DESIGN AND SYNTHESIS OF NADH MIMICS THAT TARGET MITOCHONDRIAL ELECTRON TRANSPORT OF PLASMODIUM PARASITE

by

ALI IBRAHIM ALTHARAWI

(Under the Direction of J. Warren Beach)

ABSTRACT

Malaria, particularly that caused by *Plasmodium falciparum*, remains one of the most deadly infectious diseases worldwide. Unfortunately, multi–drug resistance (MDR) to currently used antimalarials has spread worldwide and led to a failure to control malaria. Therefore, new chemotherapeutic agents that are affordable, easy to synthesize, and overcome resistant strains of parasites are urgently needed. Plasmodium mitochondria has attracted the attention of many projects as a potential target for malaria treatment, and provided atovaquone as treatment and prophylaxis of malaria.

In this project, the advantage of a triphenylphosphonium (TPP) moiety in delivering biomolecules to mitochondria has been investigated to deliver NADH-mimic compounds as potential inhibitors of the mitochondrial electron transport chain (mtETC). A small library of phosphonium cations based on clopidal and amodiaquine (AQ) were synthesized and evaluated for antiparasitic activity against W2

chloroquine-resistant strain. The test results revealed that the phosphonium moiety reduced antiplasmodial efficacy, and phosphonium substituents (R = Me) were as effective as (TPP) moiety. It is hypothesized that re-routing the AQ-based phosphonium cation to the negatively-charged mitochondria by electrostatic attraction resulted in moderate antiplasmodial effects and the overall activities of the compounds in this study were the result of NADH dehydrogenase (Complex I) inhibition.

INDEX WORDS: *Plasmodium falciparum*, multi-drug resistance (MDR), mitochondrial electron transport chain (mtETC), Triphenylphosphonium (TPP), 4(1*H*)-pyridinone, W2 chloroquine-resistant *Plasmodium falciparum*, IC_{50}s, NADH dehydrogenase (Complex I), food vacuole (FV)

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DEDICATION

To My Family and My Wife

I could not have achieved my goals without your love, inspiration, and support.

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I would like to express my sincere gratitude to my major advisor Dr. Timothy E. Long. This work would not have been possible without his support, guidance and motivation. I would like also to extend my appreciation to Dr. Michael G. Bartlett and Dr. J. Warren Beach for serving in my committee.

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CHