
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 4137.985 | 10.347 | 31.1 |
| 2 | 3570.284 | 8.927 | 27.4 |
| 3 | 3410.045 | 8.526 | 19.0 |
| 4 | 3401.805 | 8.506 | 20.8 |
| 5 | 3269.219 | 8.174 | 31.8 |
| 6 | 3077.299 | 7.694 | 16.3 |
| 7 | 3010.640 | 7.528 | 20.0 |
| 8 | 3002.583 | 7.508 | 23.3 |
| 9 | 2868.349 | 7.172 | 23.2 |
| 10 | 2860.108 | 7.151 | 22.2 |
| 11 | 1565.750 | 3.915 | 36.4 |
| 12 | 1437.376 | 3.594 | 33.3 |
| 13 | 996.217 | 2.491 | 91.2 |



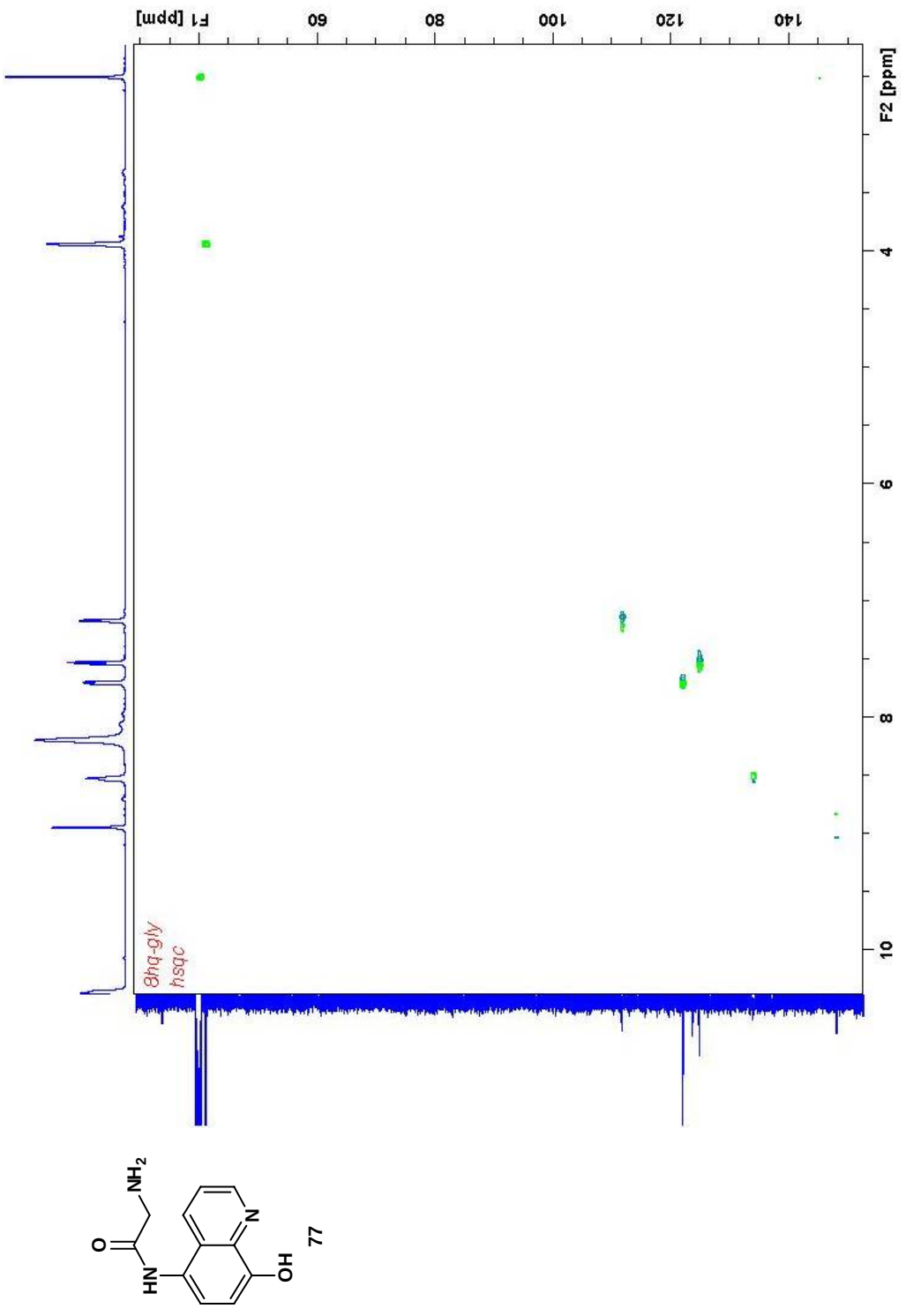

**Spectrum 46 $^{1}$H NMR spectrum of 77 in DMSO-$d_6$.**



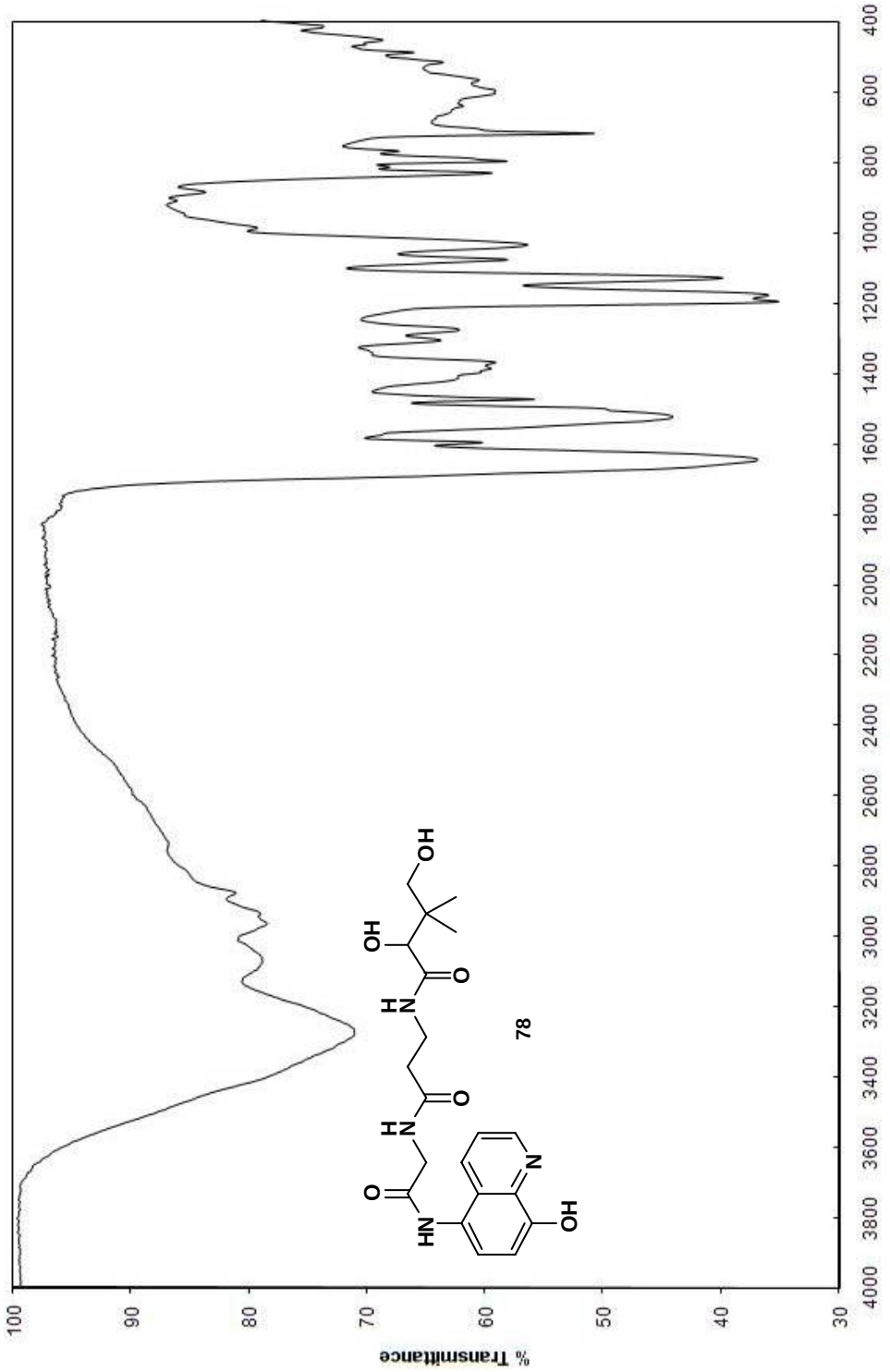

**Spectrum 47 $^{13}$C NMR spectrum of 77 in DMSO-$d_6$.**



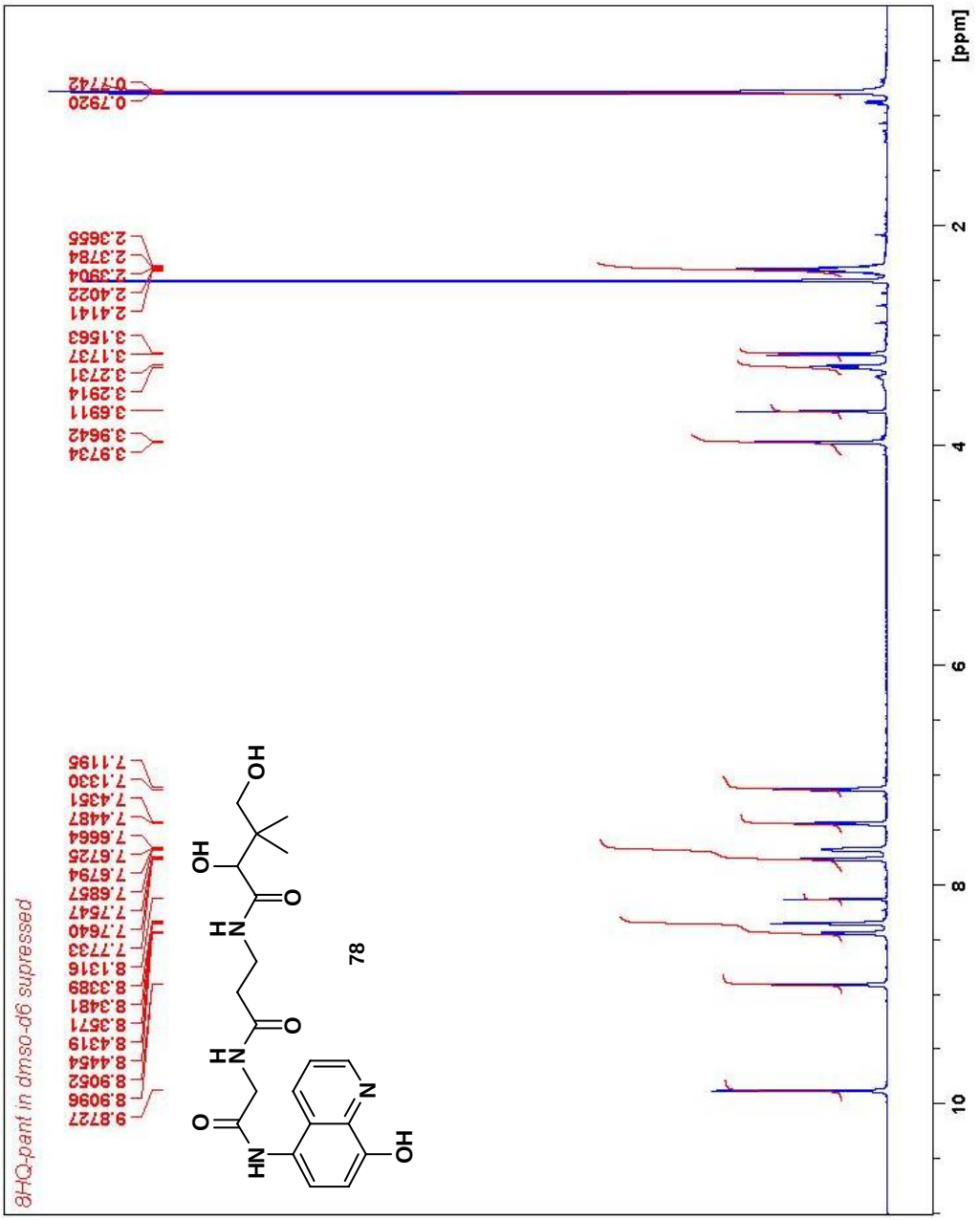

**Spectrum 48 COSY NMR spectrum of 77 in DMSO-$d_6$.**



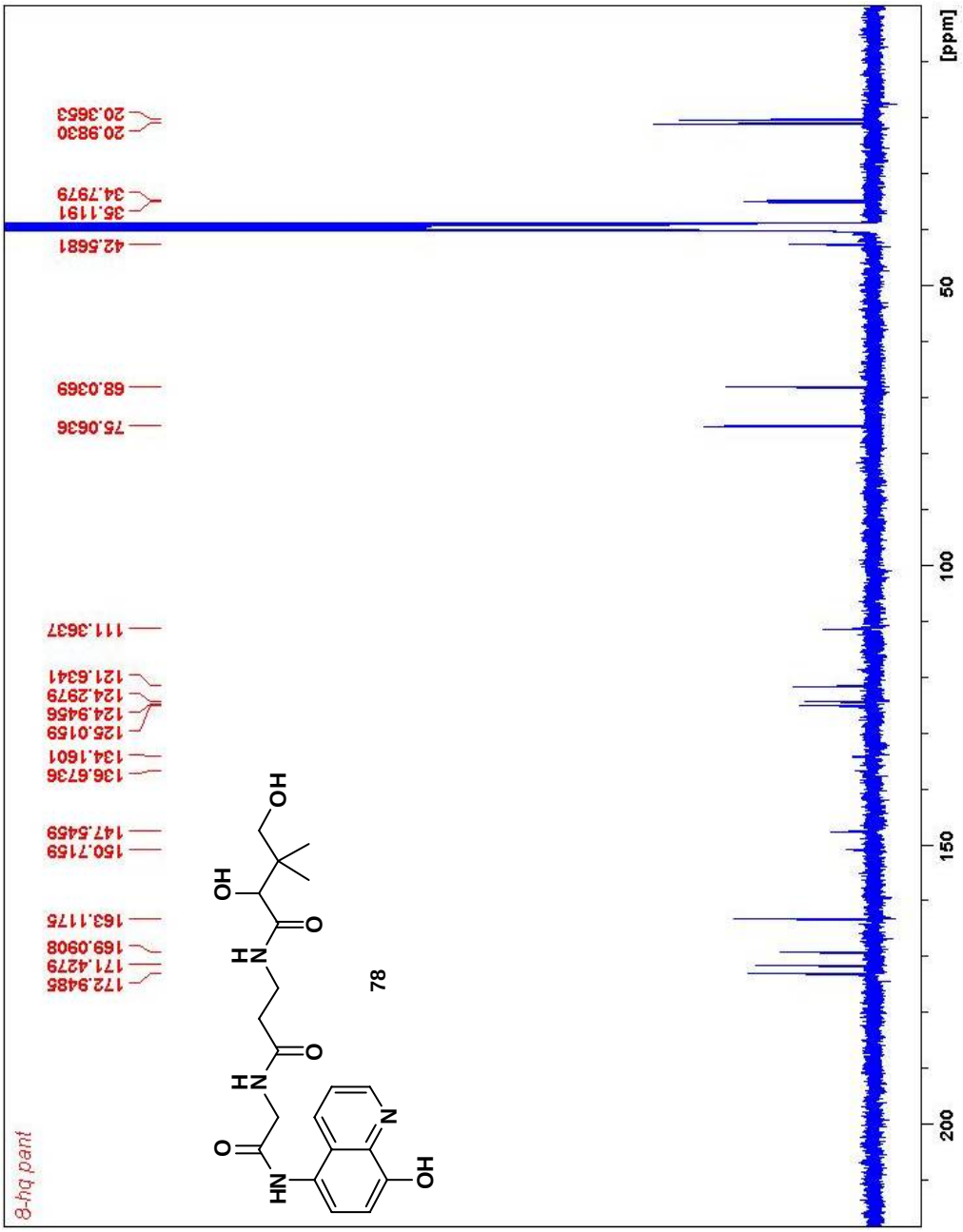

**Spectrum 49 HSQC NMR spectrum of 77 in DMSO-$d_6$.**



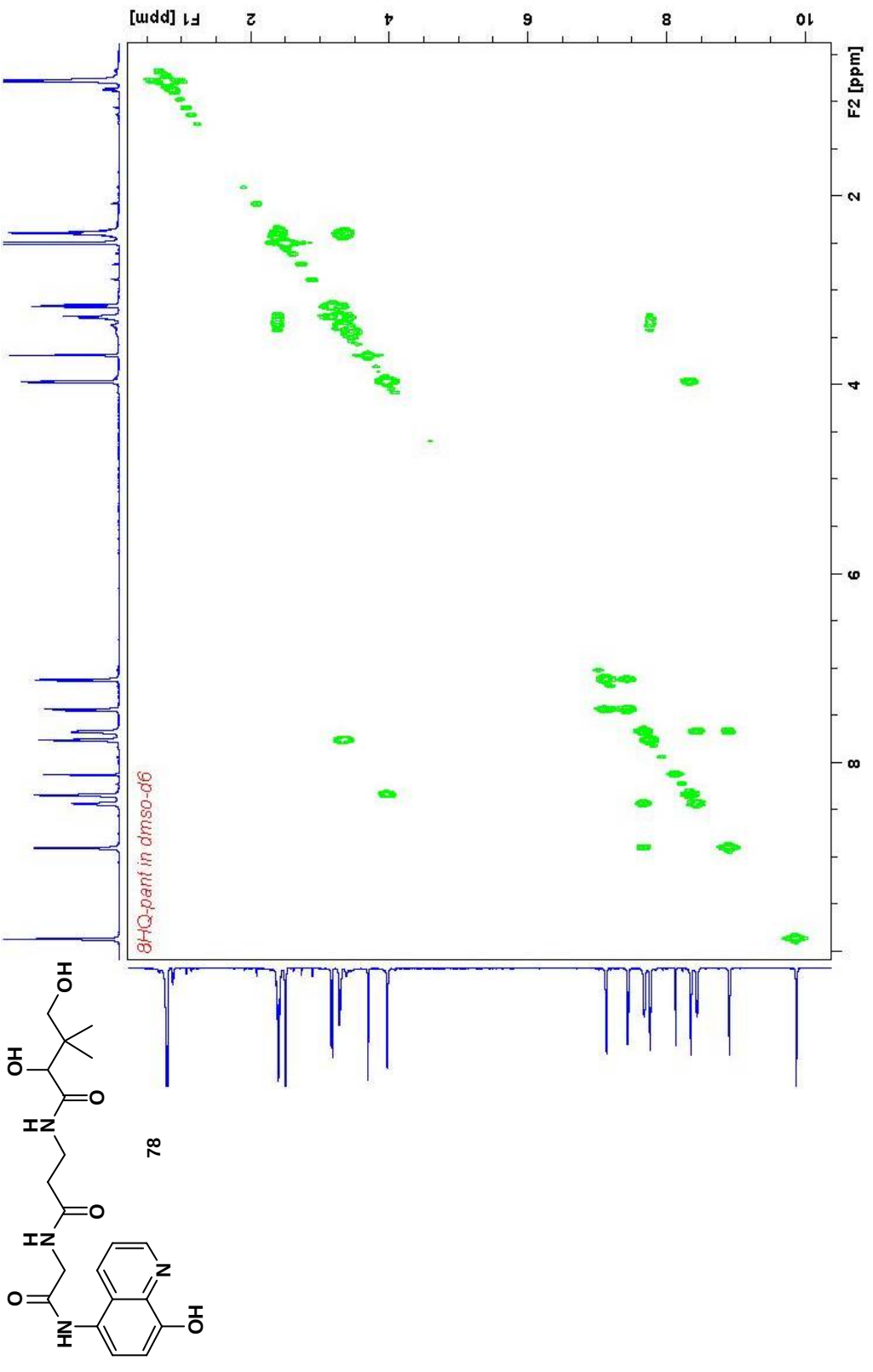

**Spectrum 50 IR spectrum of 78.**



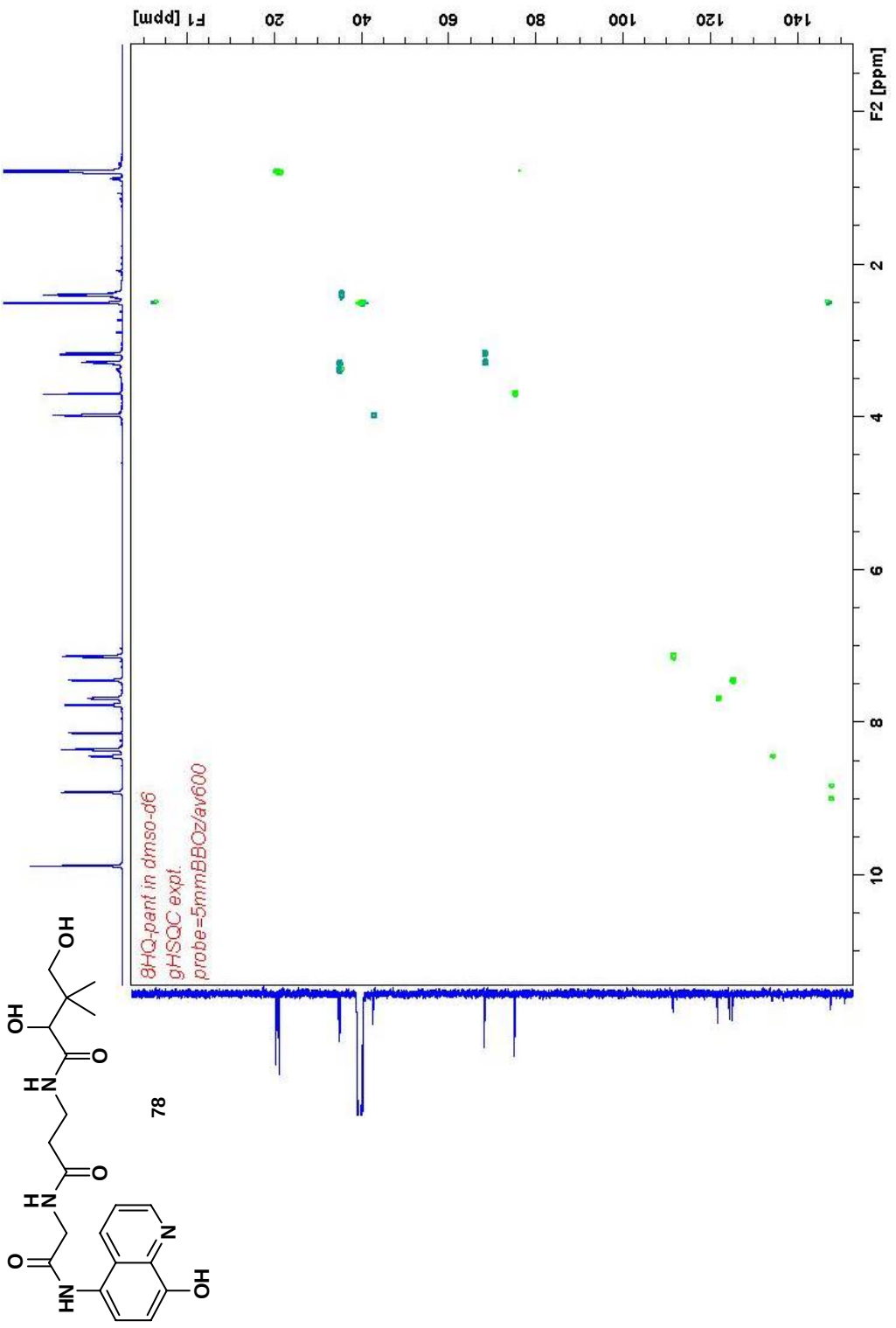

**Spectrum 51 $^{1}$H NMR spectrum of 78 in DMSO-$d_6$.**



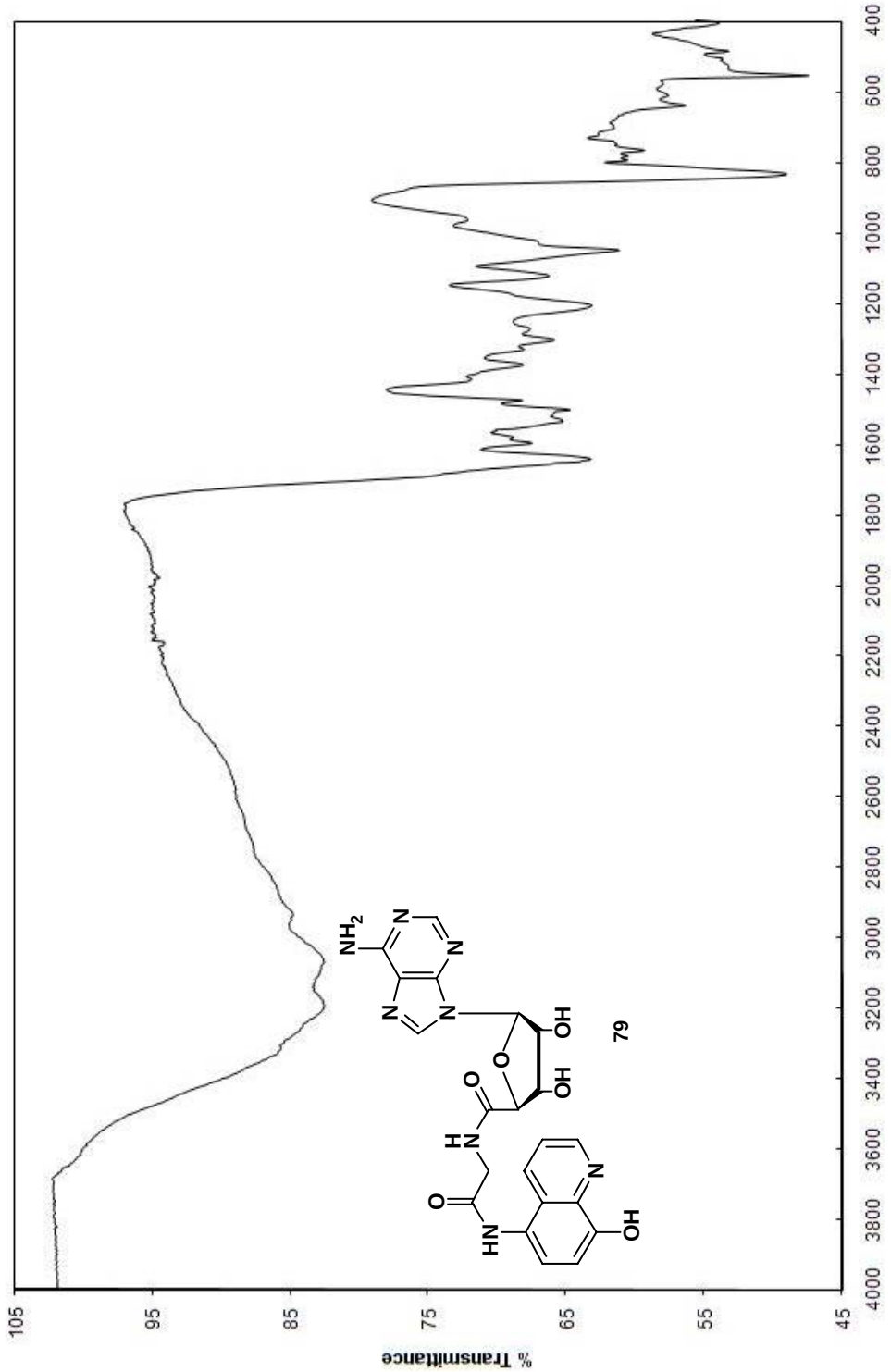

**Spectrum 52 $^{13}$C NMR spectrum of 78 in DMSO-$d_6$.**



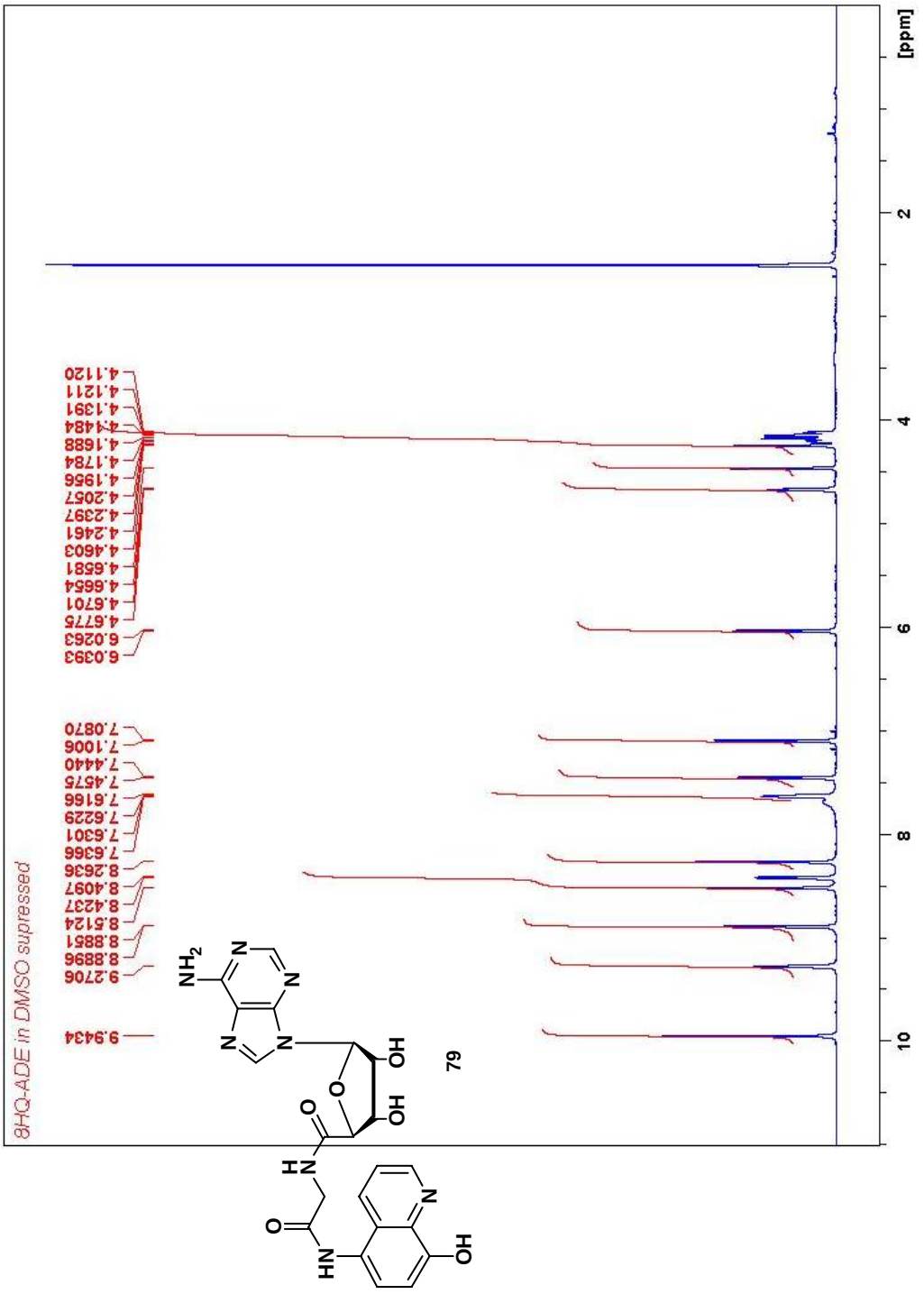

**Spectrum 53 COSY NMR spectrum of 78 in DMSO-$d_6$.**



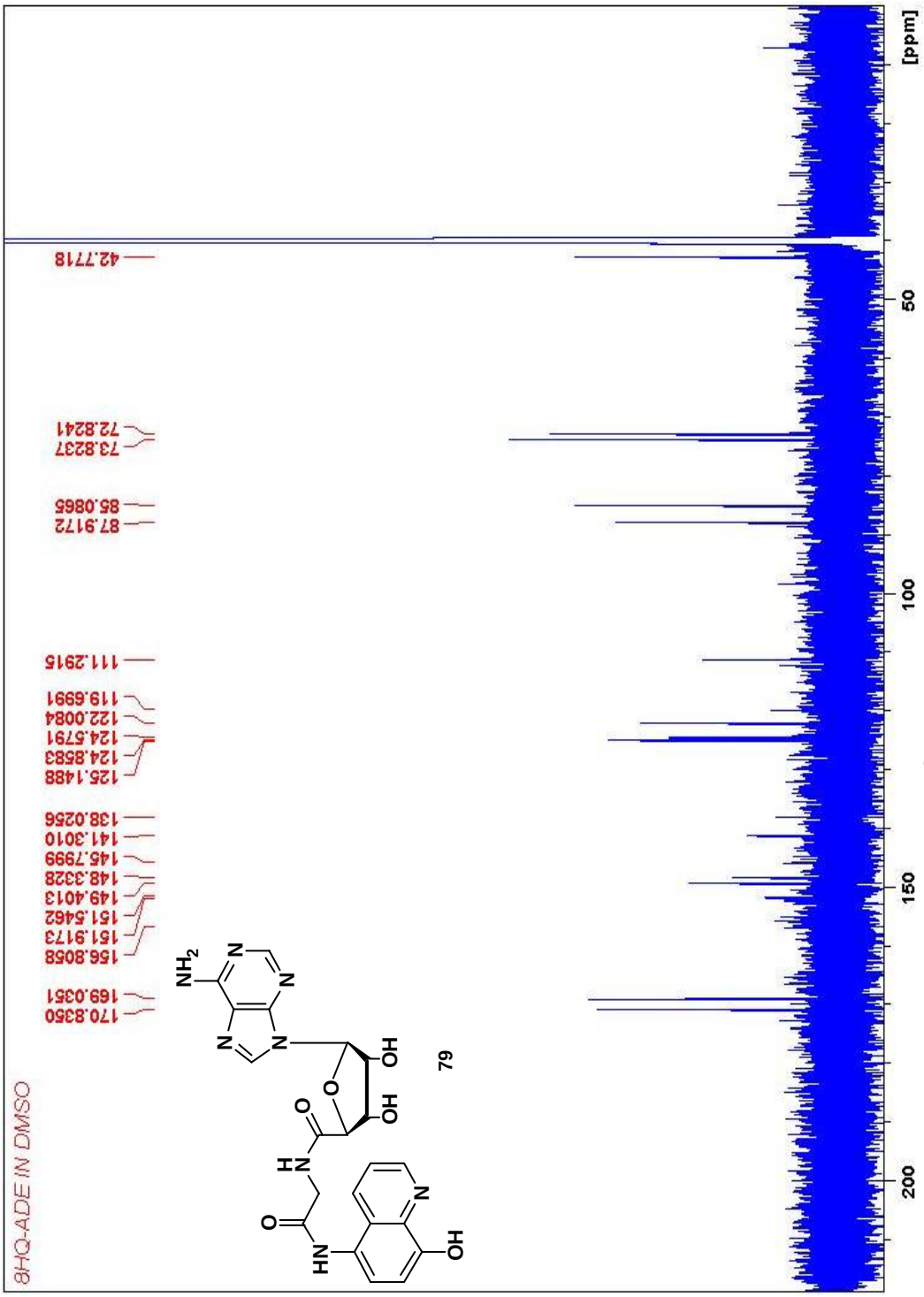

**Spectrum 54 HSQC NMR spectrum of 78 in DMSO-$d_6$.**



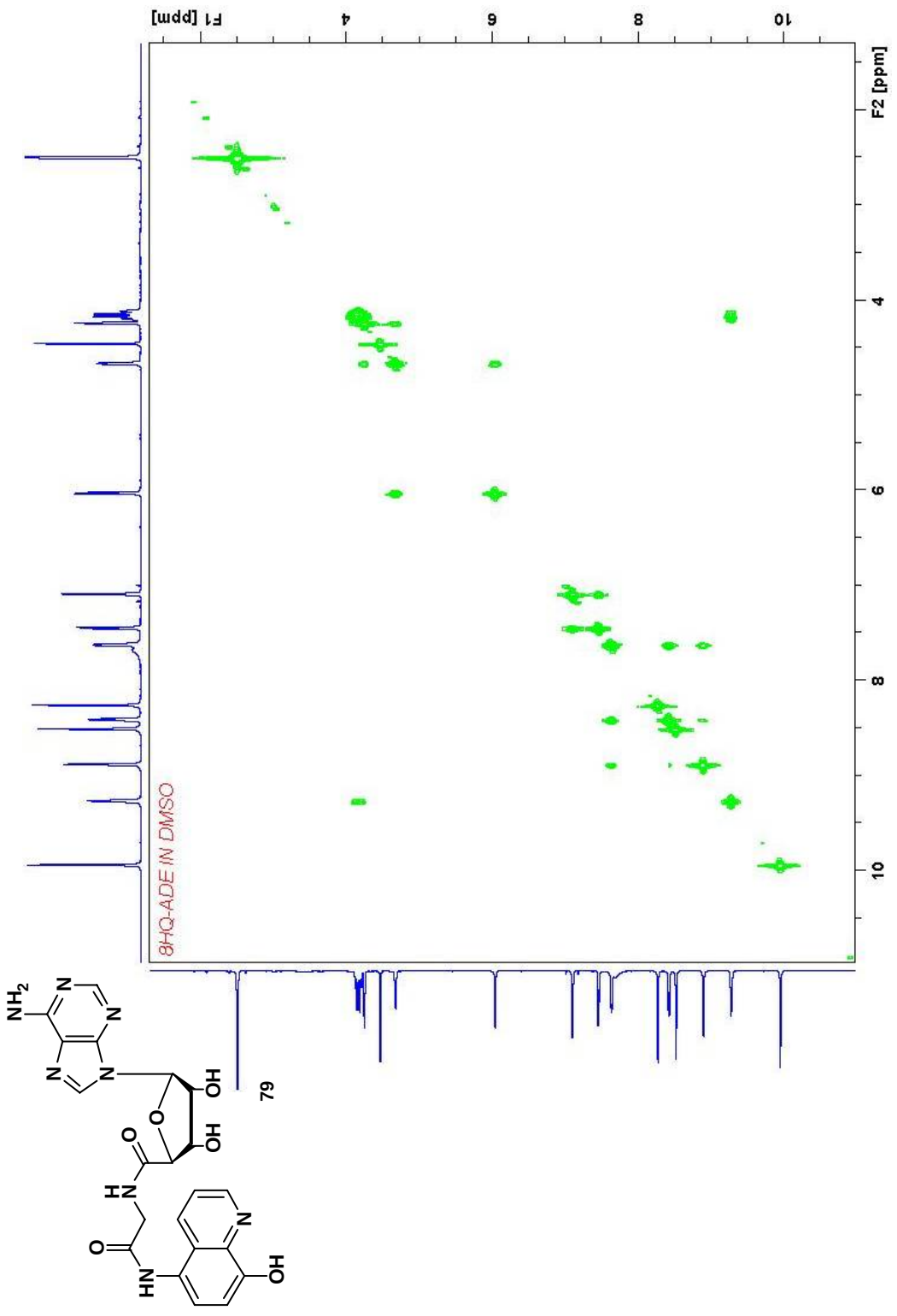

**Spectrum 55 IR spectrum of 79.**



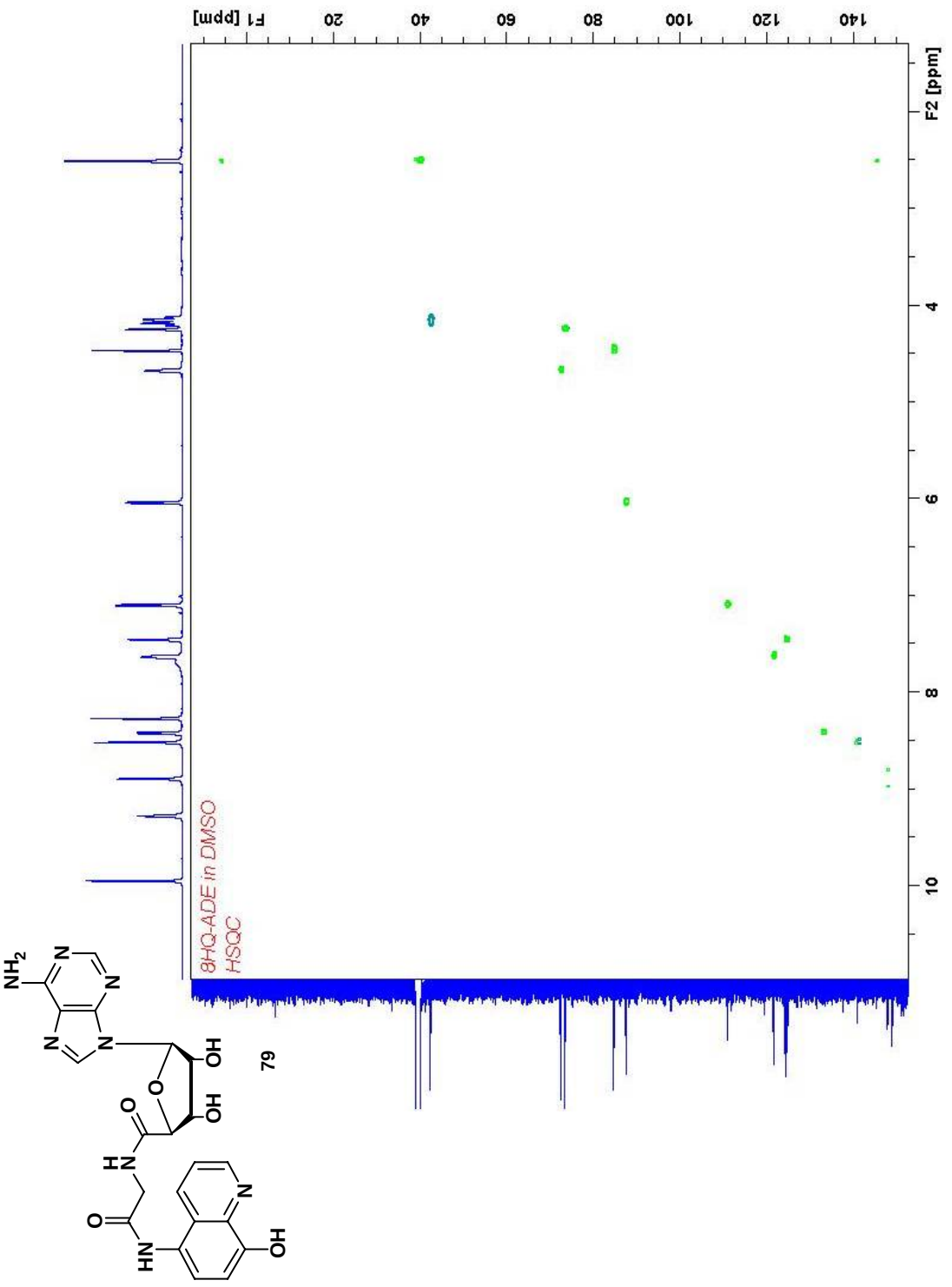

**Spectrum 56 $^{1}$H NMR spectrum of 79 in DMSO-$d_6$.**



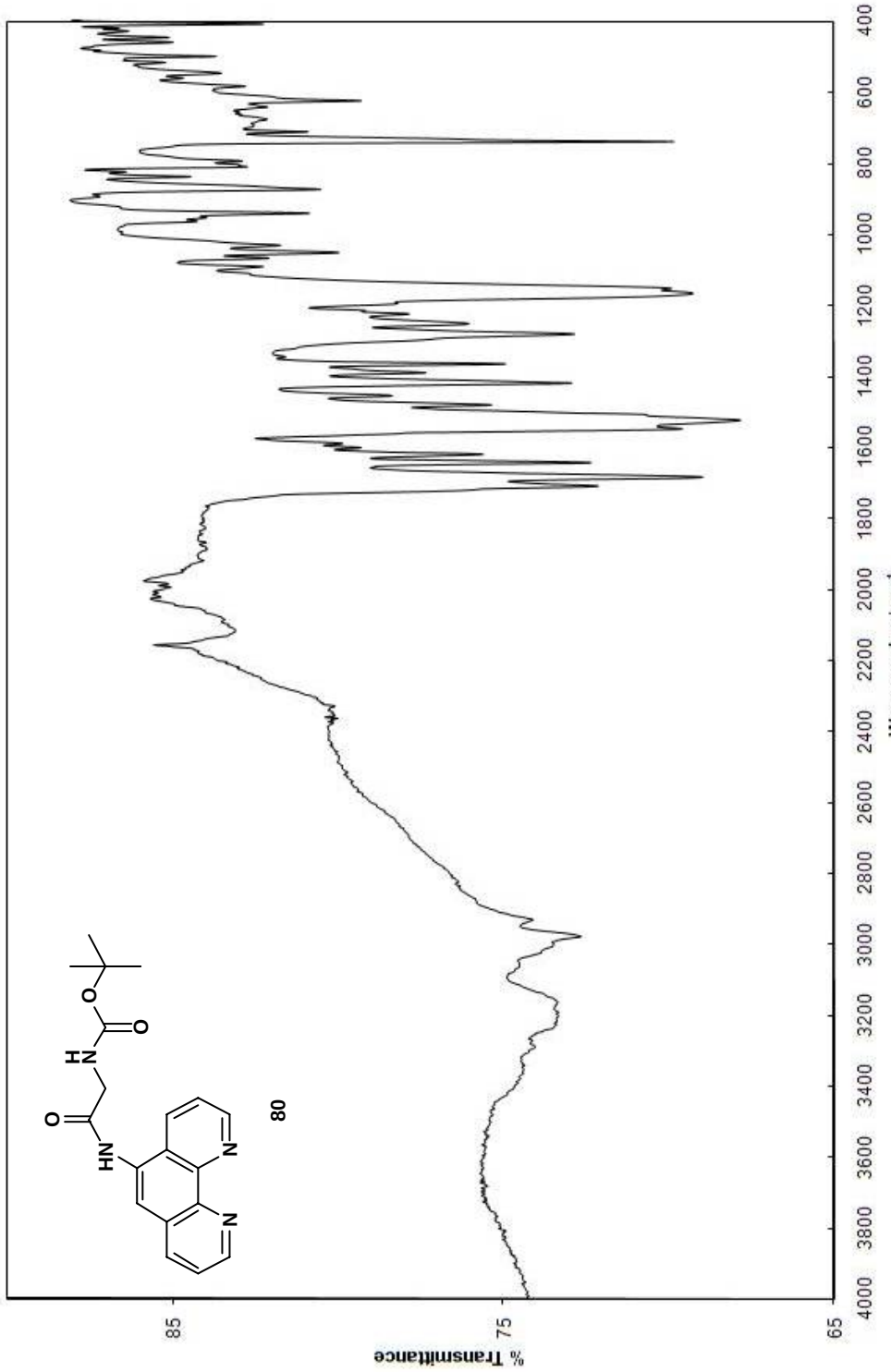

**Spectrum 57 $^{13}$C NMR spectrum of 79 in DMSO-$d_6$.**



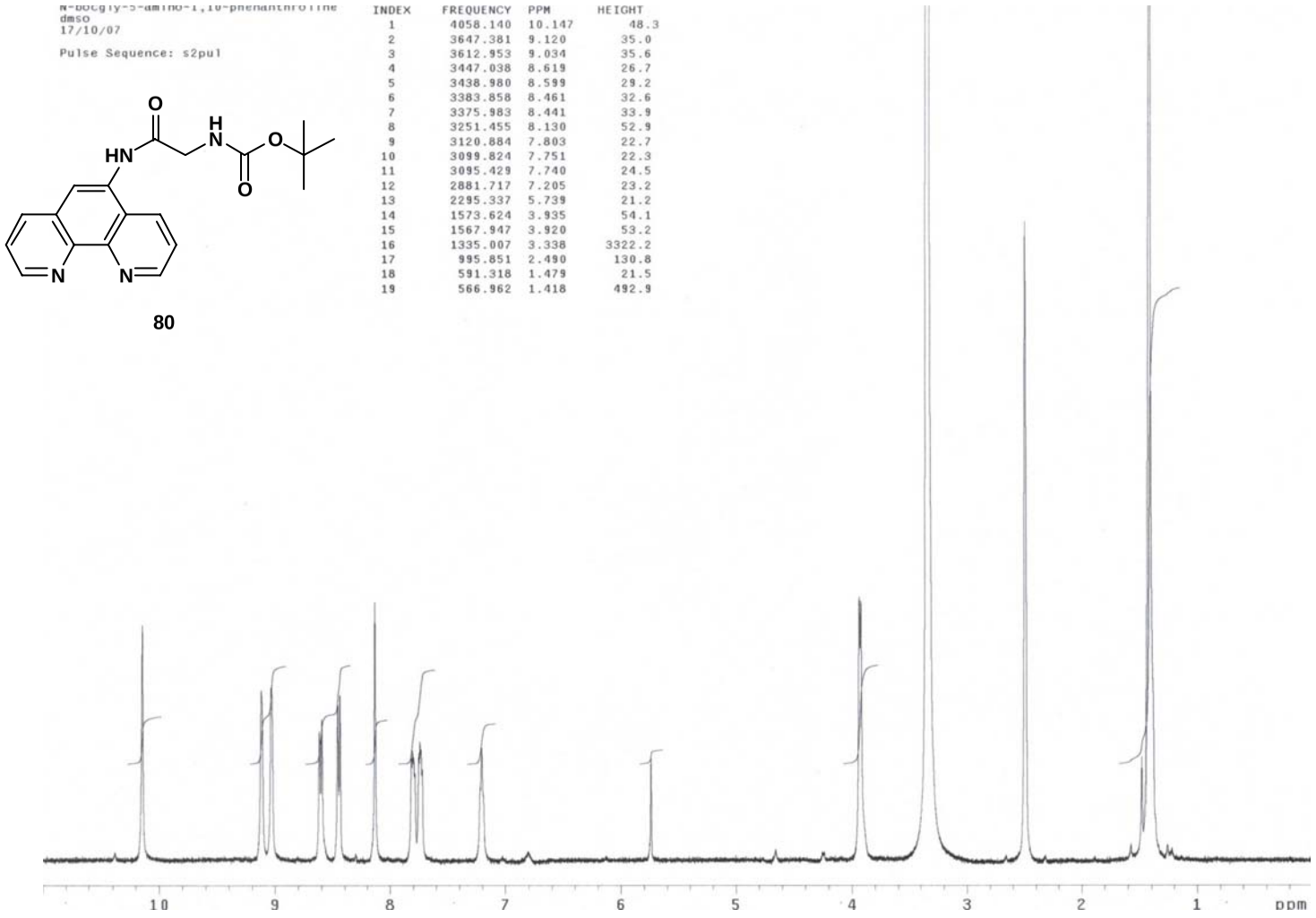

**Spectrum 58 COSY NMR spectrum of 79 in DMSO-$d_6$.**




**Spectrum 59 HSQC NMR spectrum of 79 in DMSO-$d_6$.**




**Spectrum 60 IR spectrum of 80.**

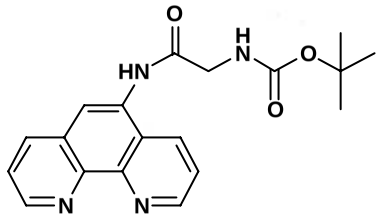



80

**Spectrum 61 $^{1}$H NMR spectrum of 80 in DMSO-$d_6$.**

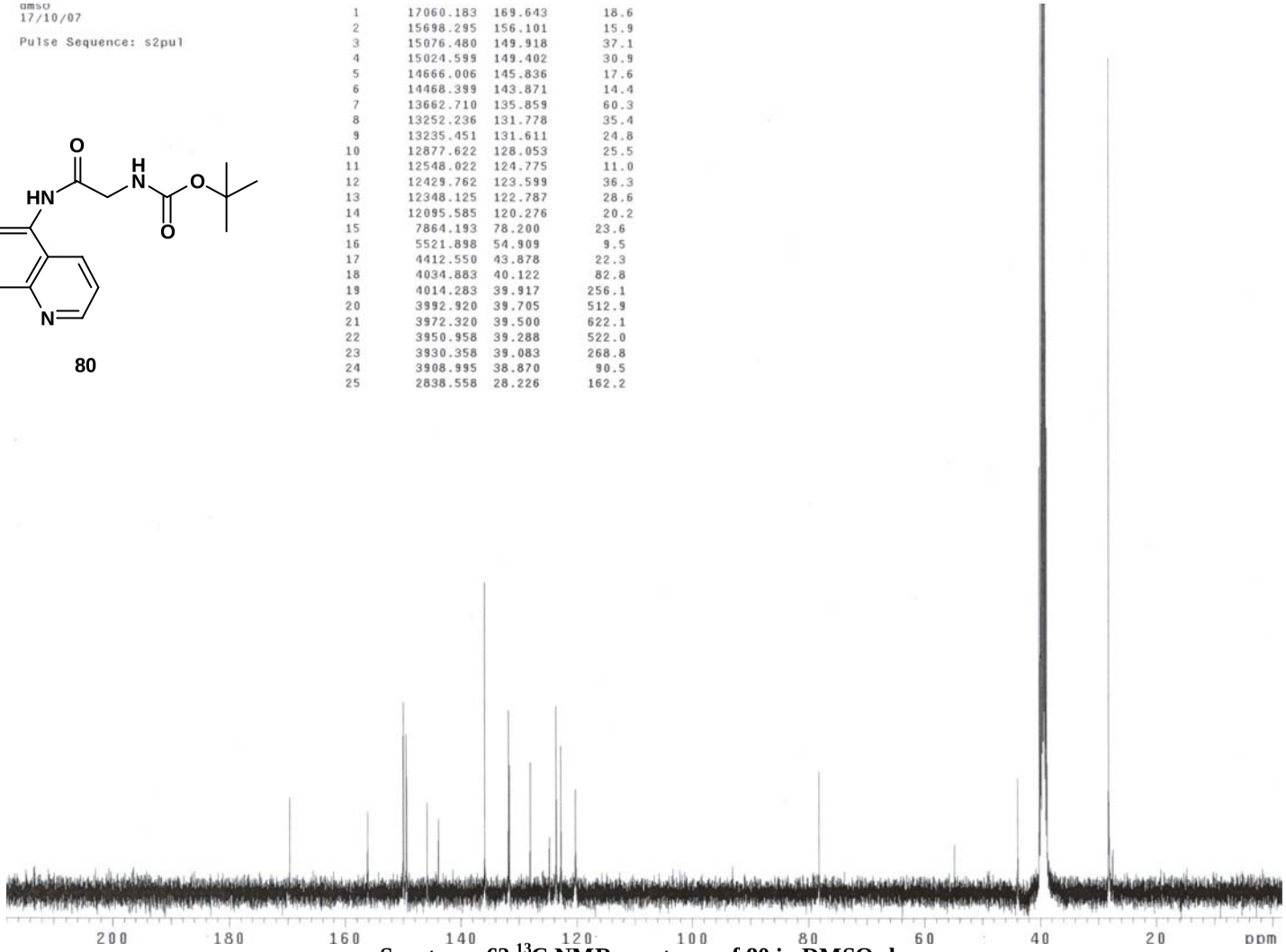
dmso
17/10/07

Pulse Sequence: s2pul

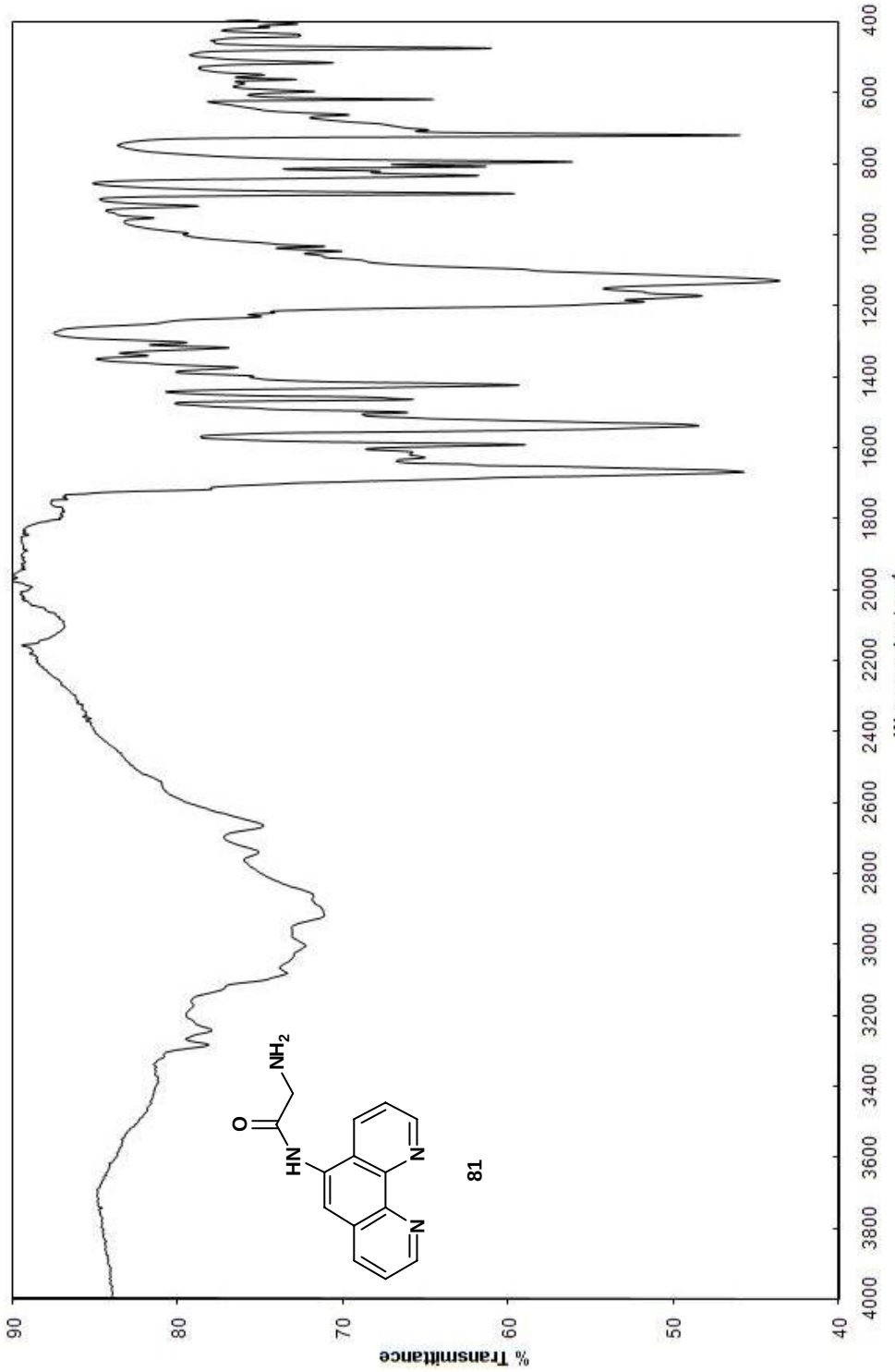



**80**

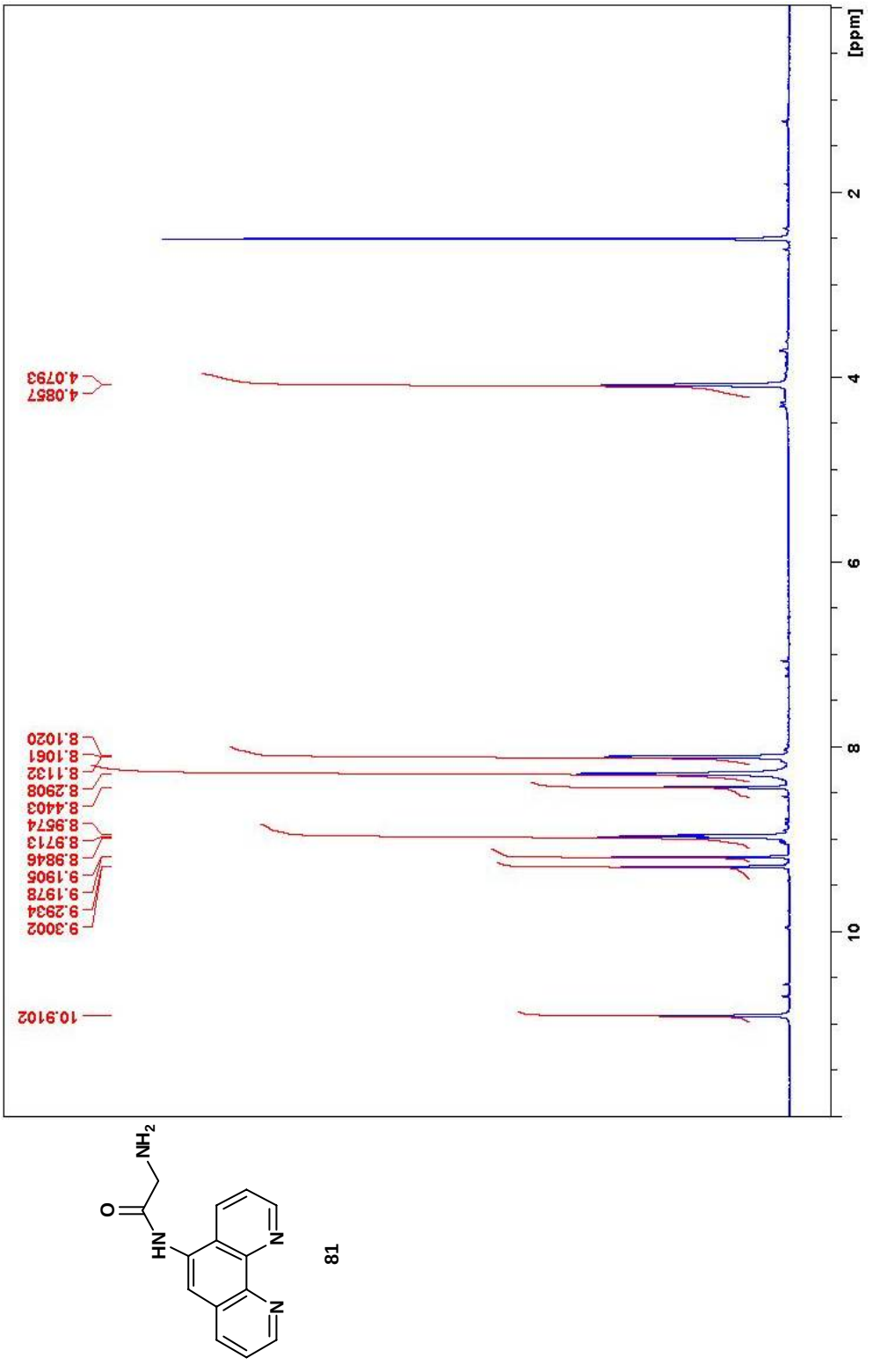
| | | | |
|---|---|---|---|
| 1 | 17060.183 | 169.643 | 18.6 |
| 2 | 15698.295 | 156.101 | 15.9 |
| 3 | 15076.480 | 149.918 | 37.1 |
| 4 | 15024.599 | 149.402 | 30.9 |
| 5 | 14666.006 | 145.836 | 17.6 |
| 6 | 14468.399 | 143.871 | 14.4 |
| 7 | 13662.710 | 135.859 | 60.3 |
| 8 | 13252.236 | 131.778 | 35.4 |
| 9 | 13235.451 | 131.611 | 24.8 |
| 10 | 12877.622 | 128.053 | 25.5 |
| 11 | 12548.022 | 124.775 | 11.0 |
| 12 | 12429.762 | 123.599 | 36.3 |
| 13 | 12348.125 | 122.787 | 28.6 |
| 14 | 12095.585 | 120.276 | 20.2 |
| 15 | 7864.193 | 78.200 | 23.6 |
| 16 | 5521.898 | 54.909 | 9.5 |
| 17 | 4412.550 | 43.878 | 22.3 |
| 18 | 4034.883 | 40.122 | 82.8 |
| 19 | 4014.283 | 39.917 | 256.1 |
| 20 | 3992.920 | 39.705 | 512.9 |
| 21 | 3972.320 | 39.500 | 622.1 |
| 22 | 3950.958 | 39.288 | 522.0 |
| 23 | 3930.358 | 39.083 | 268.8 |
| 24 | 3908.995 | 38.870 | 90.5 |
| 25 | 2838.558 | 28.226 | 162.2 |




**Spectrum 62 $^{13}$C NMR spectrum of 80 in DMSO-$d_6$.**

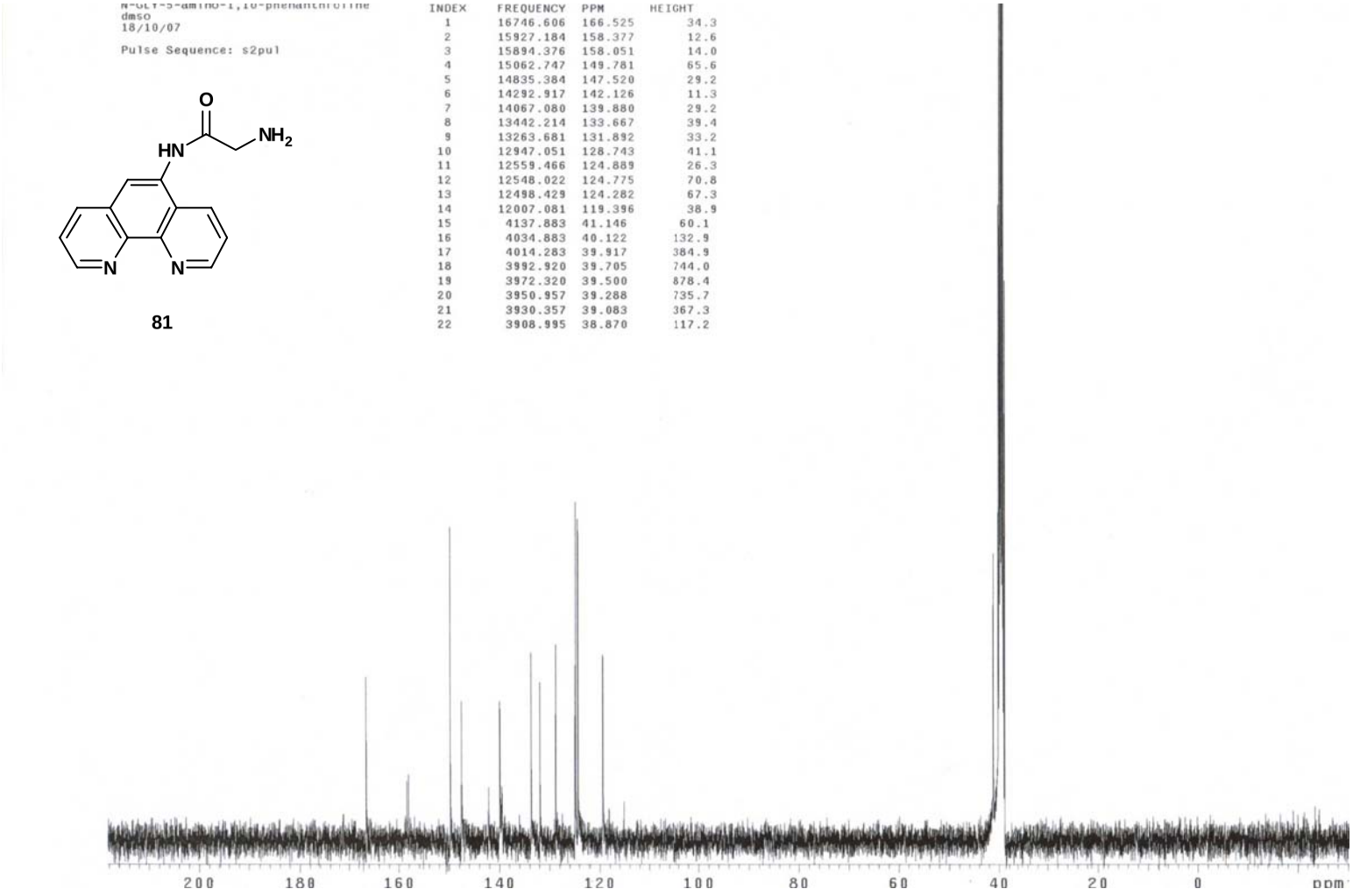



**Spectrum 63 IR spectrum of 81.**

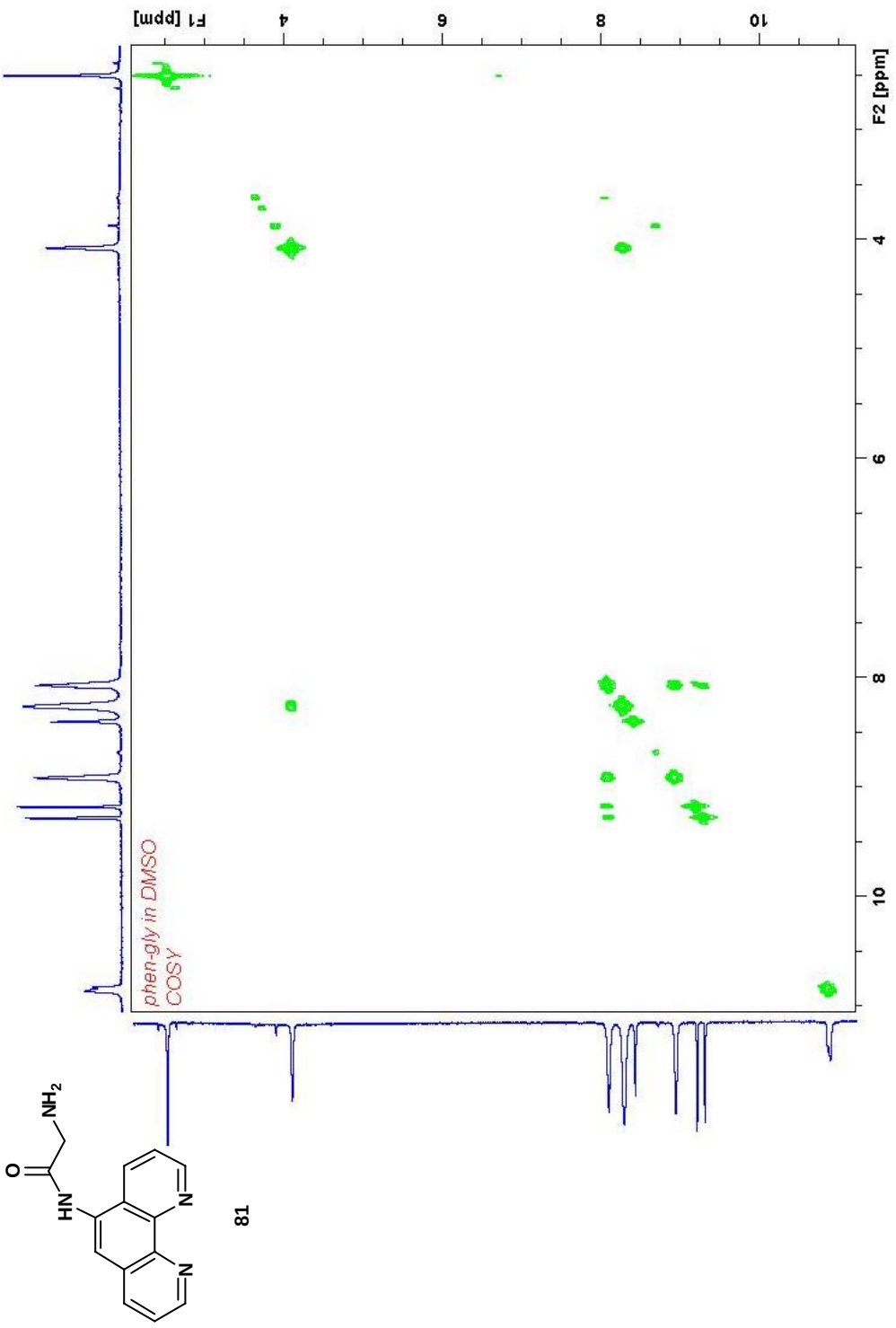



81

**Spectrum 64 $^{1}$H NMR of 81 in DMSO-$d_6$.**

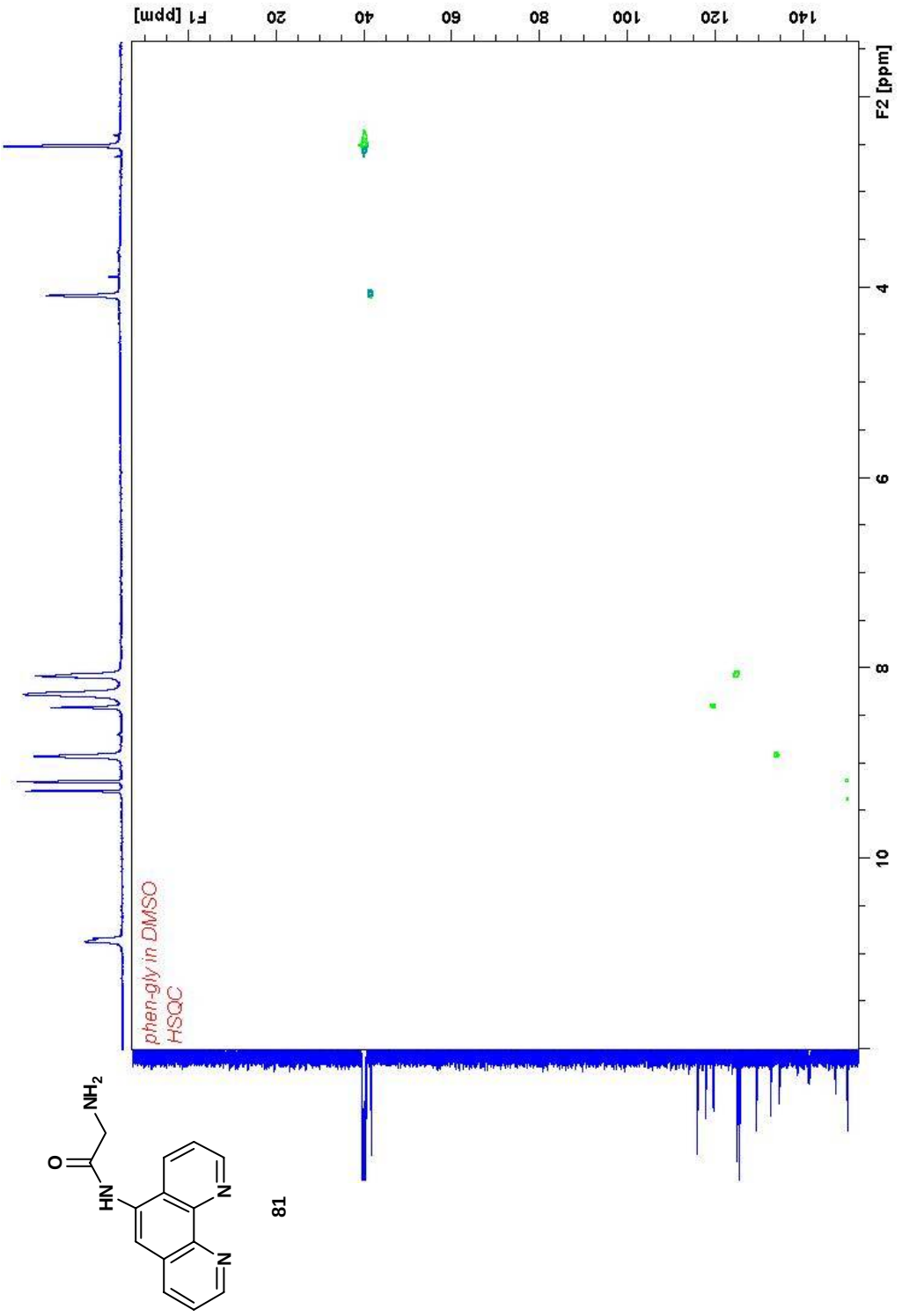



**Spectrum 65 $^{13}$C NMR spectrum of 81 in DMSO-$d_6$.**



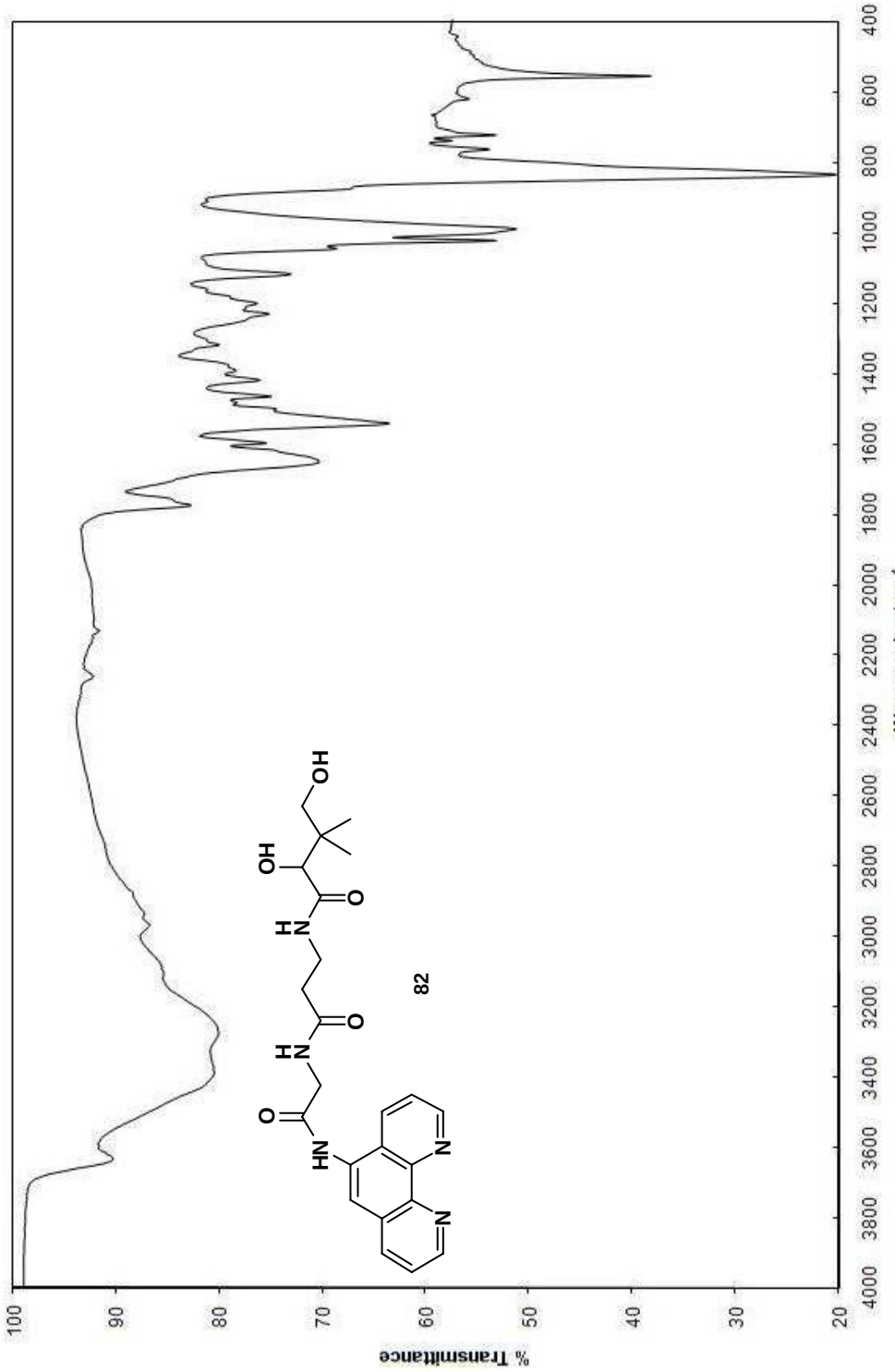

81

**Spectrum 66 COSY NMR spectrum of 81 in DMSO-$d_6$.**

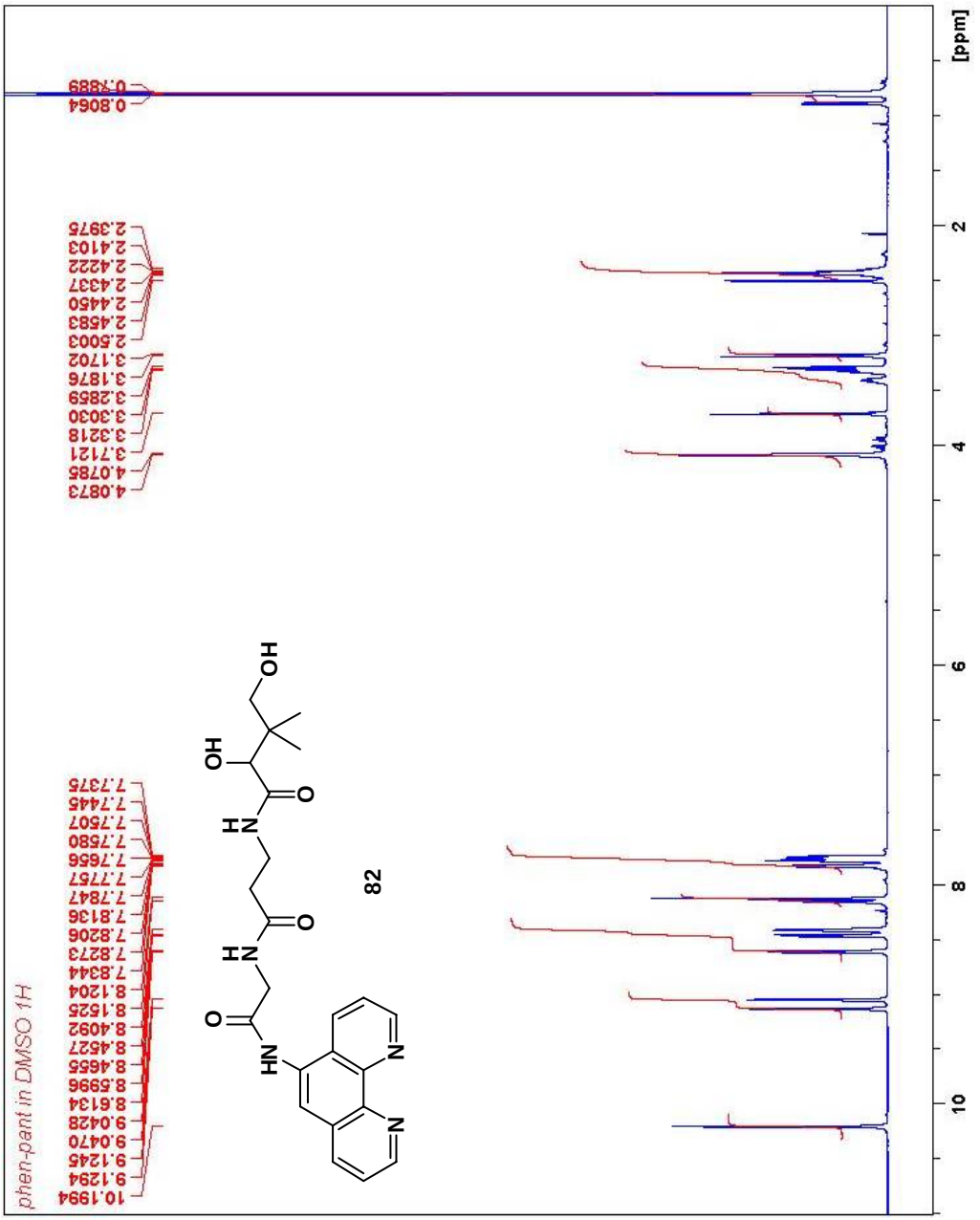



81

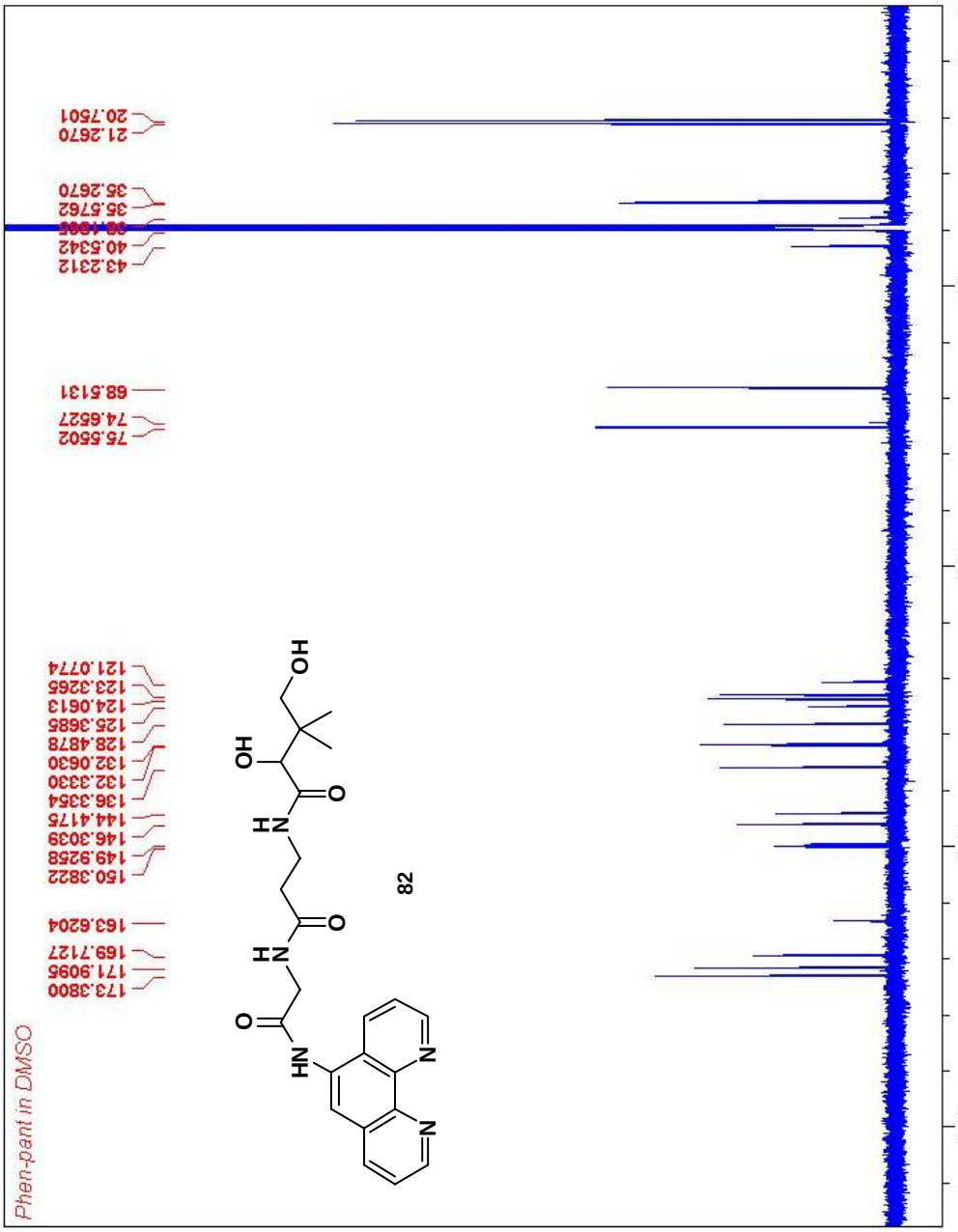
**Spectrum 67 HSQC NMR spectrum of 81 in DMSO-$d_6$.**



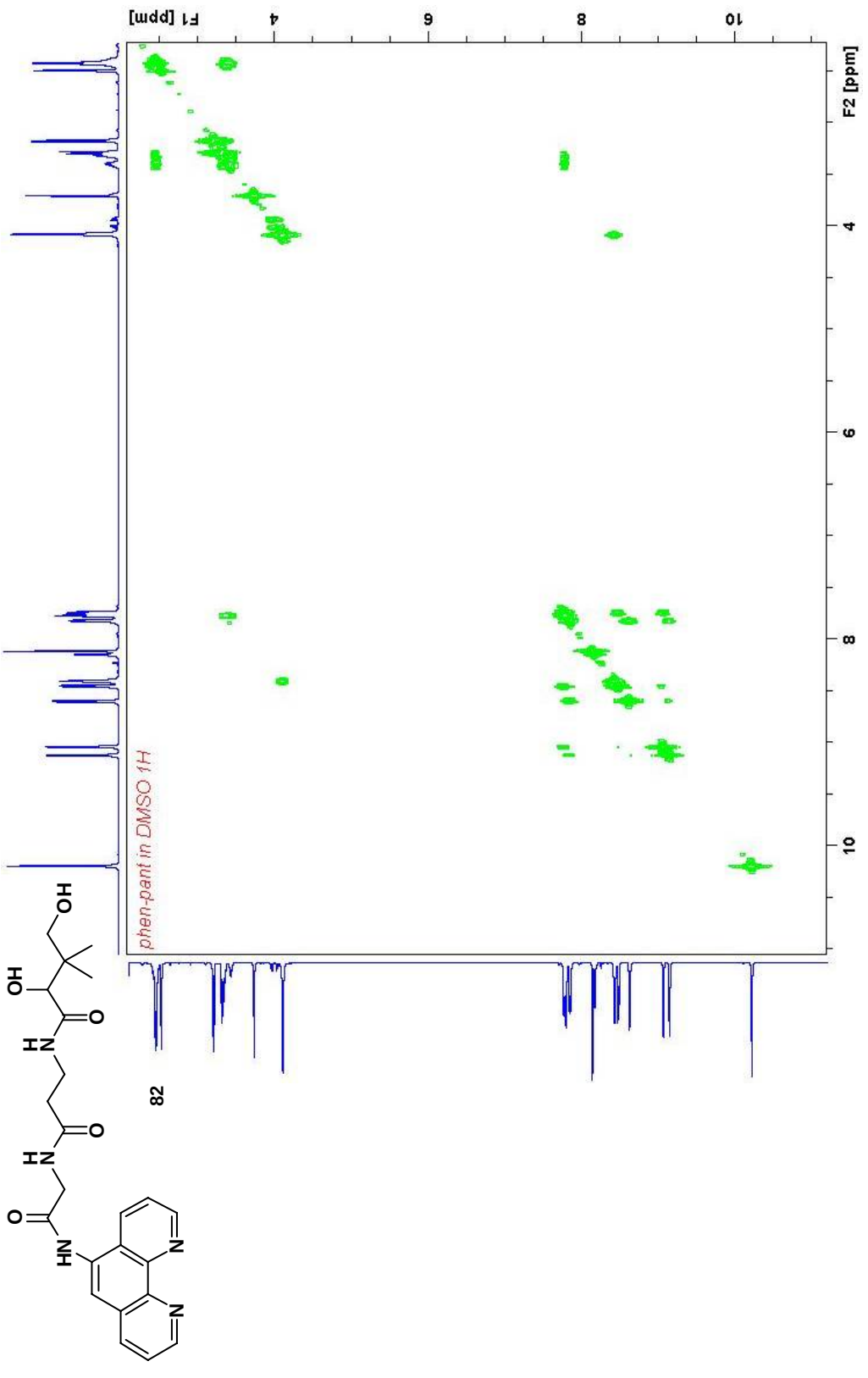

**Spectrum 68 IR spectrum of 82.**



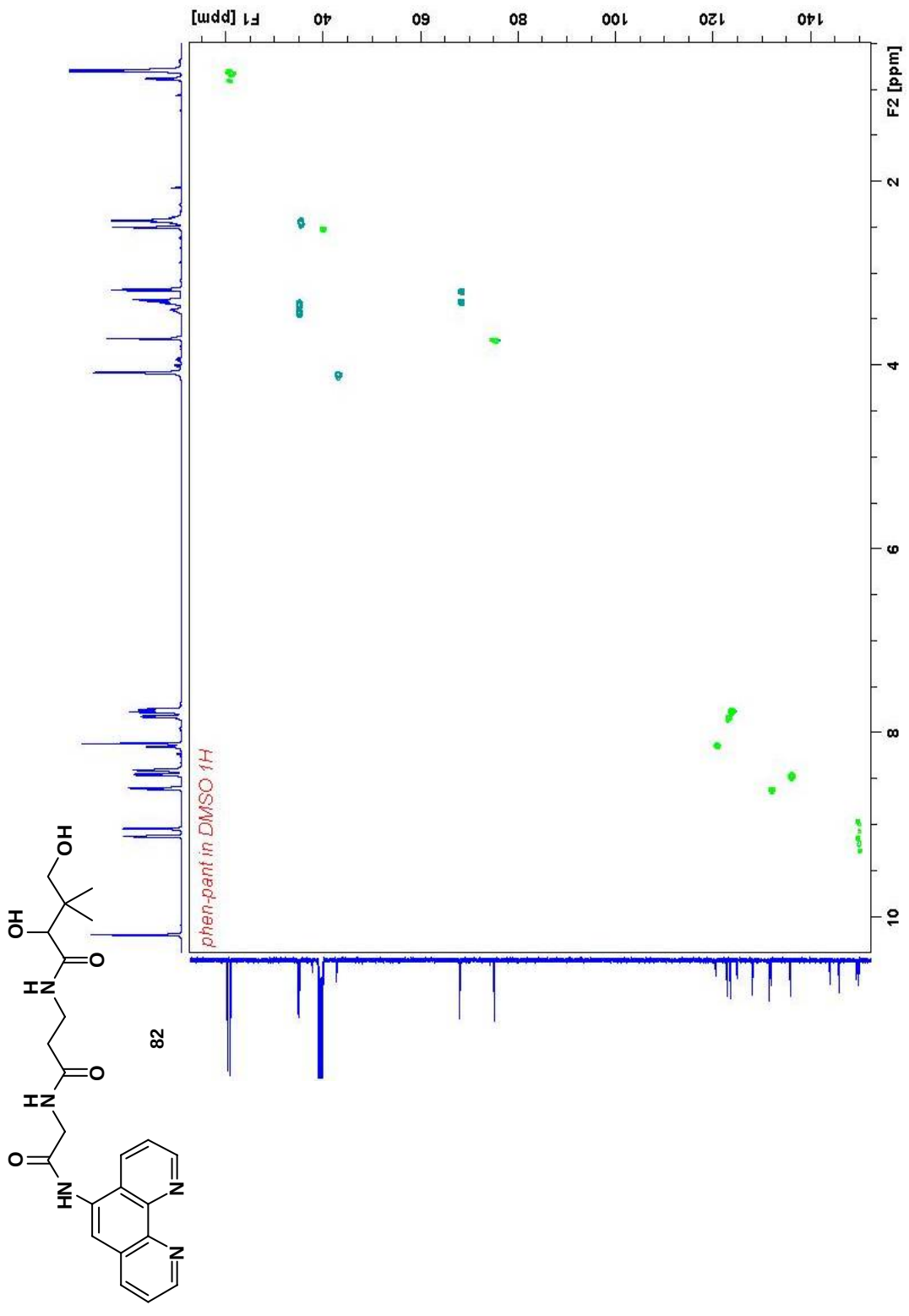

**Spectrum 69 $^{1}$H NMR spectrum of 82 in DMSO-$d_6$.**



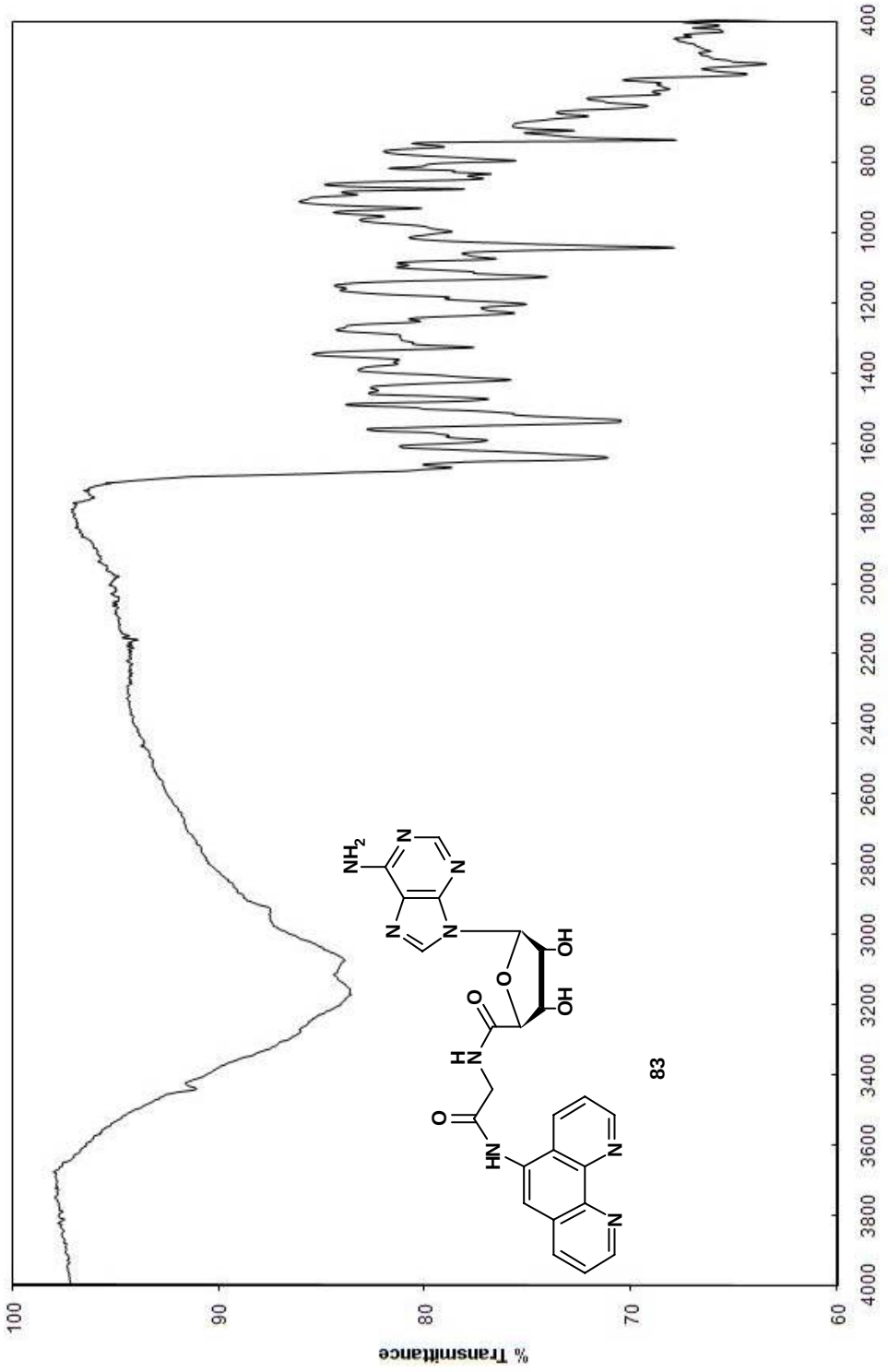

**Spectrum 70 $^{13}$C NMR spectrum of 82 in DMSO-$d_6$.**



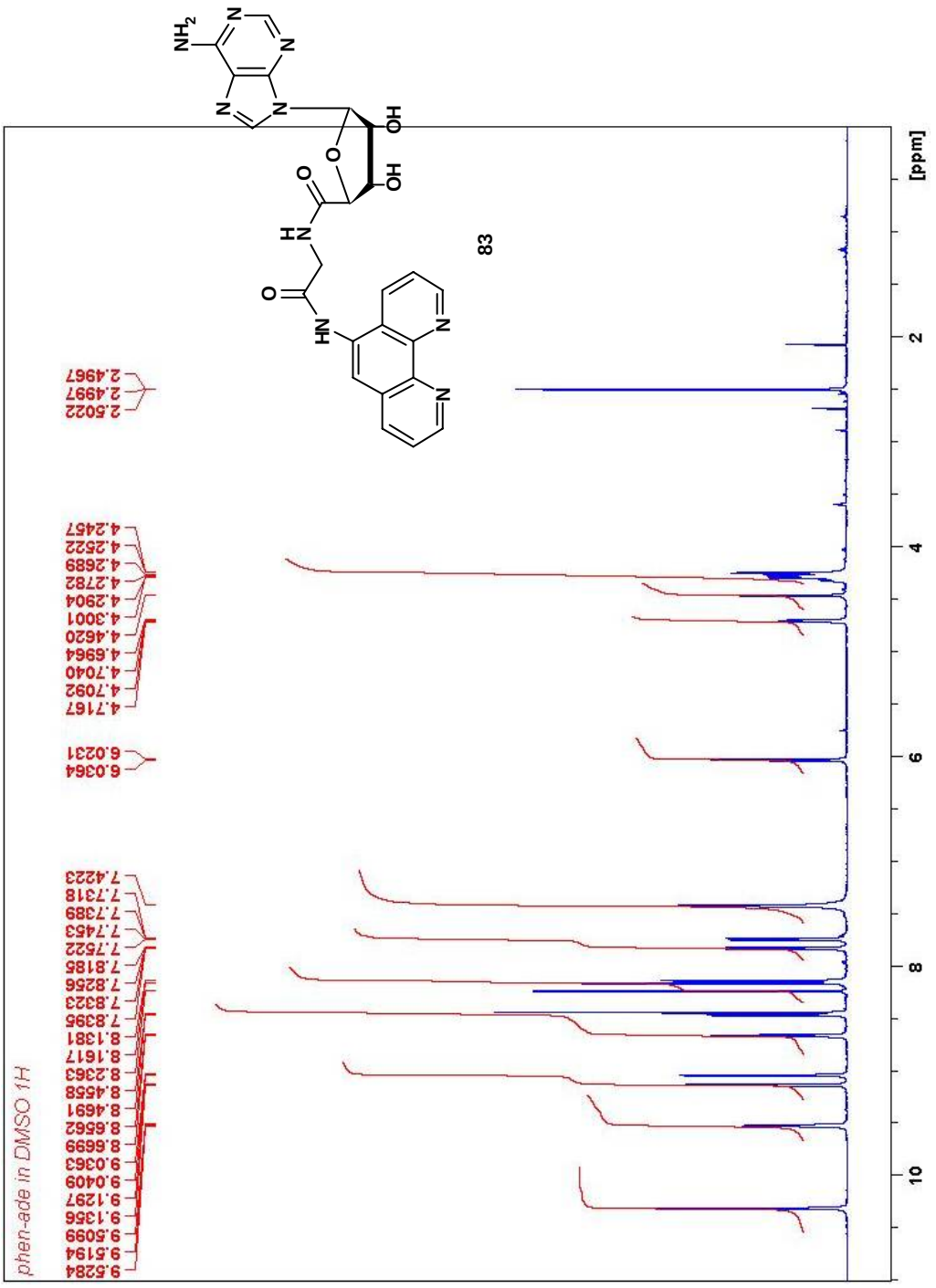

**Spectrum 71 COSY NMR spectrum of 82 in DMSO-$d_6$.**



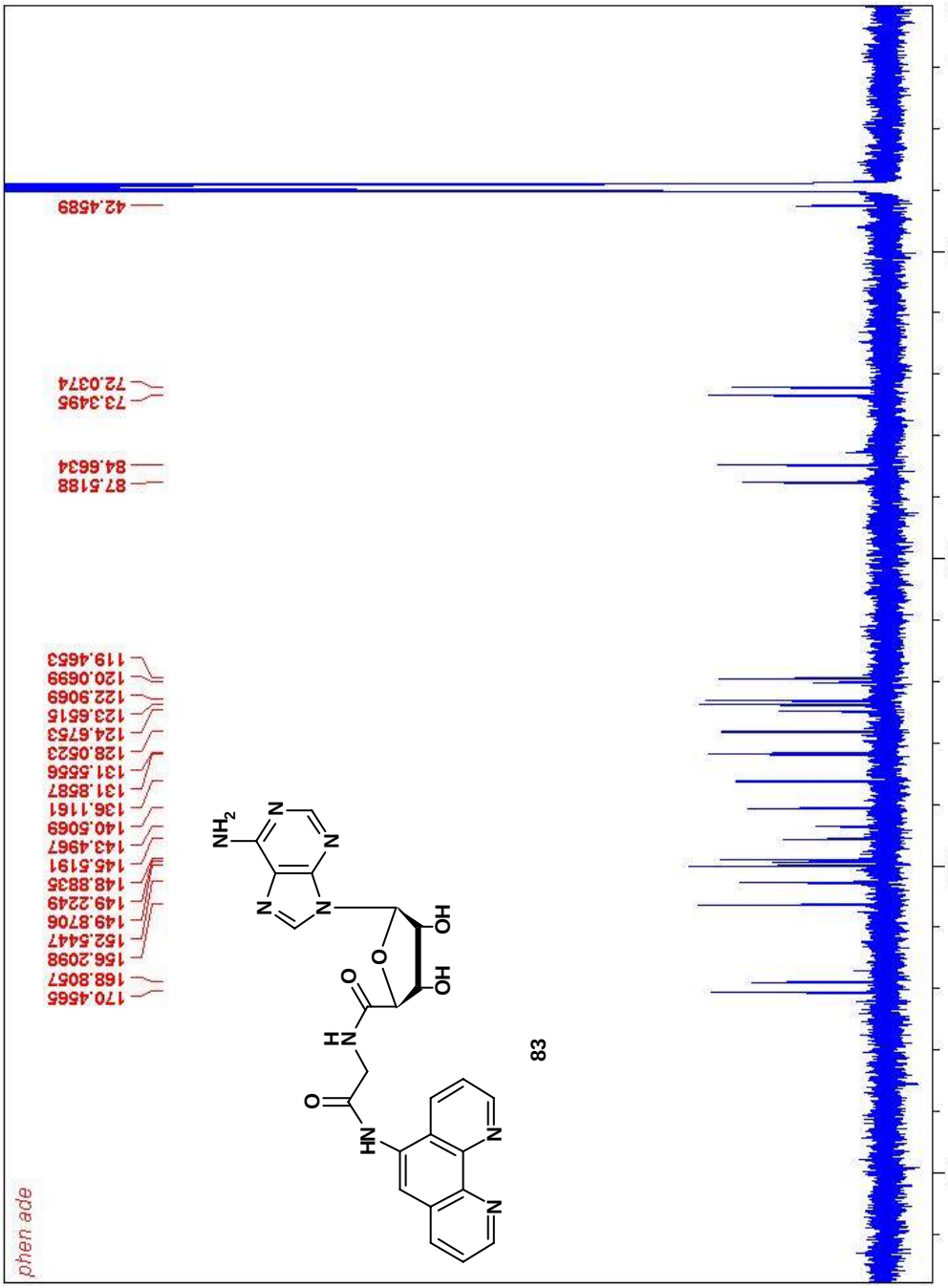

**Spectrum 72 HSQC NMR spectrum of 82 in DMSO-$d_6$.**



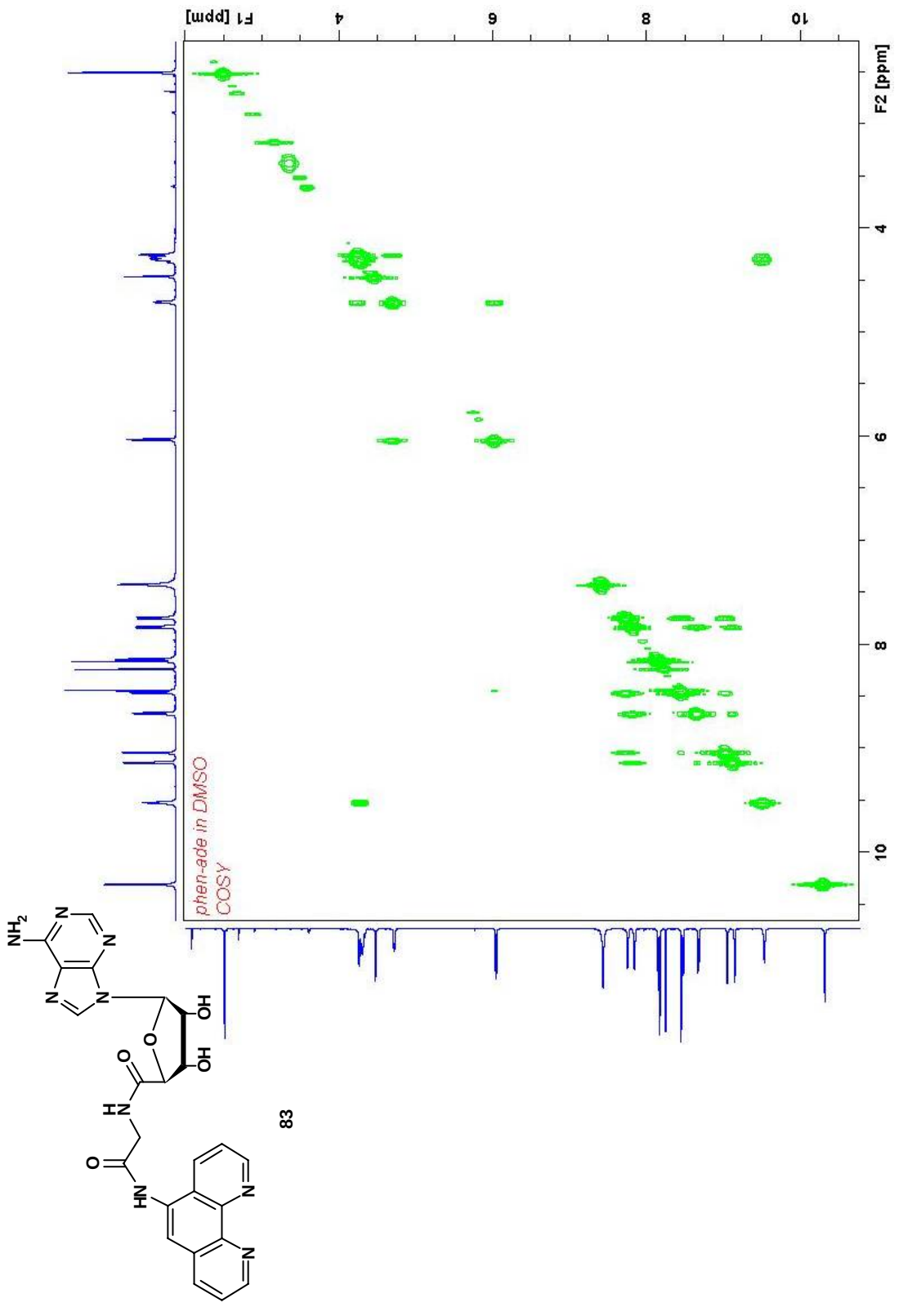

**Spectrum 73 IR spectrum of 83.**



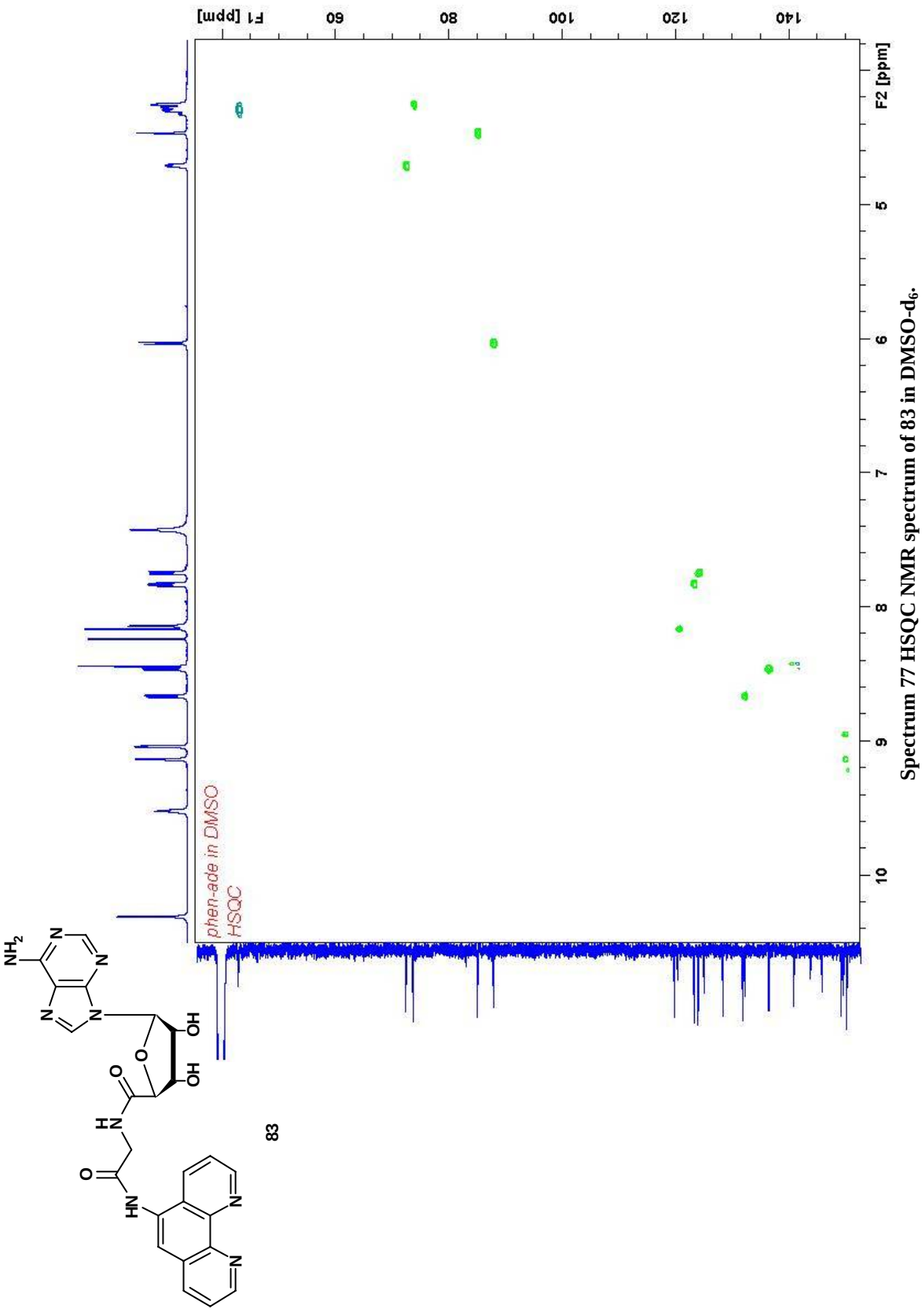

**Spectrum 74 $^{1}$H NMR spectrum of 83 in DMSO-$d_6$.**



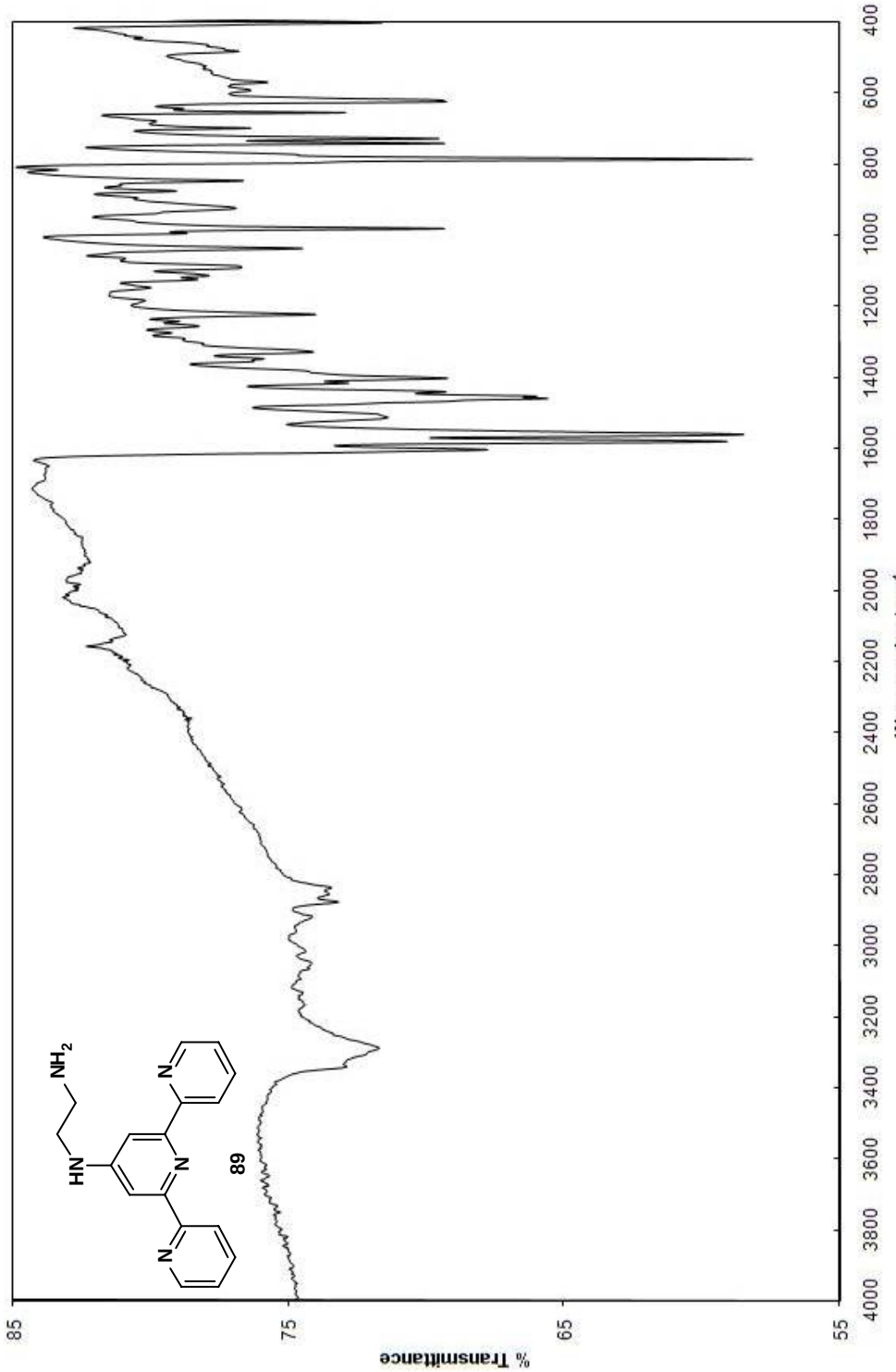

**Spectrum 75 $^{13}$C NMR spectrum of 83 in DMSO-$d_6$.**



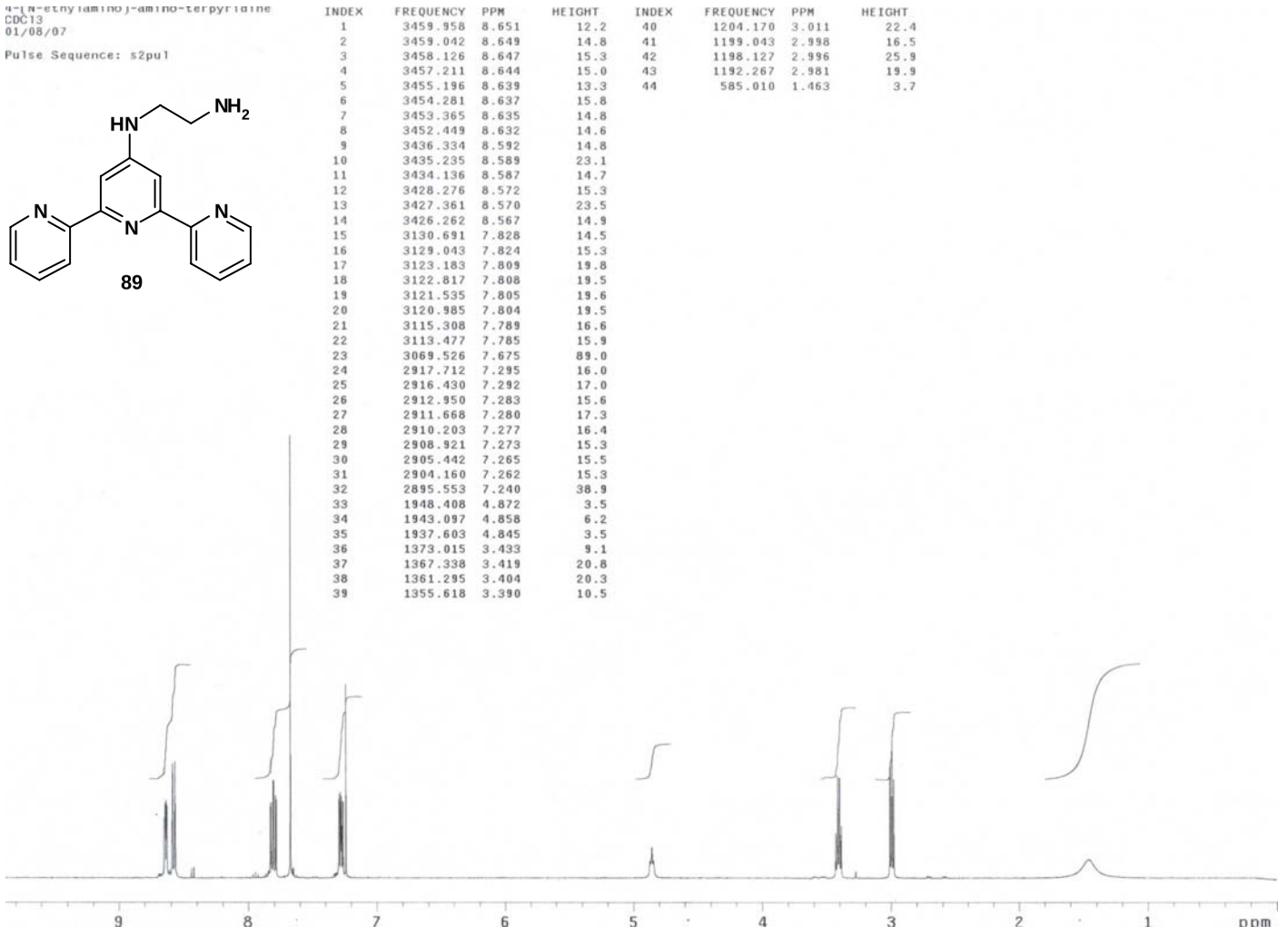

**Spectrum 76 COSY NMR spectrum of 83 in DMSO-$d_6$.**




**Spectrum 77 HSQC NMR spectrum of 83 in DMSO-$d_6$.**



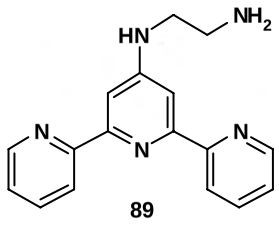

**Spectrum 78 IR spectrum of 89.**




**Spectrum 79 $^{1}$H NMR spectrum of 89 in $CDCl_3$.**

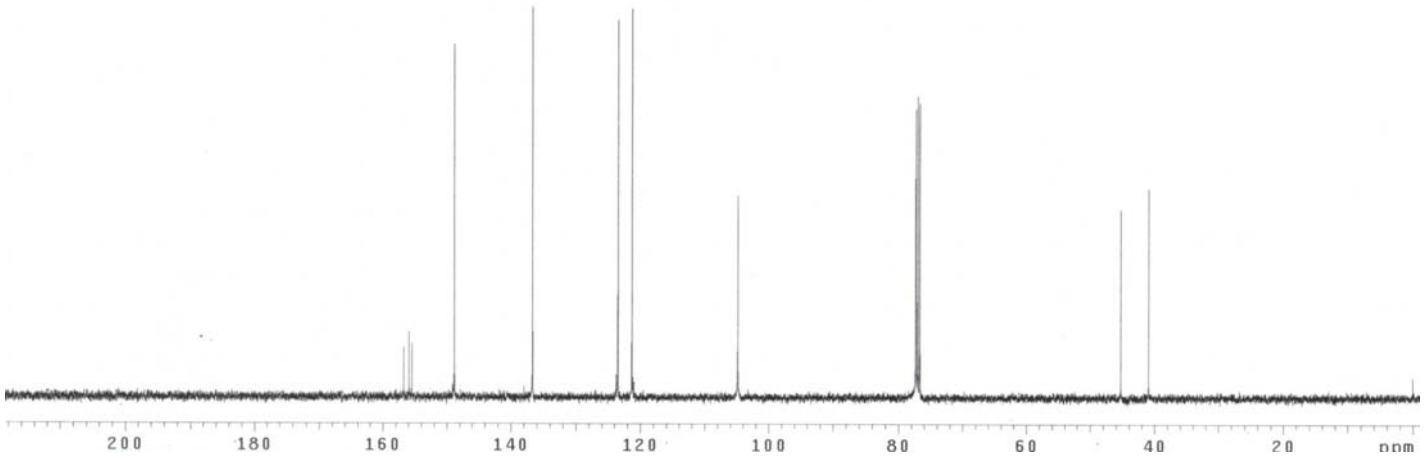
4-(N-ethylamino)-amino-terpyridine
CDCl3
01/08/07

Pulse Sequence: s2pul

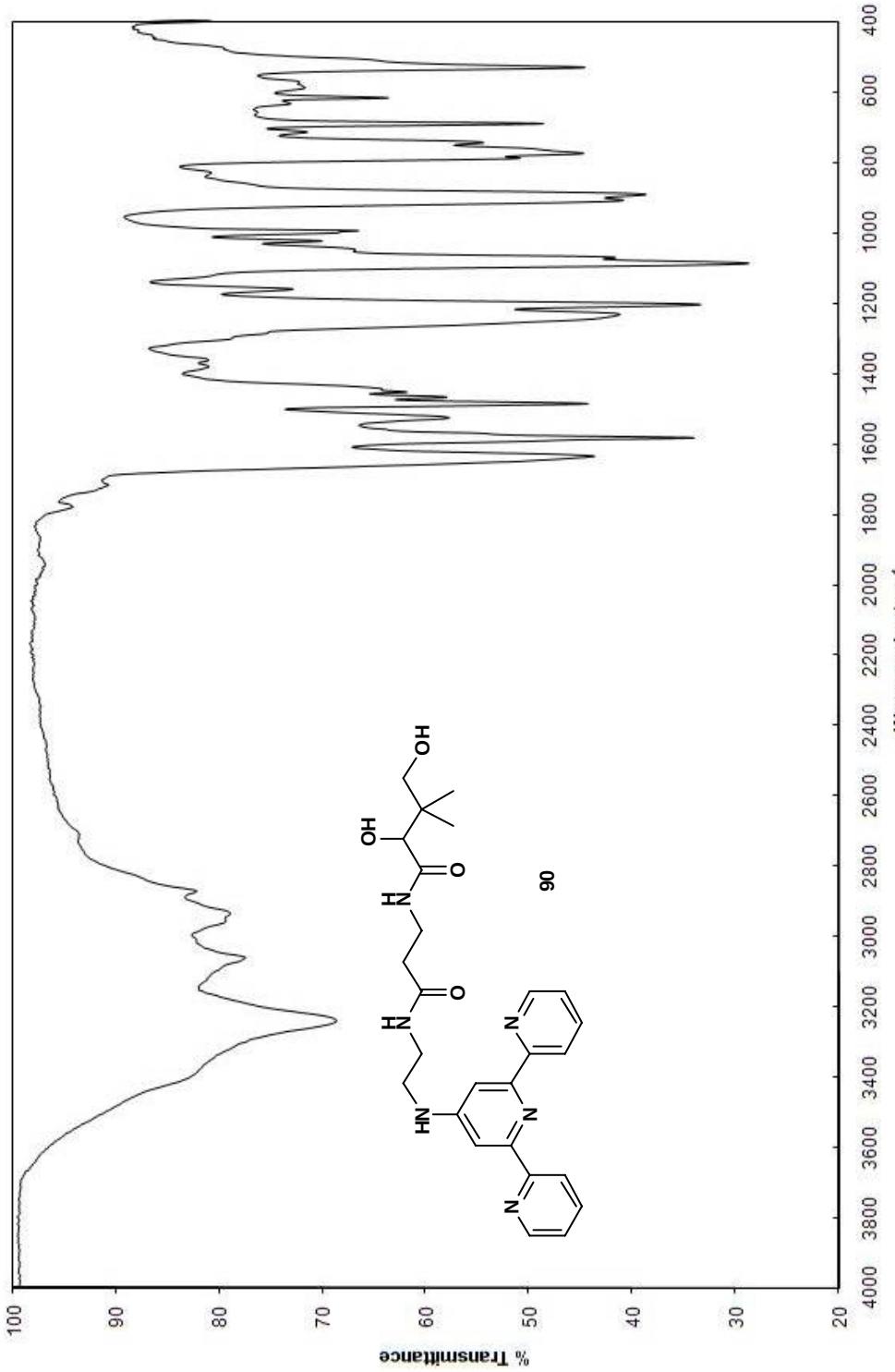



89

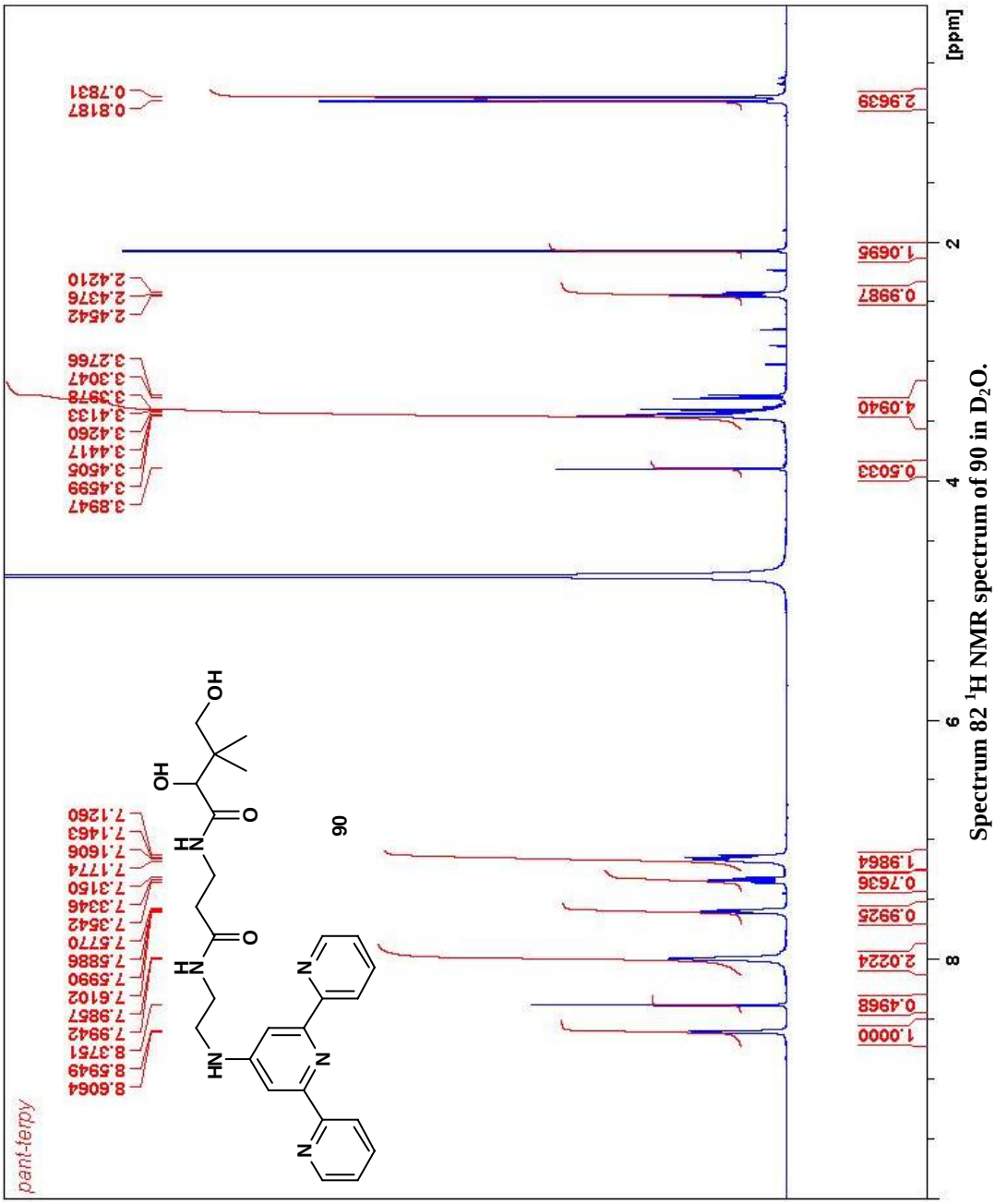
| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 15763.735 | 156.752 | 9.3 |
| 2 | 15677.520 | 155.895 | 12.3 |
| 3 | 15635.557 | 155.478 | 10.1 |
| 4 | 14966.439 | 148.824 | 65.9 |
| 5 | 13748.750 | 136.716 | 72.9 |
| 6 | 12418.143 | 123.484 | 70.5 |
| 7 | 12199.936 | 121.314 | 72.6 |
| 8 | 10541.255 | 104.821 | 37.7 |
| 9 | 7775.515 | 77.319 | 53.9 |
| 10 | 7743.471 | 77.000 | 56.3 |
| 11 | 7711.426 | 76.681 | 54.9 |
| 12 | 4551.998 | 45.264 | 35.2 |
| 13 | 4118.635 | 40.955 | 39.0 |



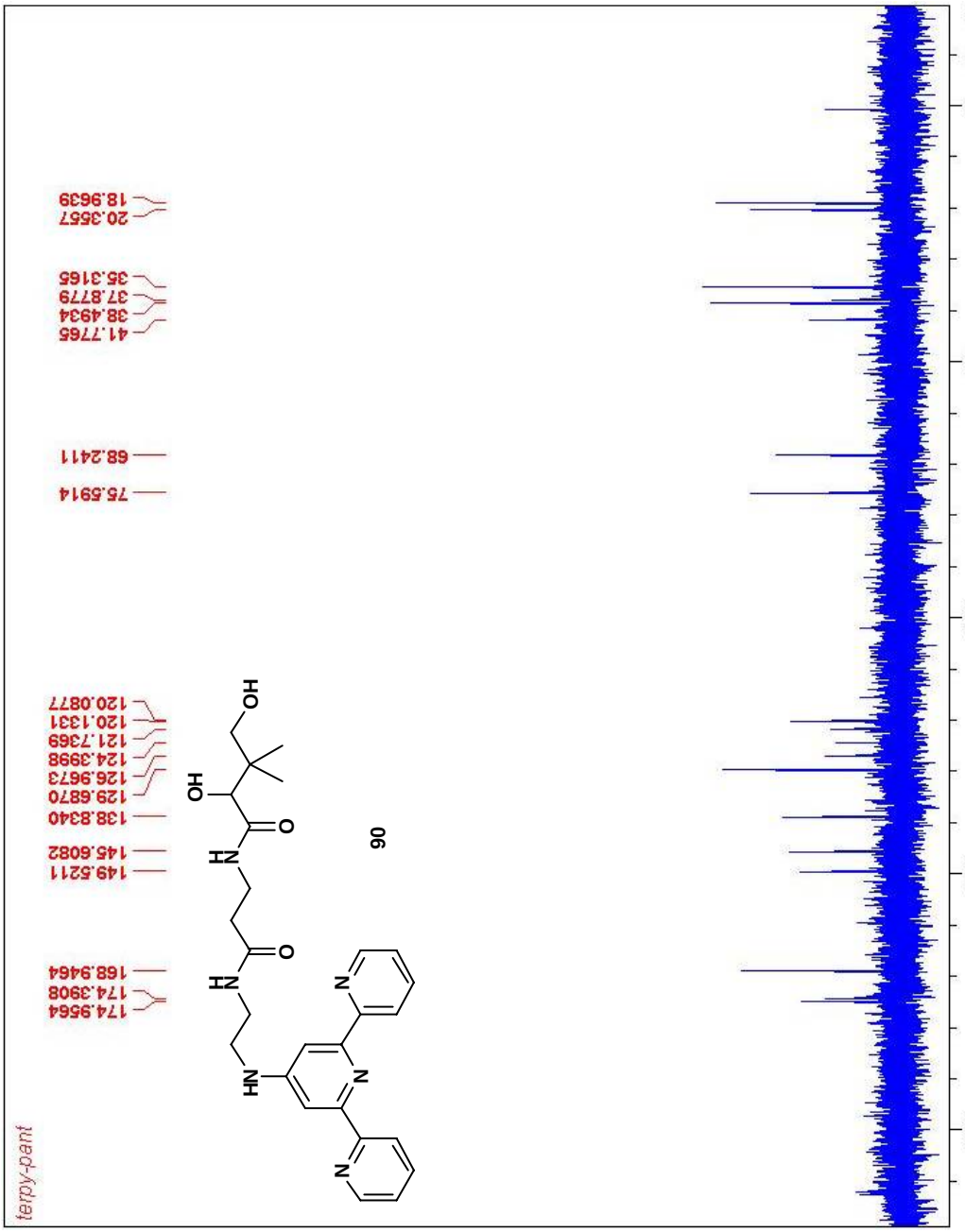

**Spectrum 80 $^{13}C$ NMR spectrum of 89 in $CDCl_3$.**



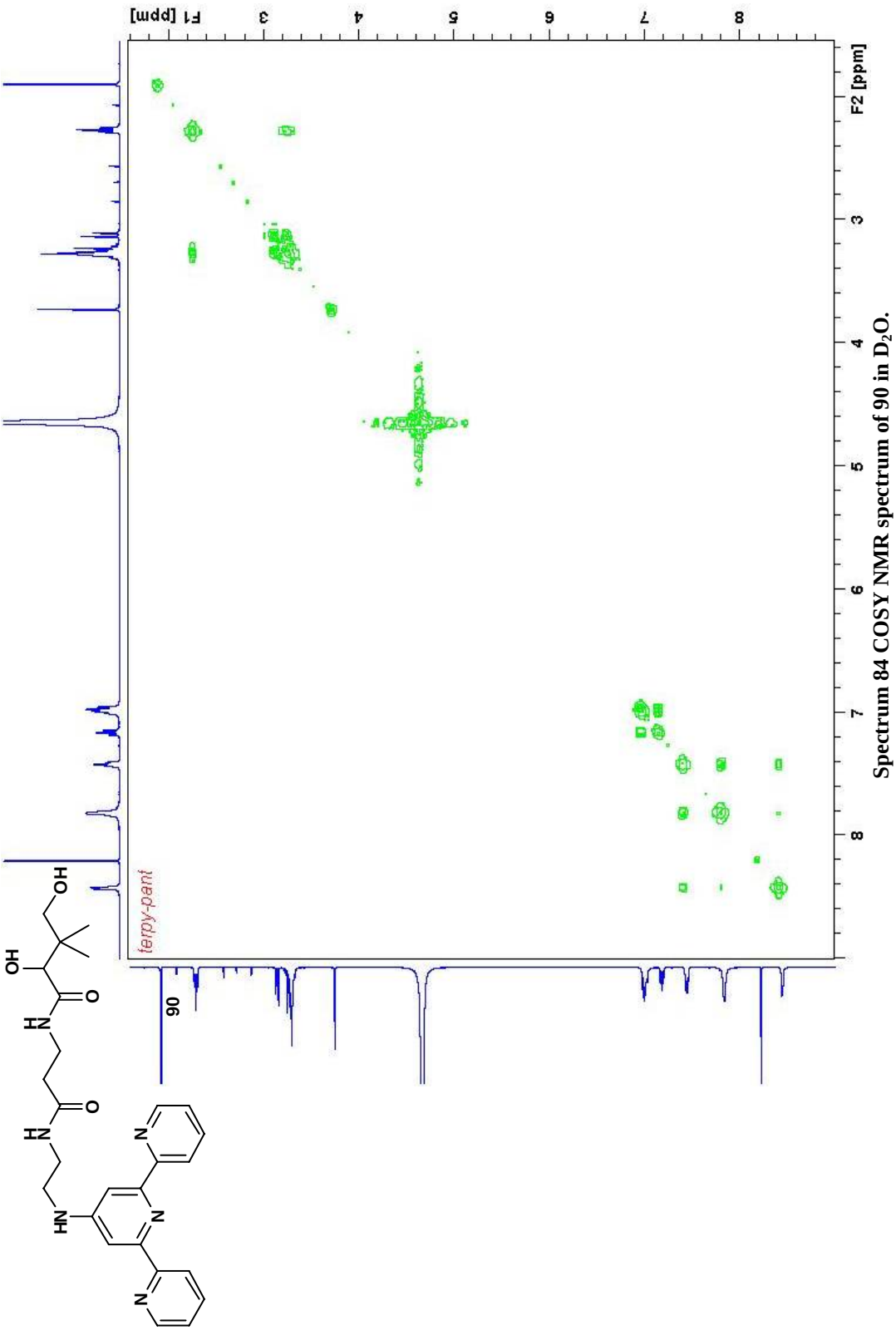

**Spectrum 81 IR spectrum of 90.**



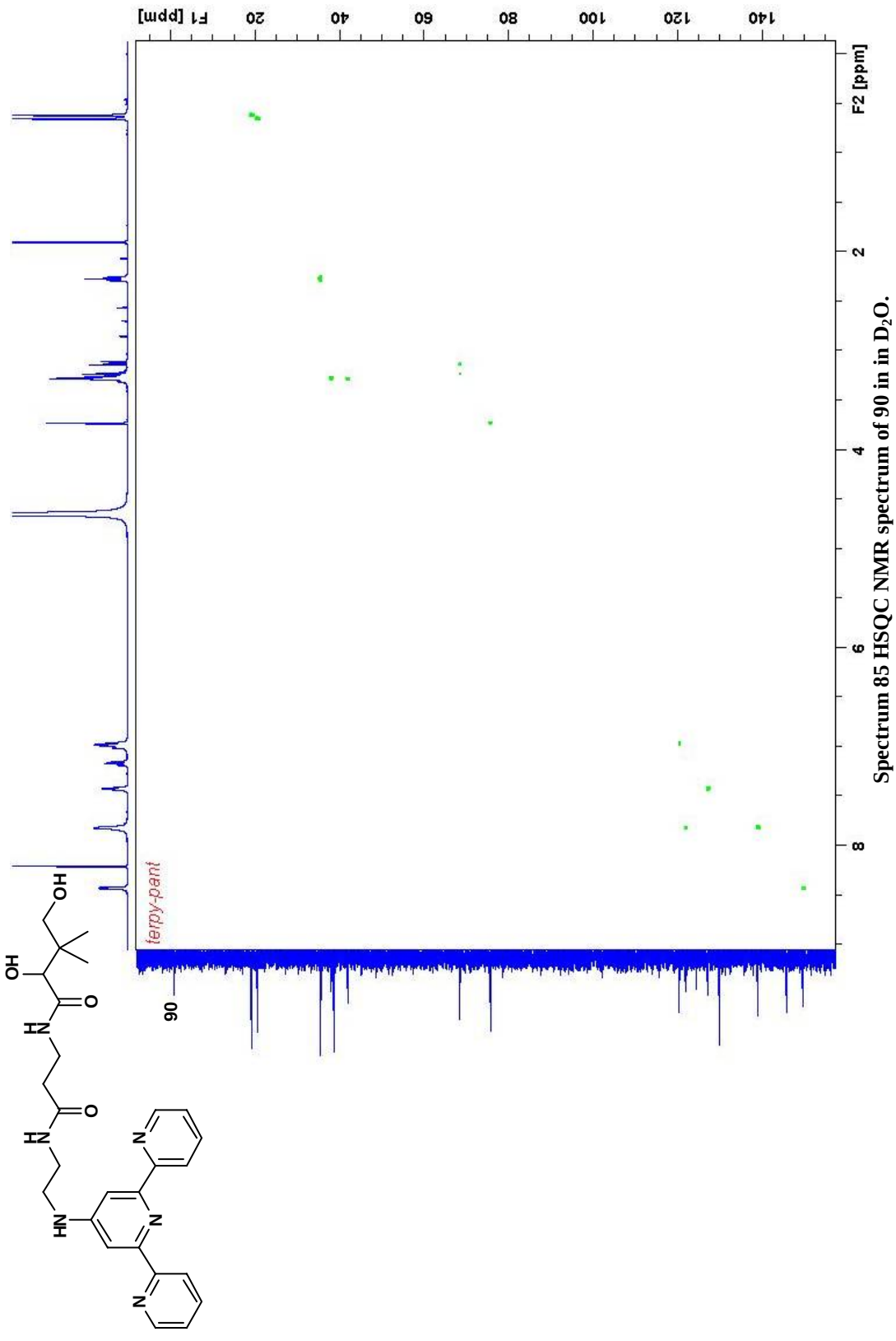

**Spectrum 82 $^1$H NMR spectrum of 90 in $D_2O$.**



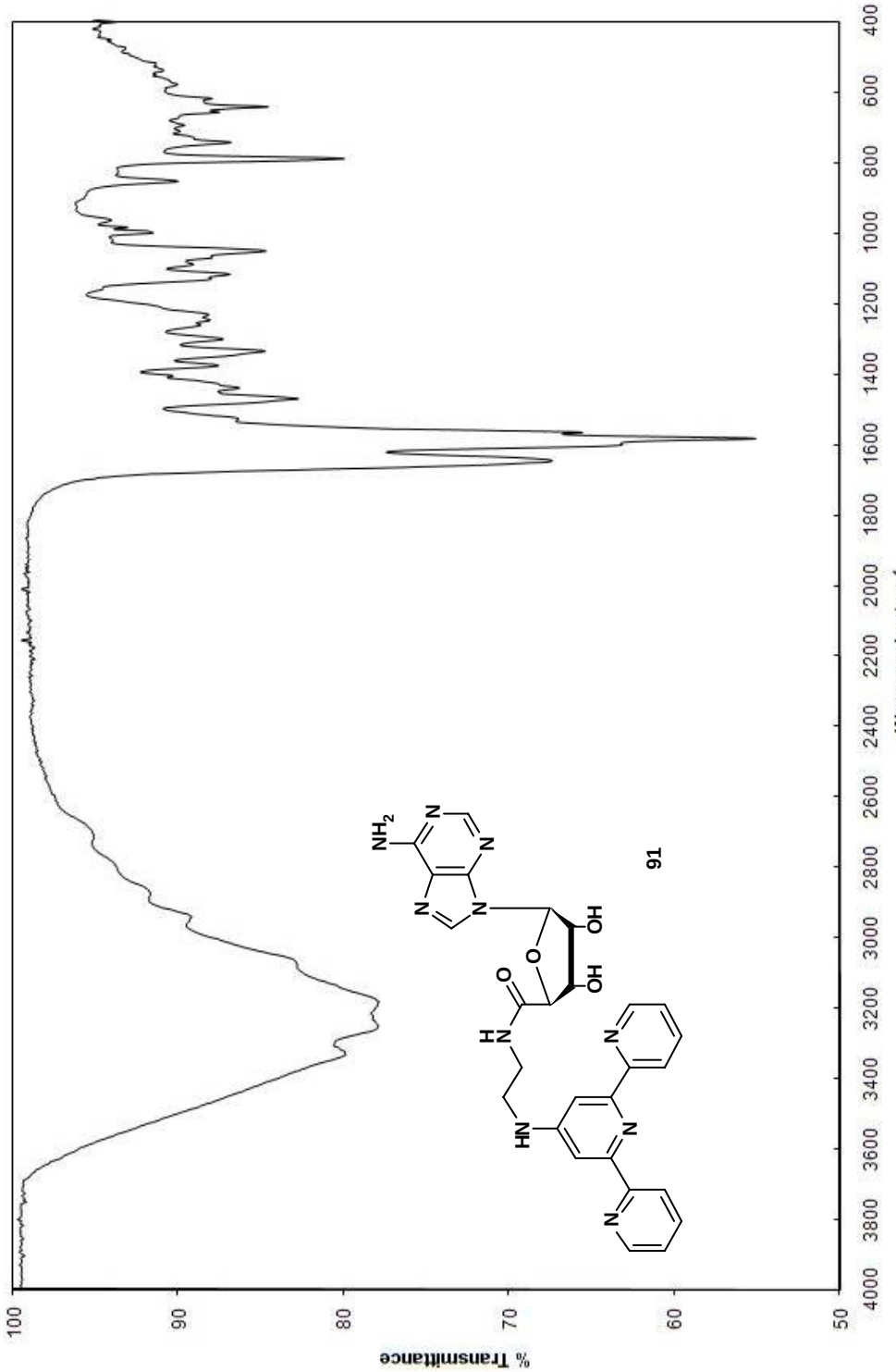

**Spectrum 83 $^{13}$C NMR spectrum of 90 in $D_2O$.**



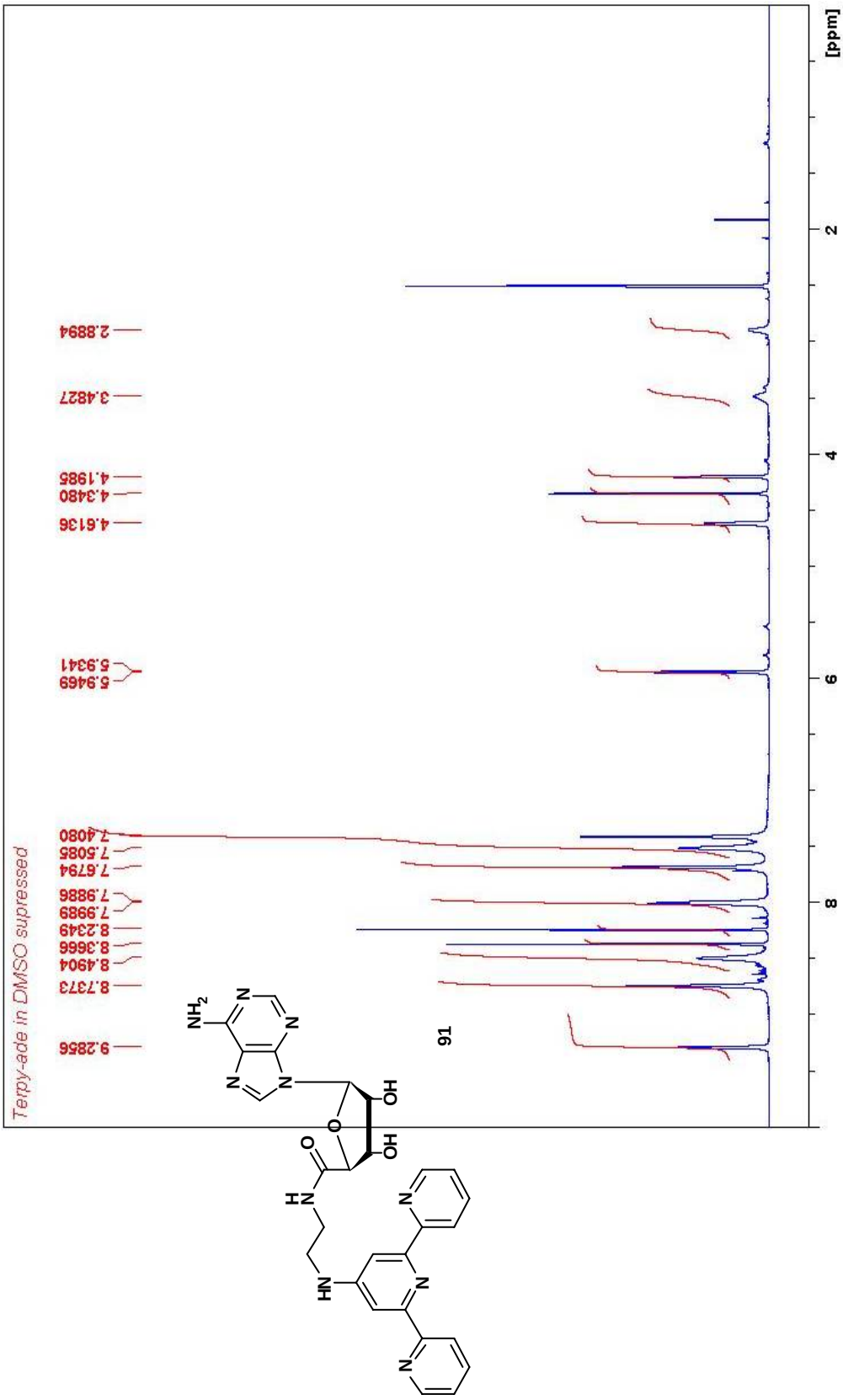

**Spectrum 84 COSY NMR spectrum of 90 in $D_2O$.**



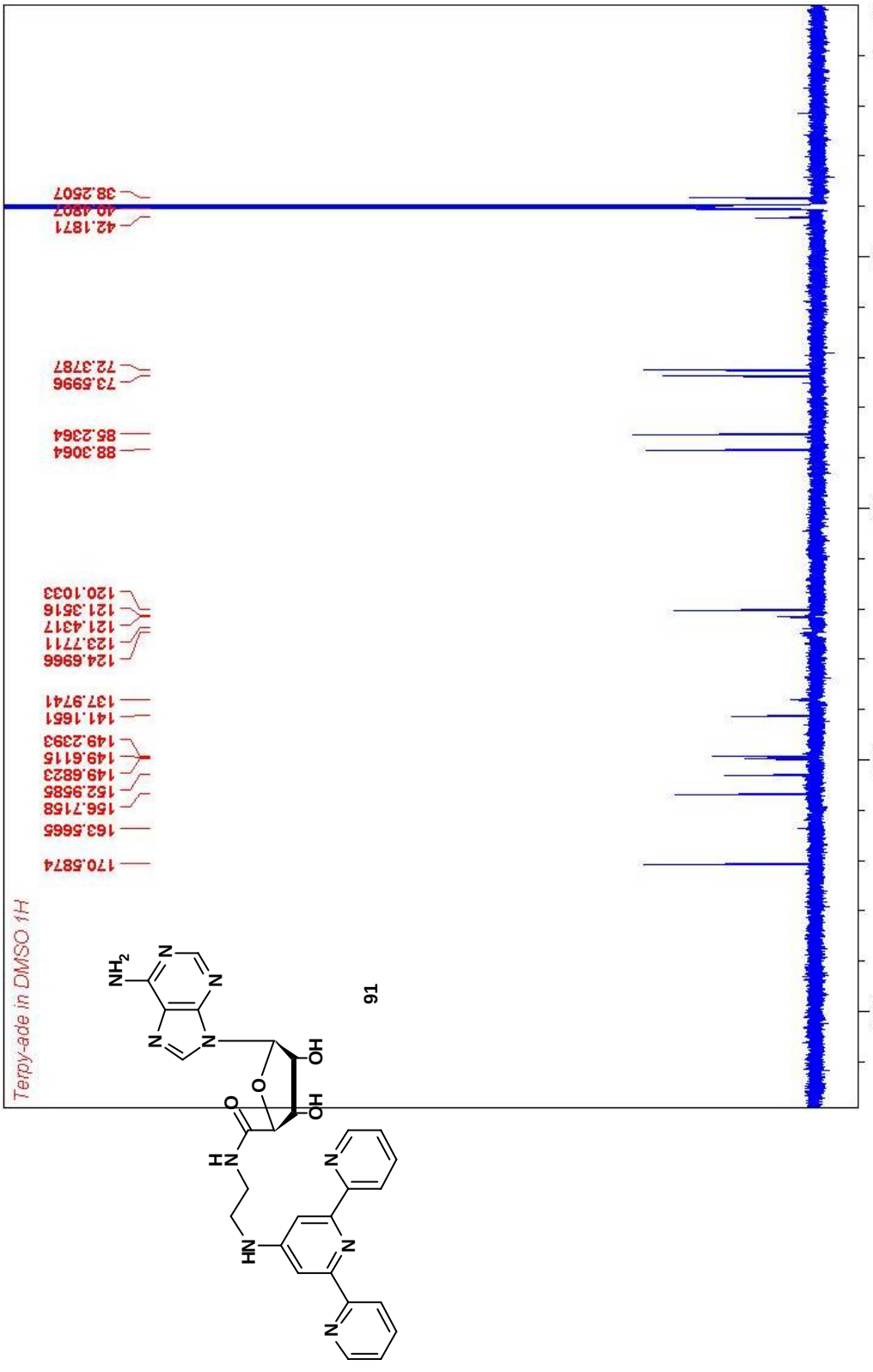

**Spectrum 85 HSQC NMR spectrum of 90 in in $D_2O$.**



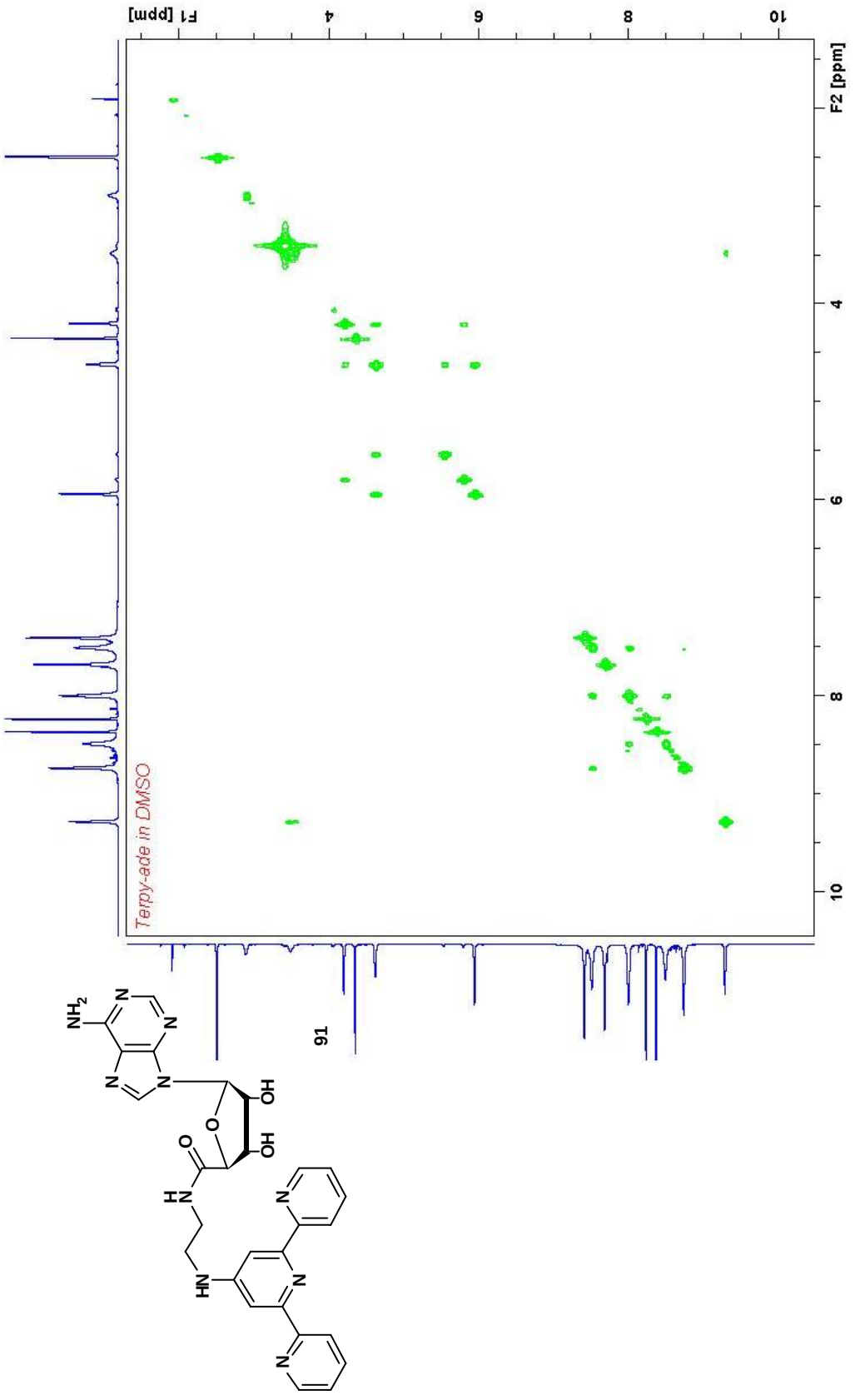

**Spectrum 86 IR spectrum of 91.**



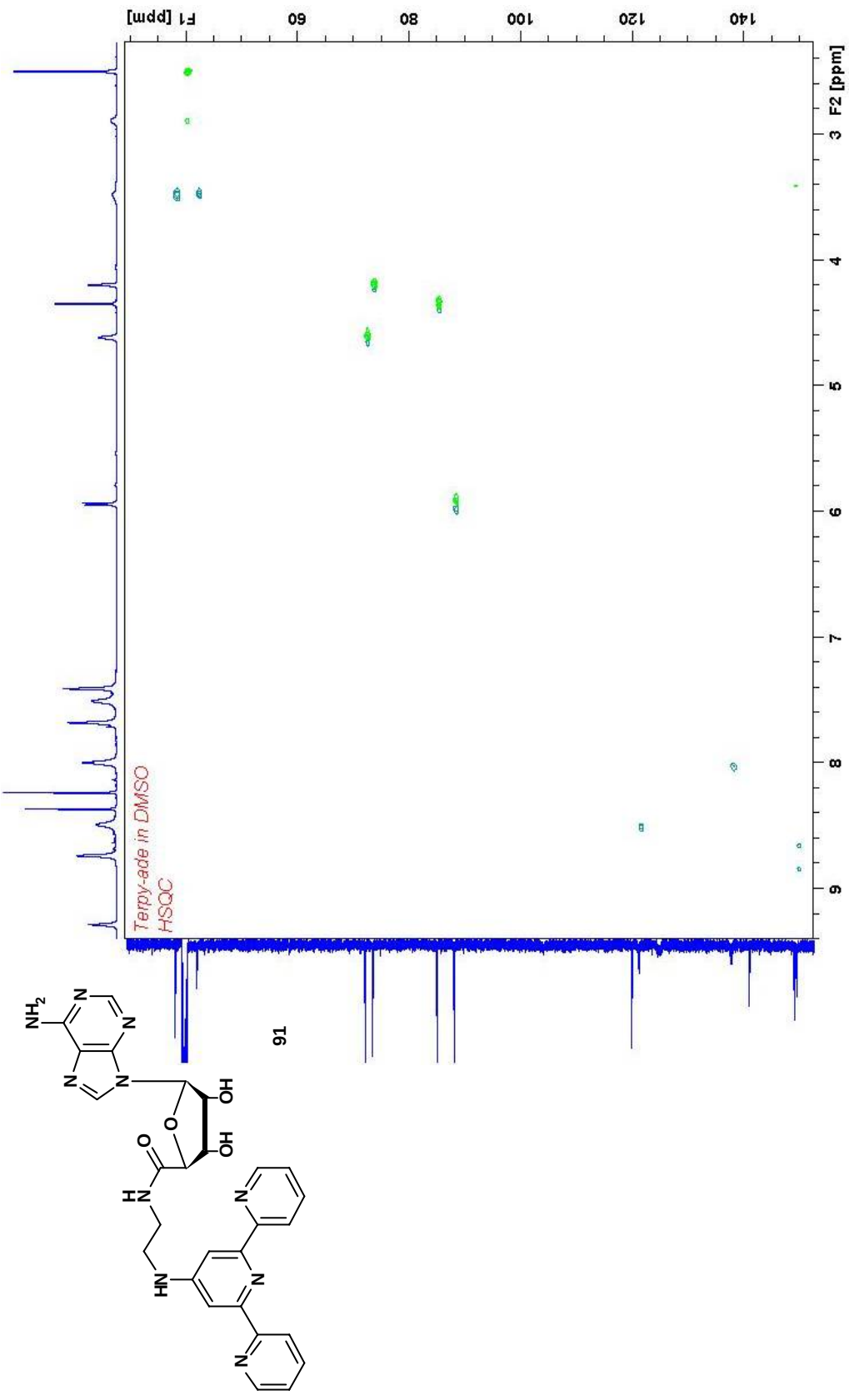

**Spectrum 87 $^{1}$H NMR spectrum of 91 in DMSO-$d_6$.**



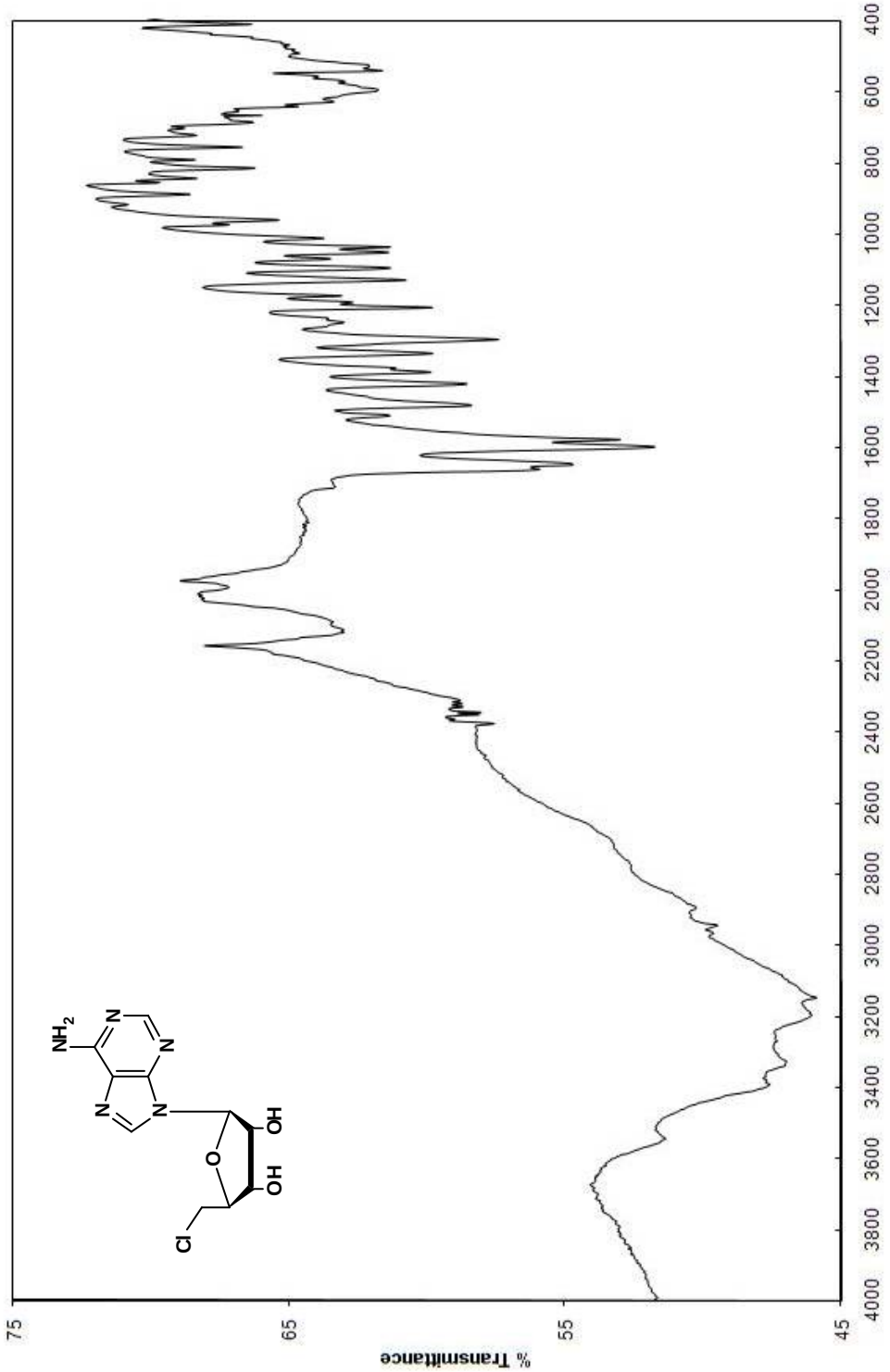

**Spectrum 88 $^{13}$C NMR spectrum of 91 in DMSO-$d_6$.**



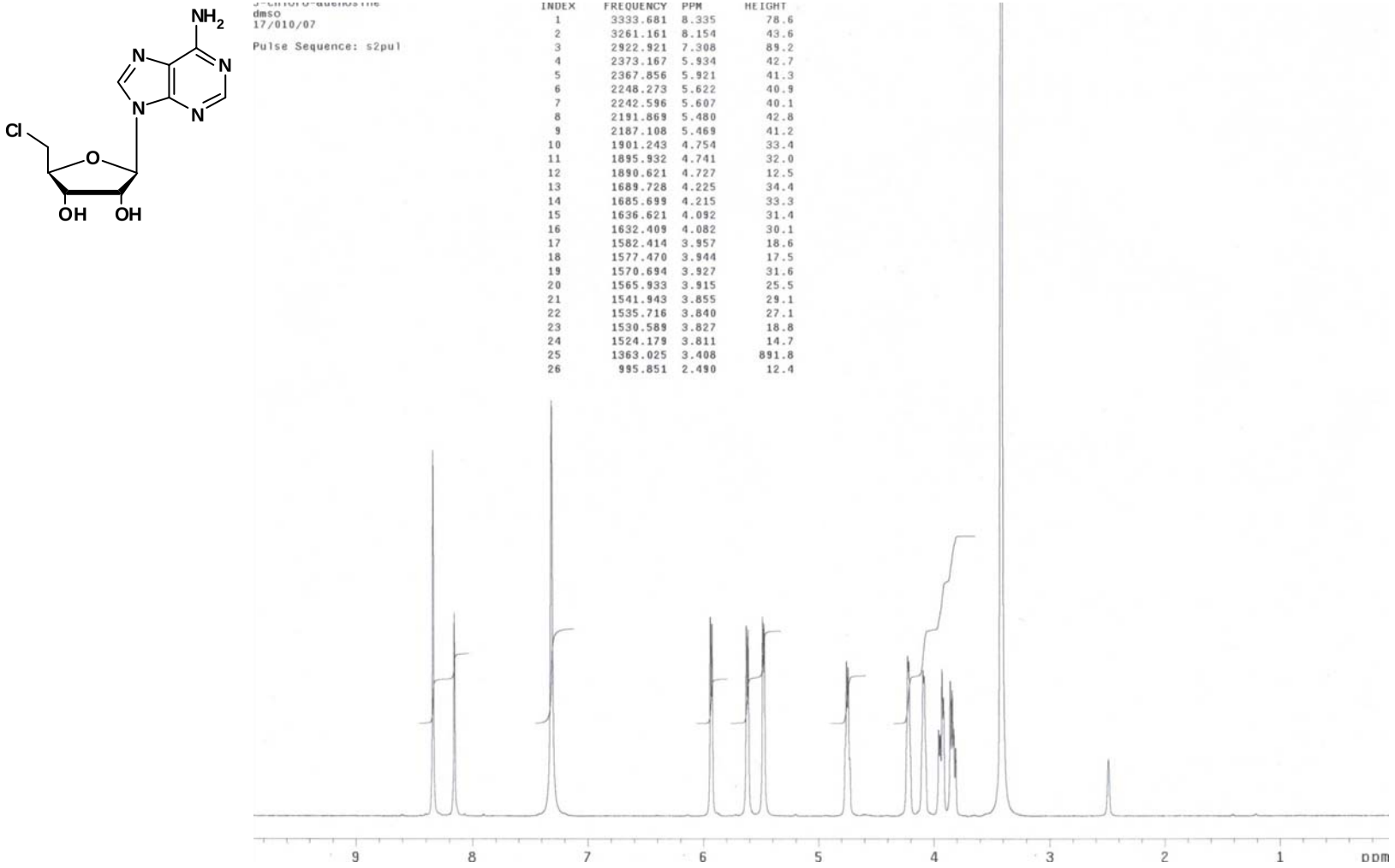

**Spectrum 89 COSY NMR spectrum of 91 in DMSO-$d_6$.**




**Spectrum 90 HSQC NMR spectrum of 91 in DMSO-$d_6$.**




**Spectrum 91 IR spectrum of (2R,3S,4R,5S)-2-(6-amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol.**

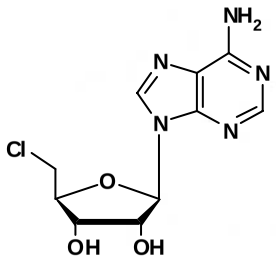


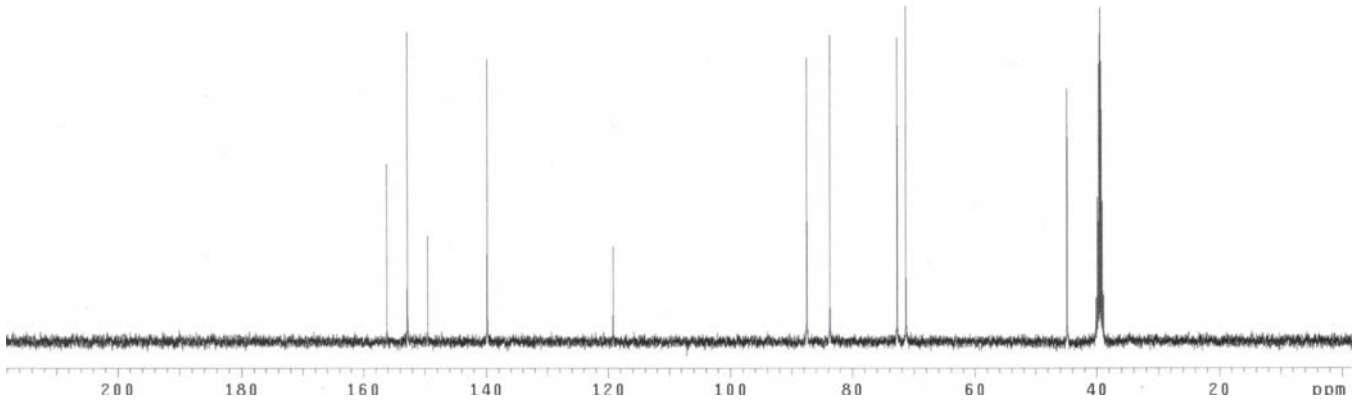

**Spectrum 92 $^{1}$H NMR spectrum of (2R,3S,4R,5S)-2-(6-amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol in DMSO-$d_6$.**

dmso
17/010/07

Pulse Sequence: s2pul

| INDEX | FREQUENCY | PPM | HEIGHT |
|---|---|---|---|
| 1 | 15702.109 | 156.139 | 32.7 |
| 2 | 15366.406 | 152.801 | 56.9 |
| 3 | 15033.754 | 149.493 | 19.6 |
| 4 | 14061.739 | 139.827 | 51.9 |
| 5 | 11984.192 | 119.169 | 17.7 |
| 6 | 8798.060 | 87.486 | 52.2 |
| 7 | 8418.104 | 83.708 | 56.4 |
| 8 | 7311.808 | 72.707 | 56.0 |
| 9 | 7170.660 | 71.304 | 61.7 |
| 10 | 4513.261 | 44.879 | 46.5 |
| 11 | 4014.283 | 39.917 | 26.6 |
| 12 | 3993.683 | 39.712 | 51.1 |
| 13 | 3972.320 | 39.500 | 61.4 |
| 14 | 3950.957 | 39.288 | 51.6 |
| 15 | 3930.357 | 39.083 | 25.9 |

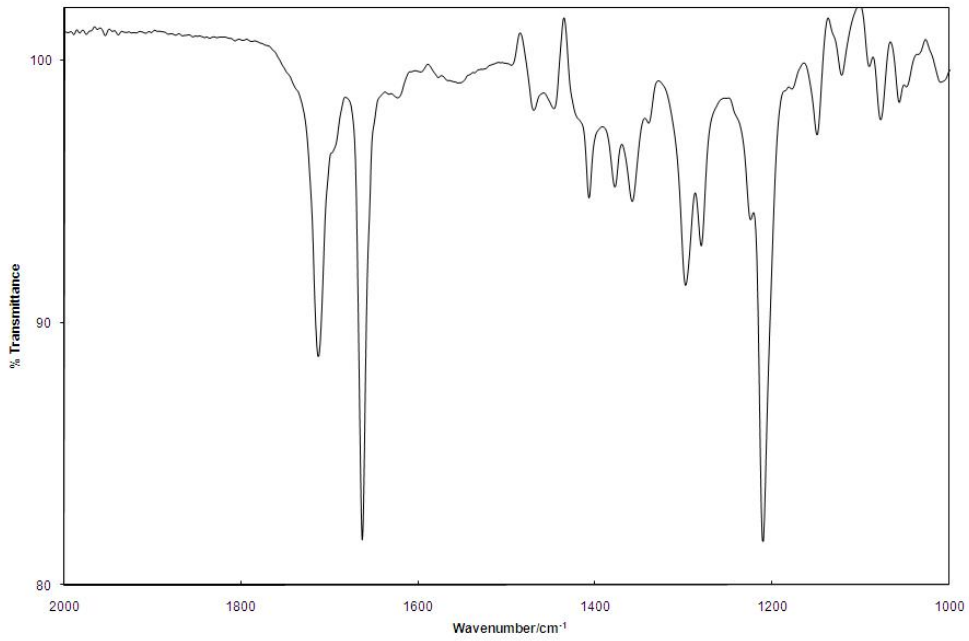


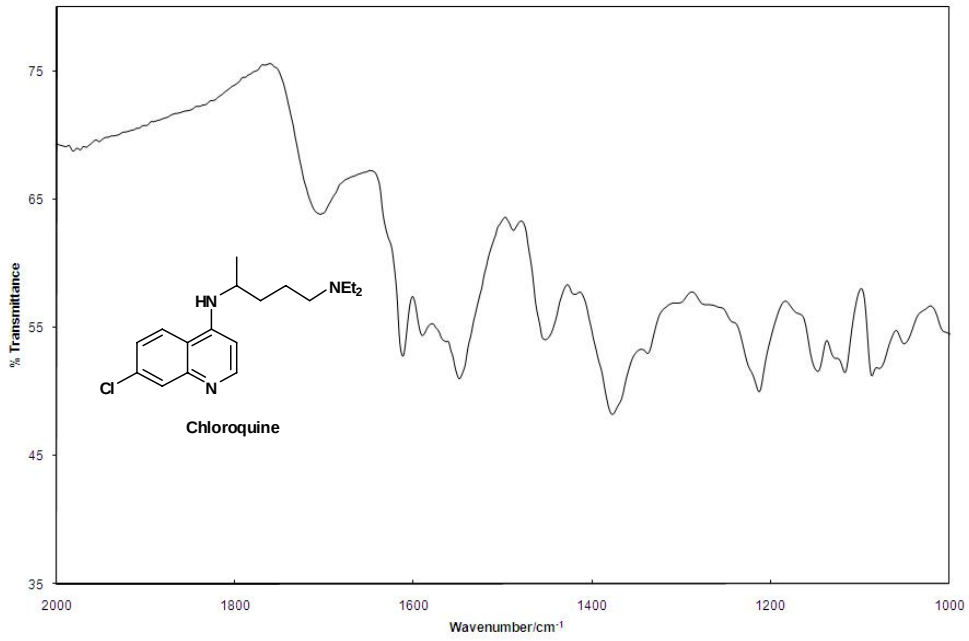

**Spectrum 93: $^{13}$C NMR spectrum of (2R,3S,4R,5S)-2-(6-amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol in DMSO-$d_6$.**

# Appendix 2

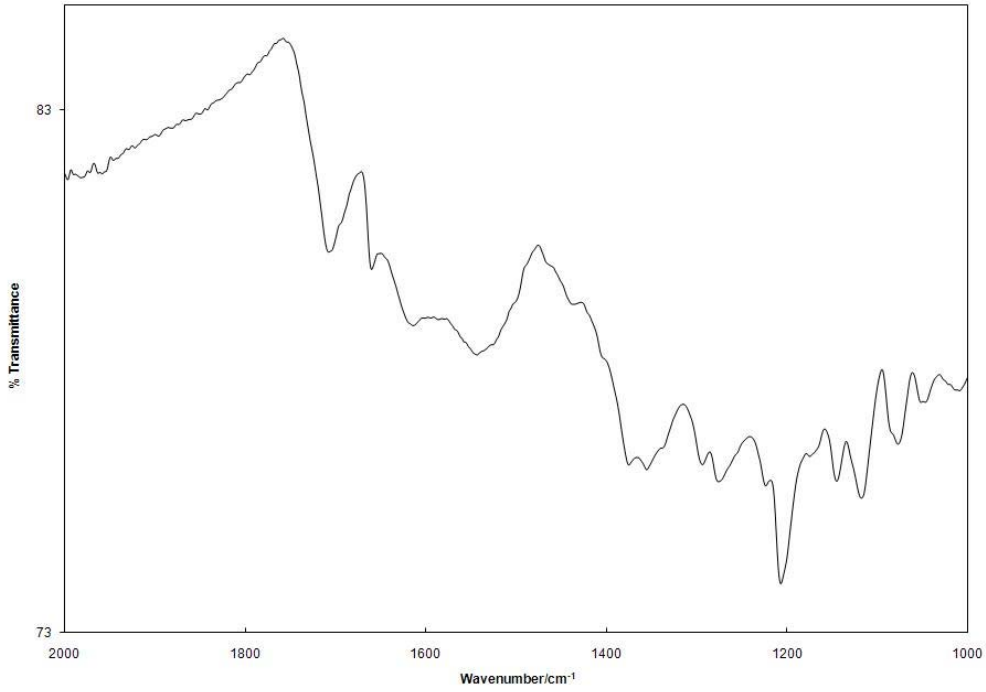
# B-Haematin Inhibition Results



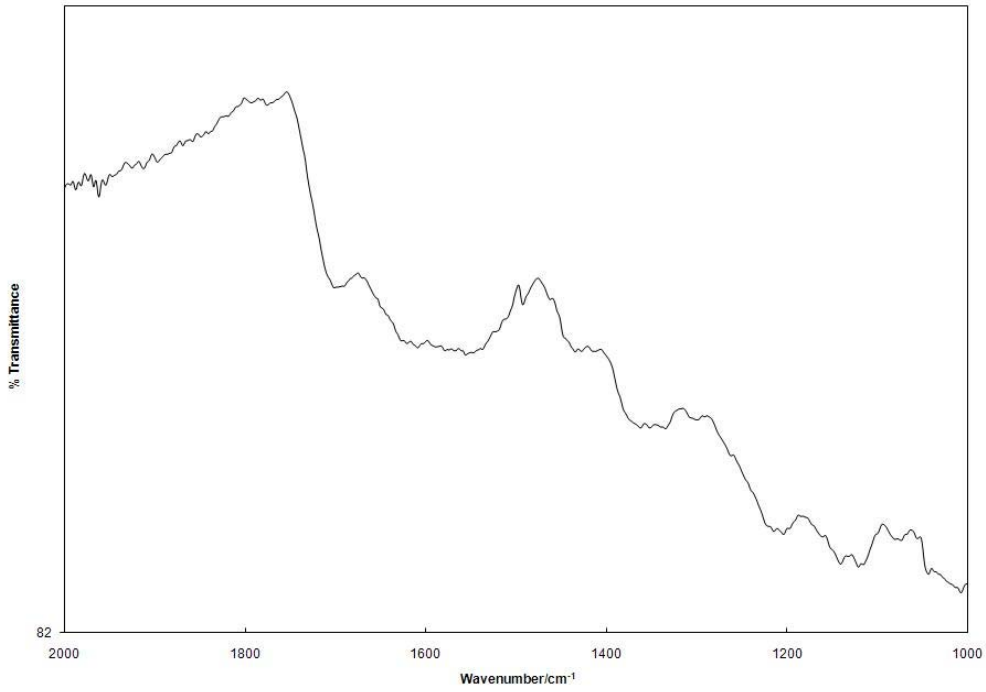

**Spectrum 94 IR spectrum of β-haematin positive control.**



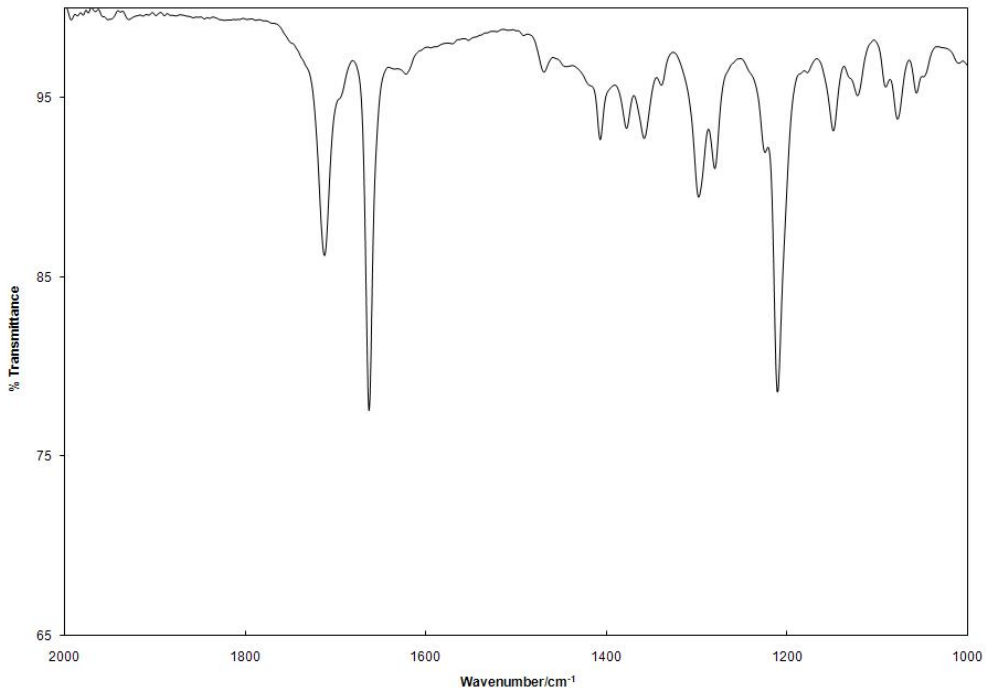

**Spectrum 95 IR spectrum of β-haematin negative control (chloroquine).**




**Spectrum 96 IR spectrum of $K_2PtCl_4$ β-haematin assay.**

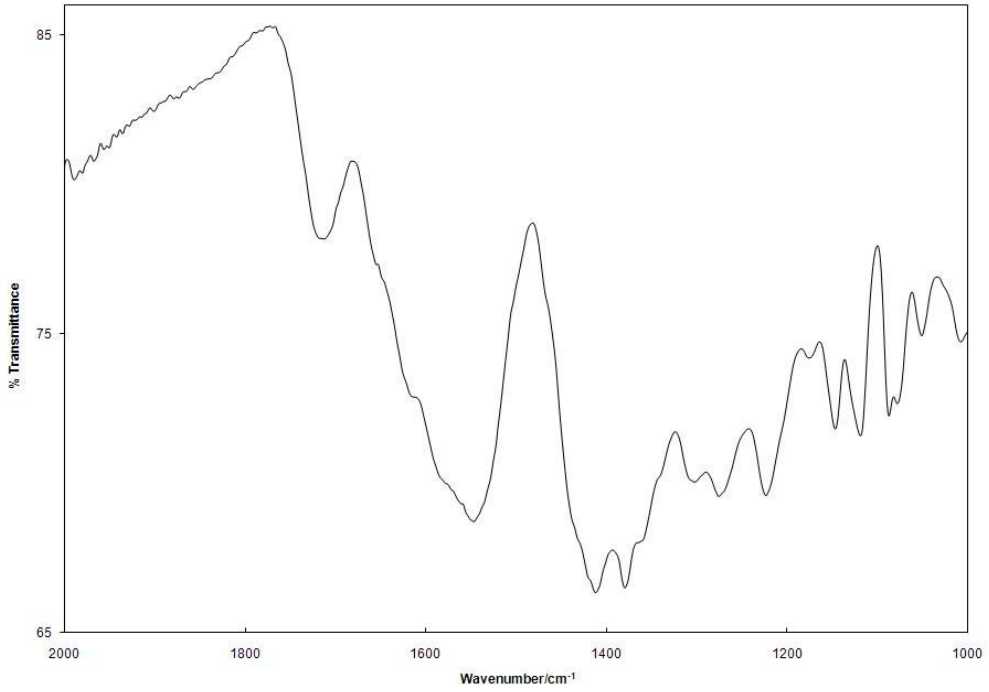



**Spectrum 97 IR spectrum of $HAuCl_4$ β-haematin assay.**

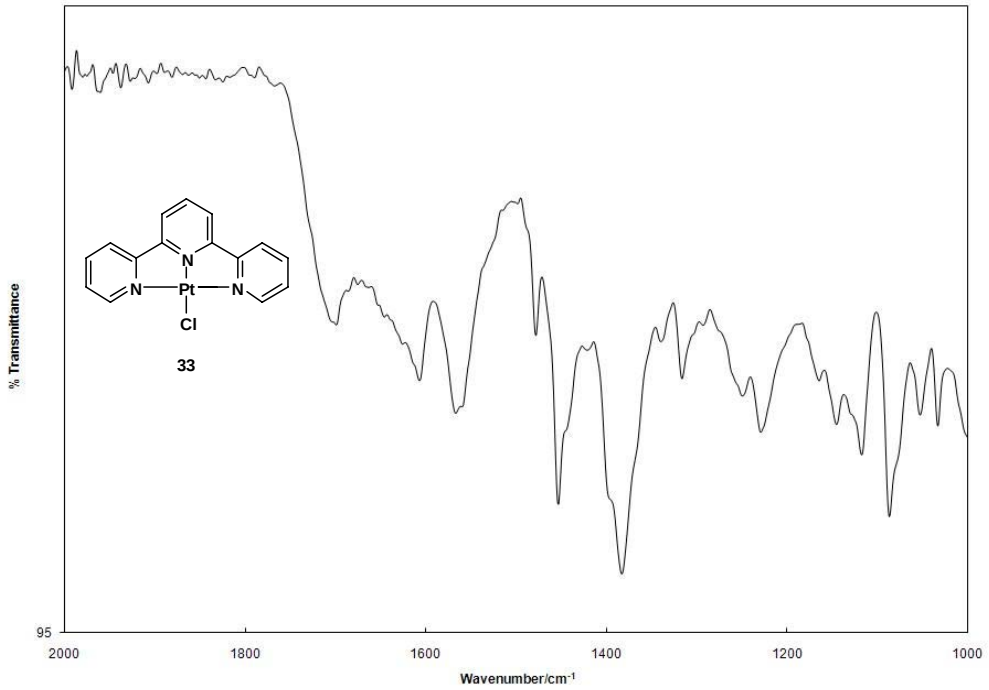



**Spectrum 98 IR spectrum of $CuCl_2$ β-haematin assay.**

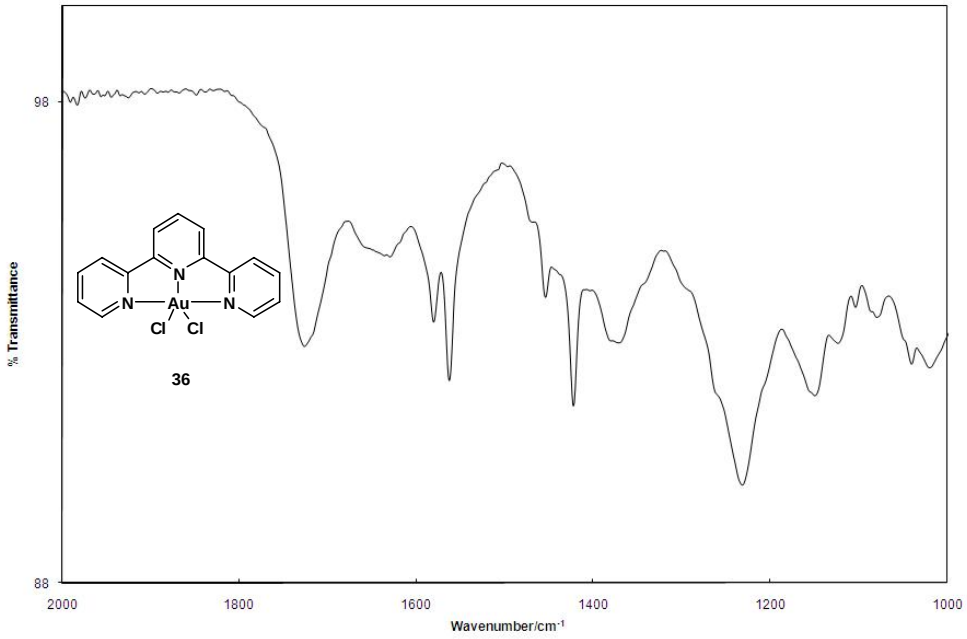

**Spectrum 99 IR spectrum of $FeCl_3$ β-haematin assay.**



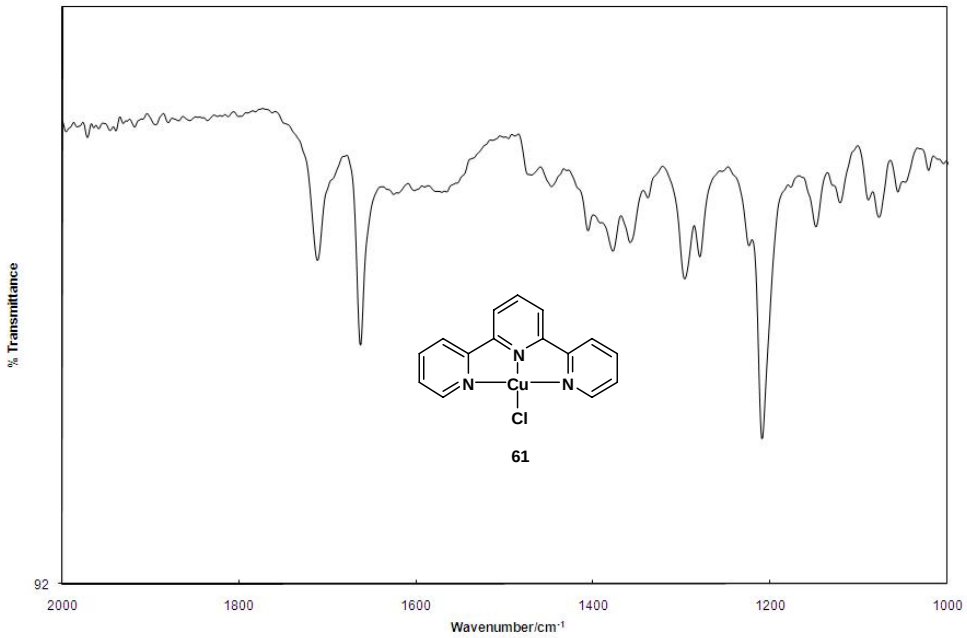

**Spectrum 100 IR spectrum of $PdCl_2$ β-haematin assay.**



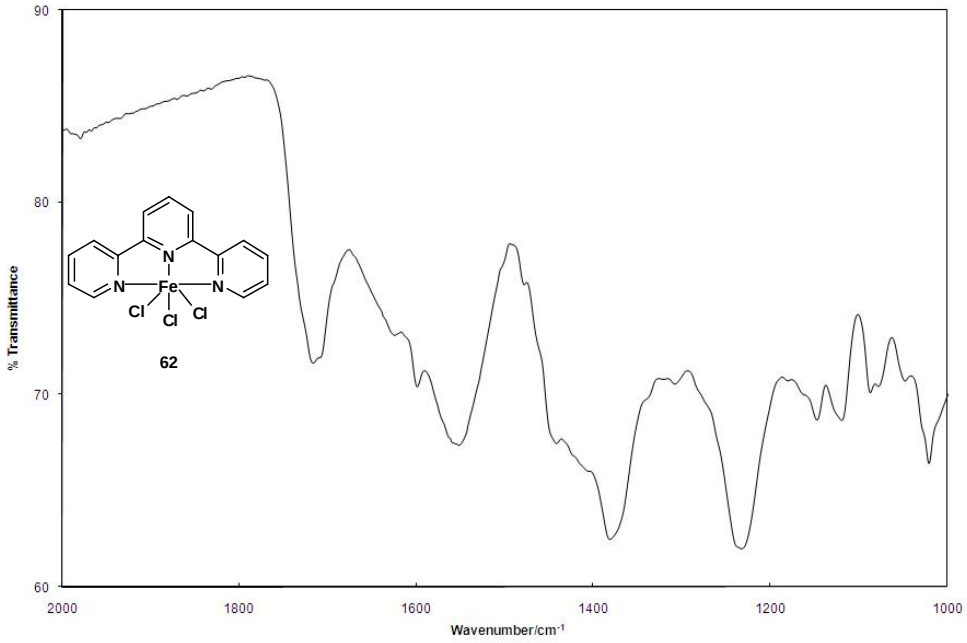

**Spectrum 101 IR spectrum of 30 β-haematin assay.**



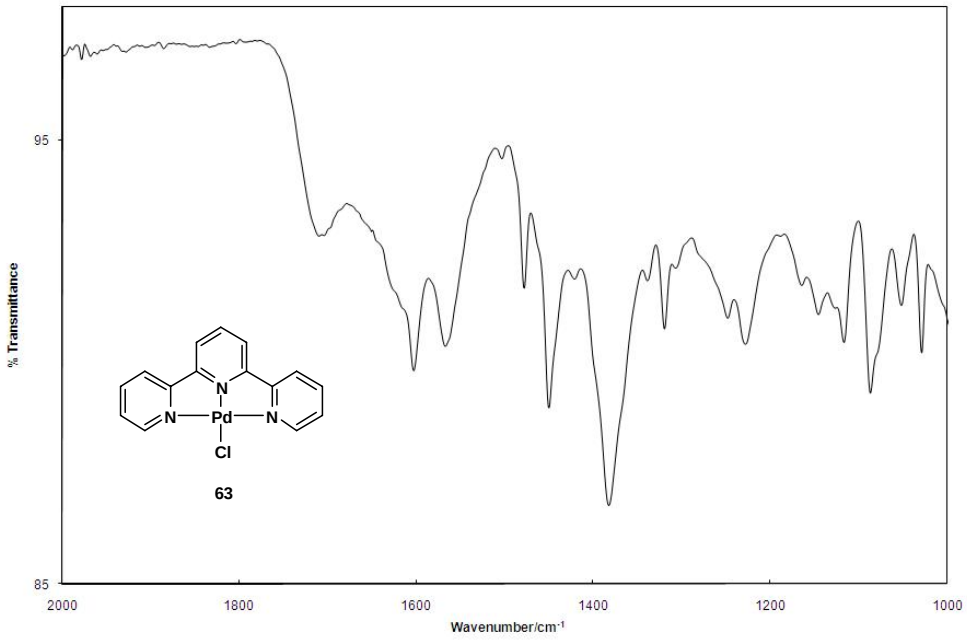

**Spectrum 102 IR spectrum of 33 β-haematin assay.**



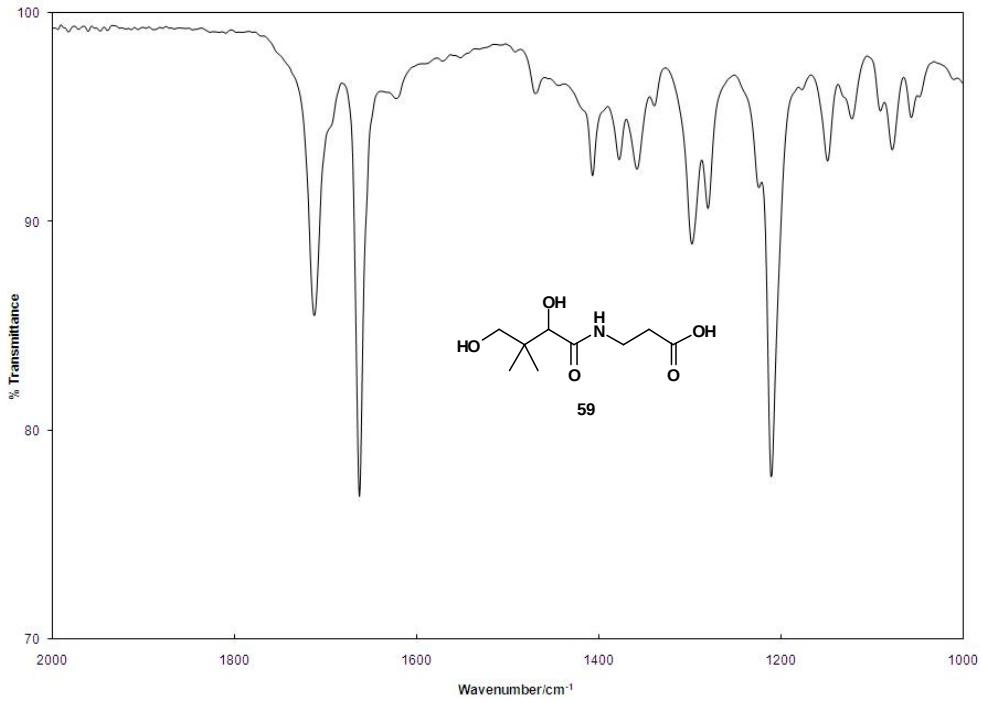

**Spectrum 103 IR spectrum of 61 β-haematin assay.**



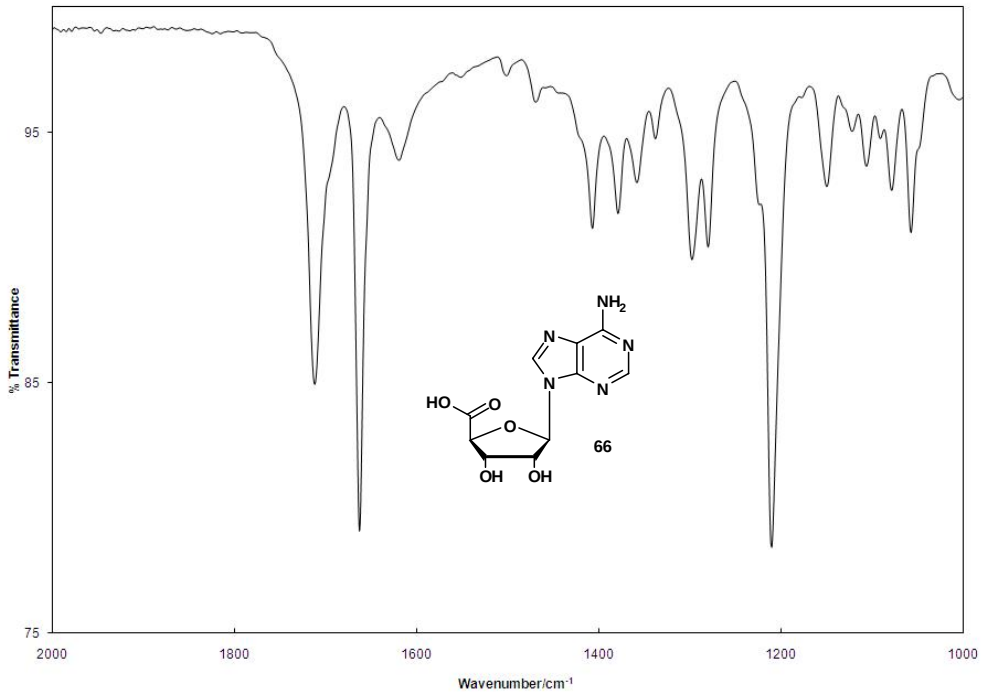

**Spectrum 104 IR spectrum of 62 β-haematin assay.**



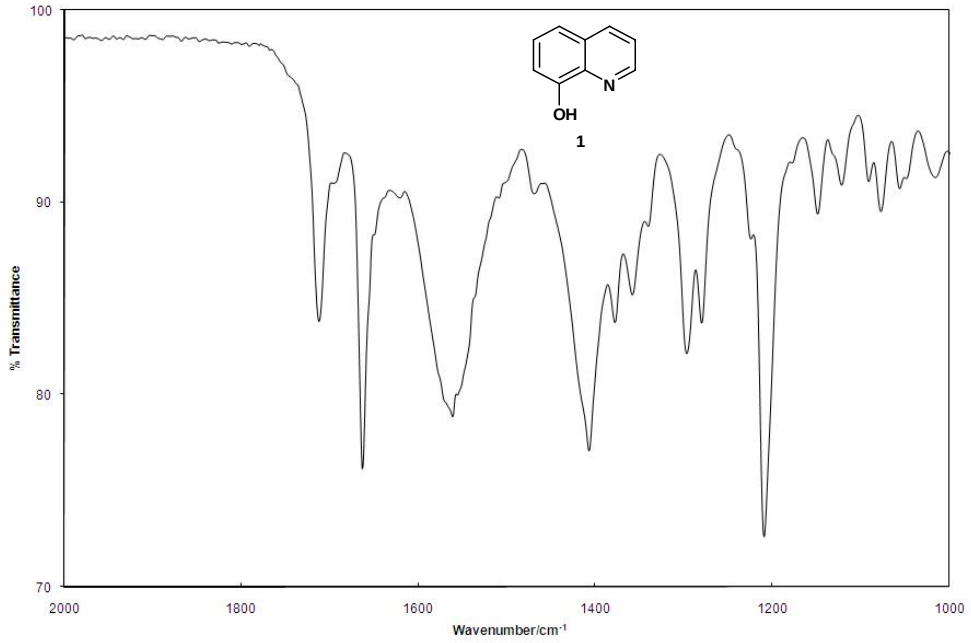

**Spectrum 105 IR spectrum of 63 β-haematin assay.**



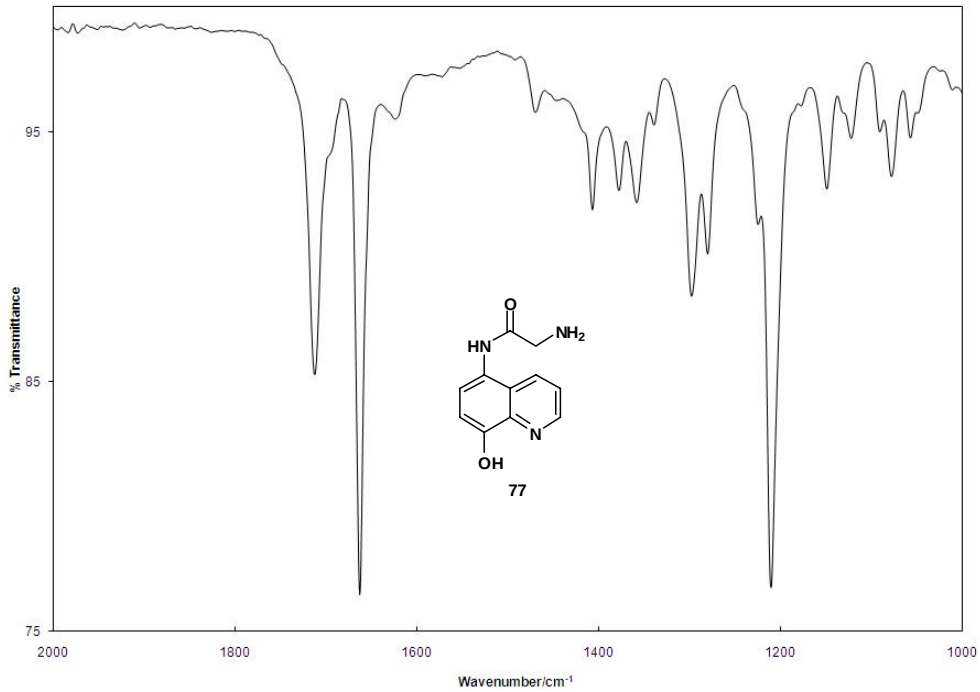

**Spectrum 106 IR spectrum of 59 β-haematin assay.**



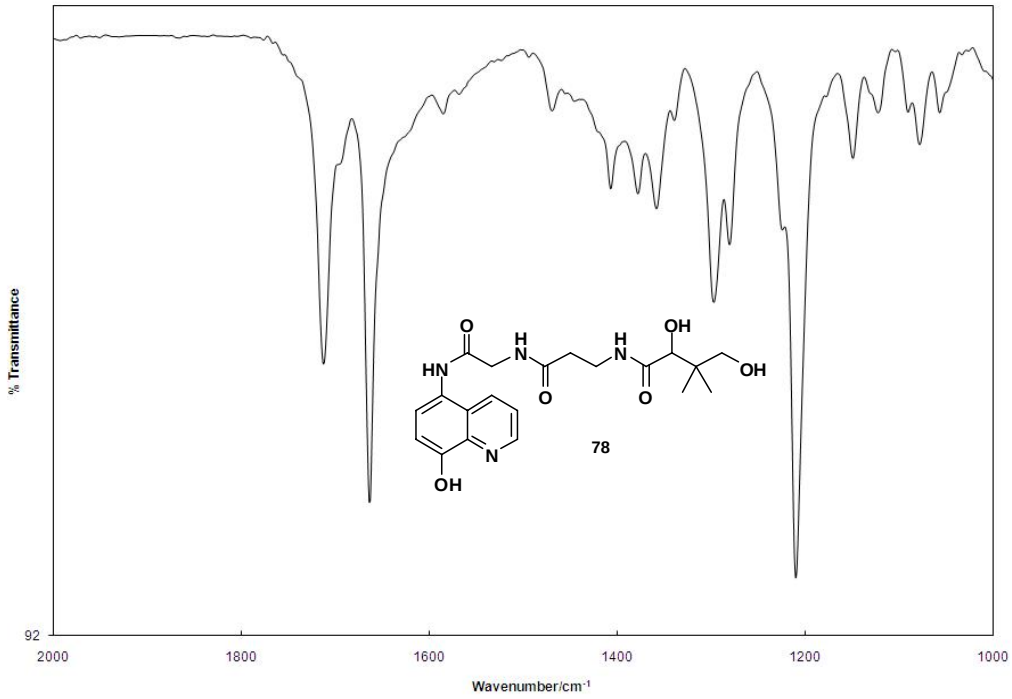

**Spectrum 107 IR spectrum of 66 β-haematin assay.**



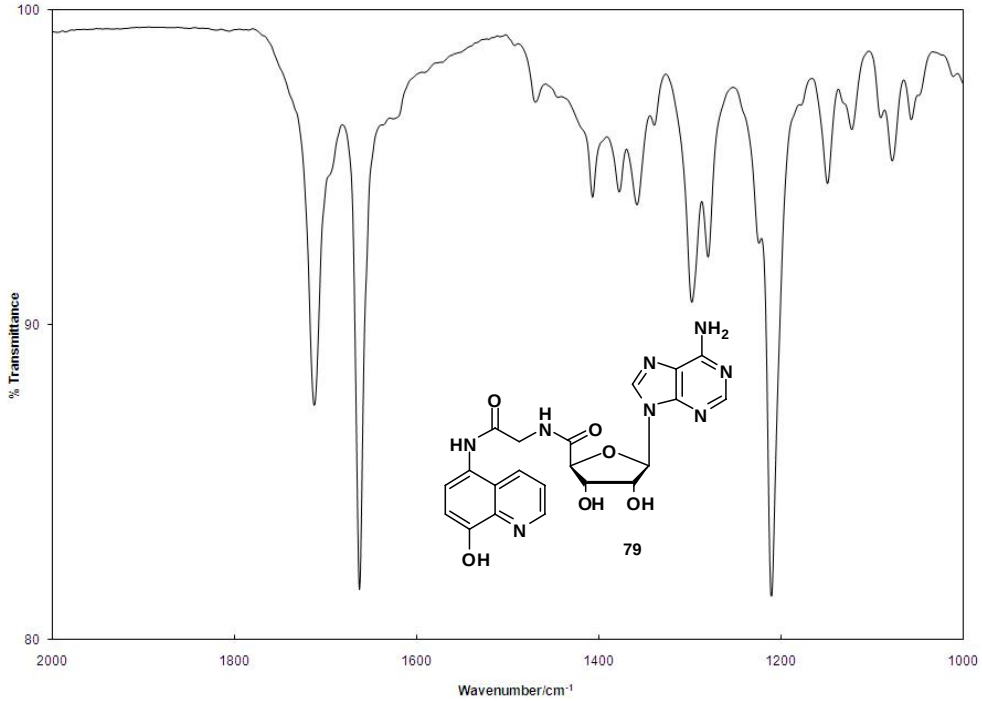

**Spectrum 108 IR spectrum of 1 β-haematin assay.**



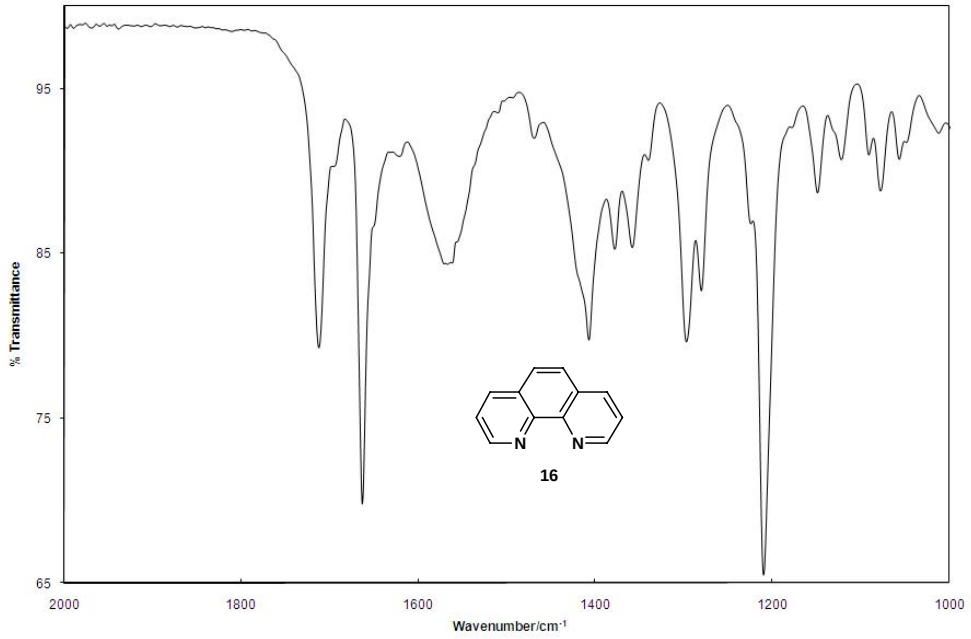

**Spectrum 109 IR spectrum of 77 β-haematin assay.**



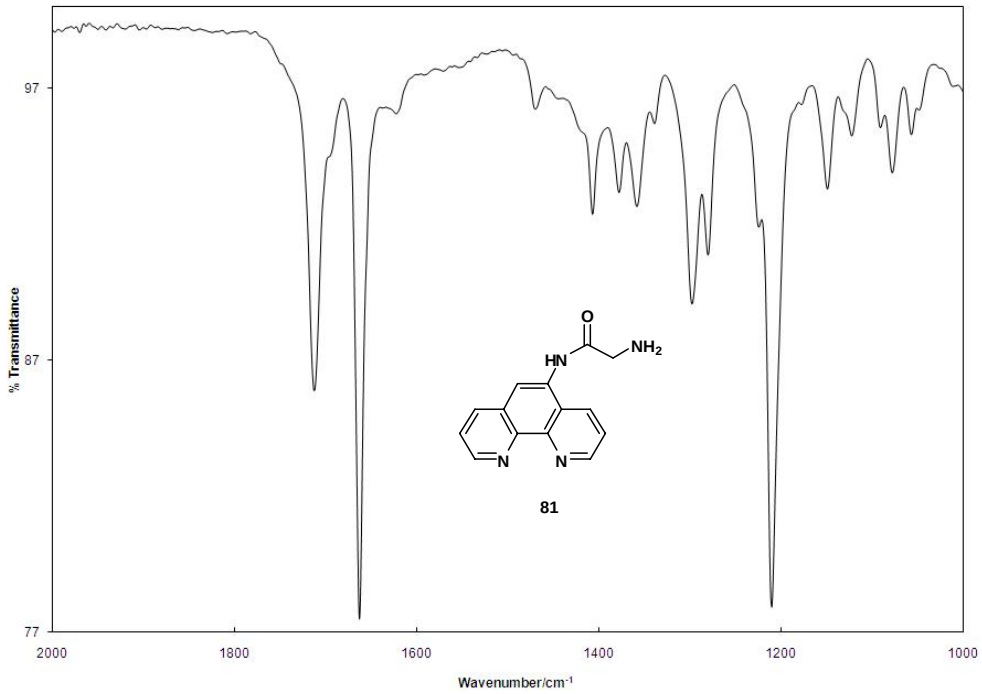

**Spectrum 110 IR spectrum of 78 β-haematin assay.**



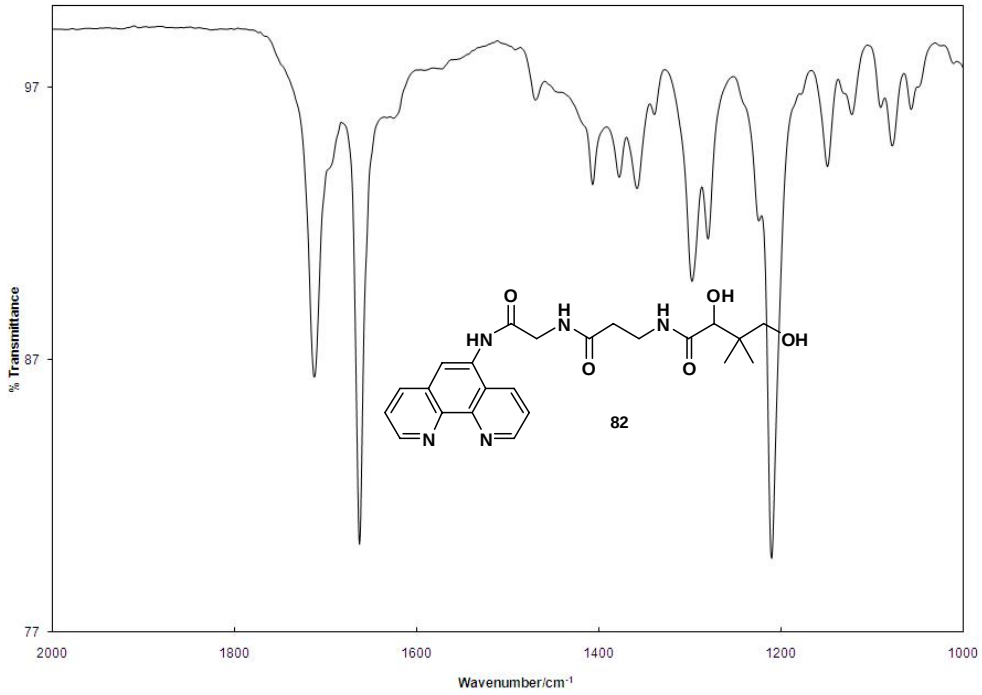

**Spectrum 111 IR spectrum of 79 β-haematin assay.**



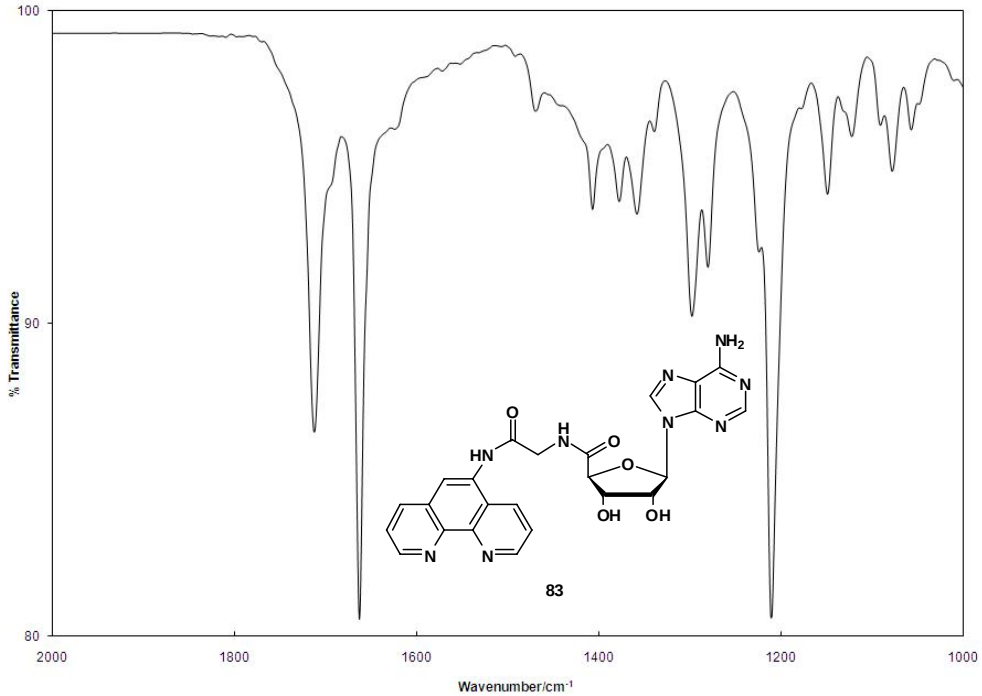

**Spectrum 112 IR spectrum of 16 β-haematin assay.**



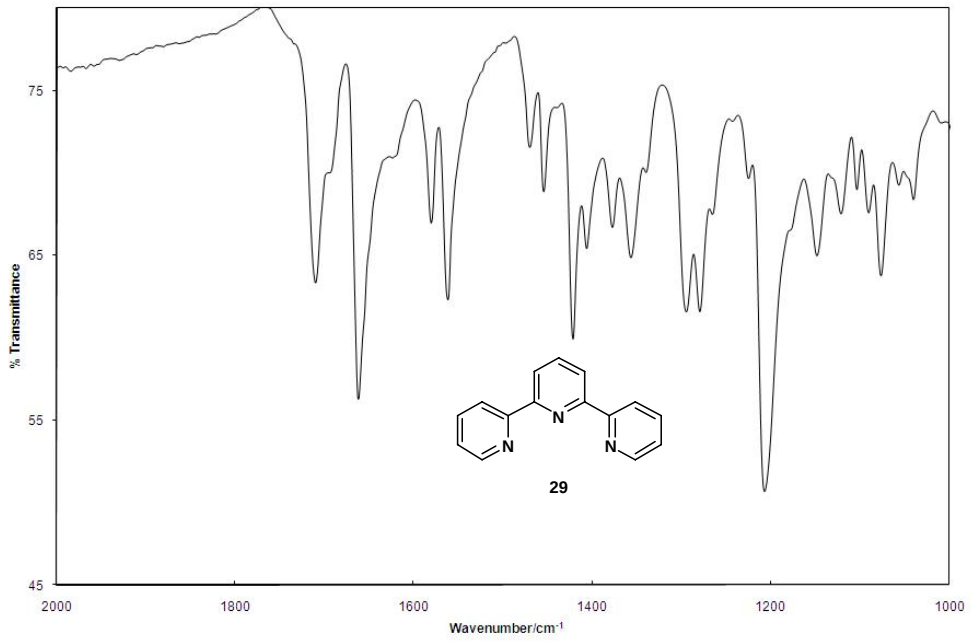

**Spectrum 113 IR spectrum of 81 β-haematin assay.**



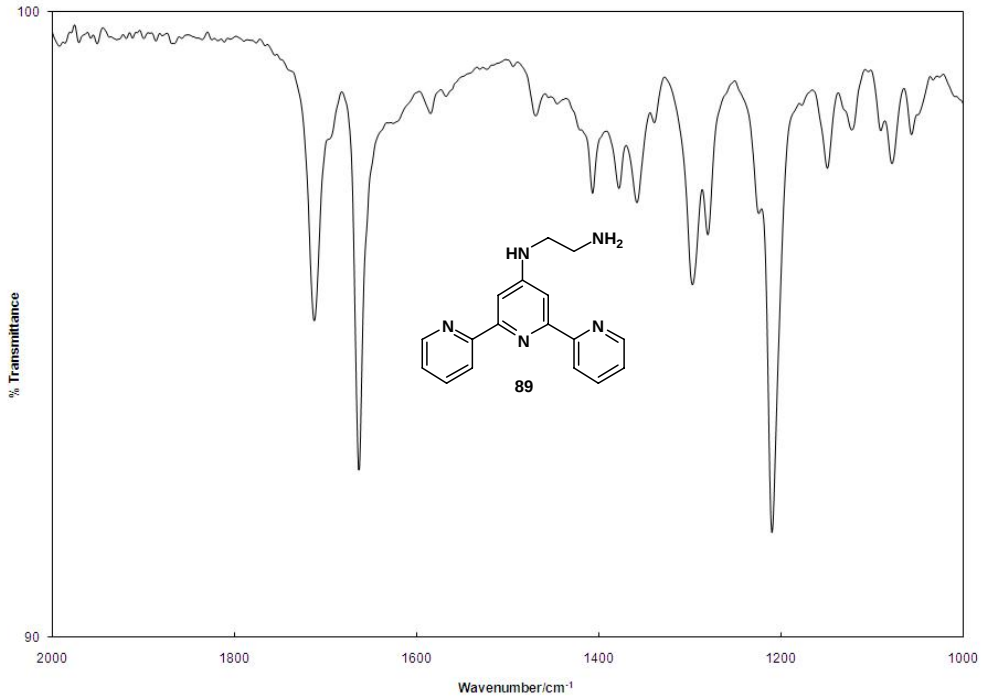

**Spectrum 114 IR spectrum of 82 β-haematin assay.**



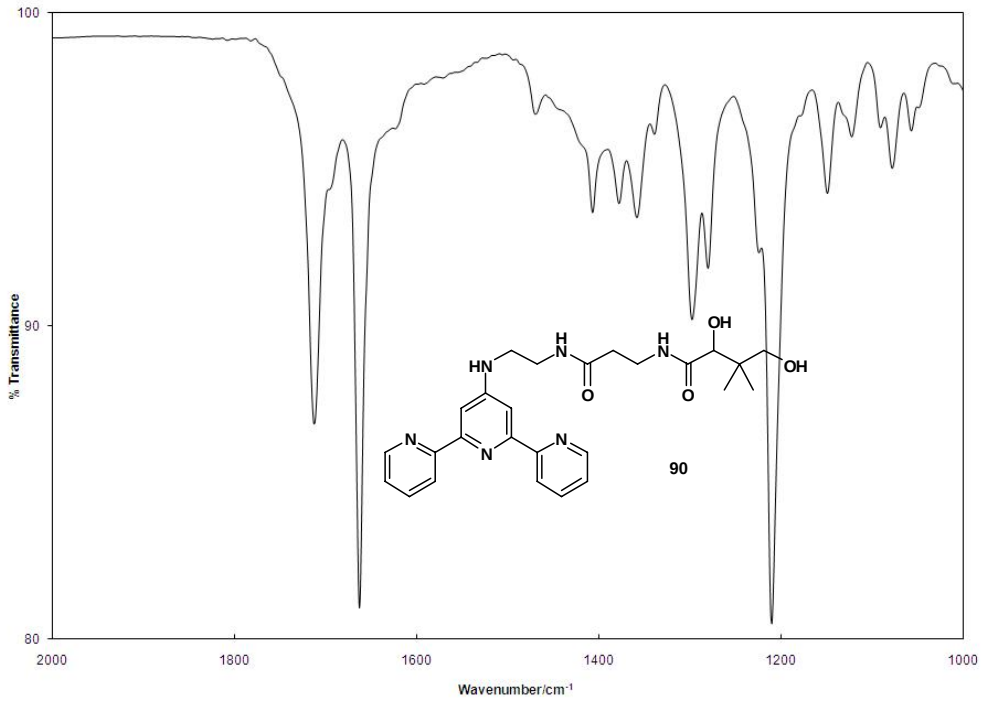

**Spectrum 115 IR spectrum of 83 β-haematin assay.**



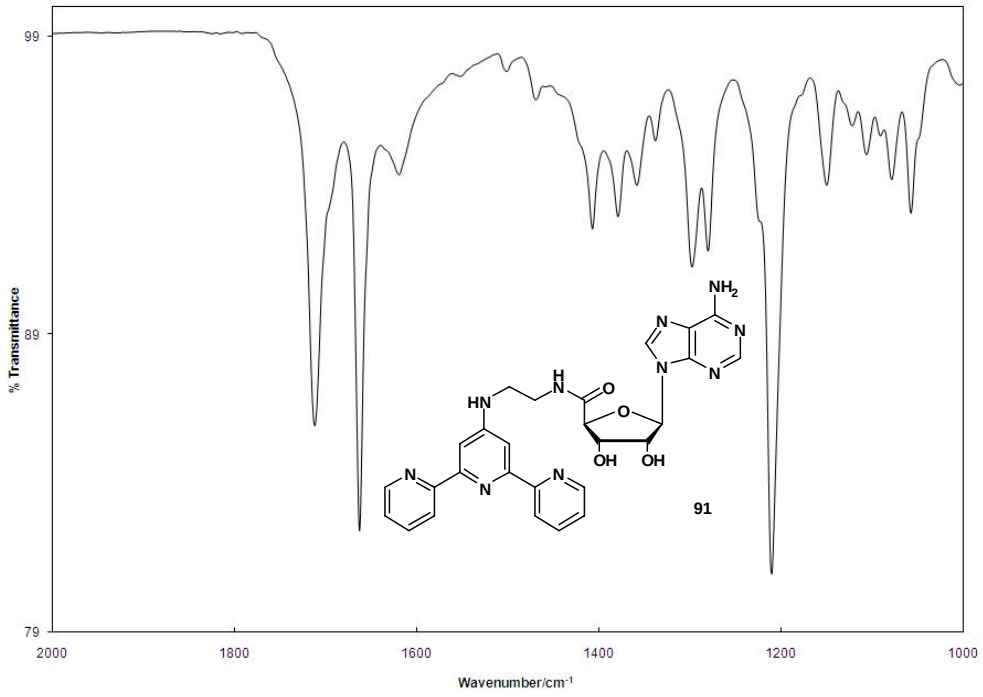

**Spectrum 116 IR spectrum of 29 β-haematin assay.**




**Spectrum 117 IR spectrum of 89 β-haematin assay.**




**Spectrum 118 IR spectrum of 90 β-haematin assay.**




**Spectrum 119 IR spectrum of 91 β-haematin assay.**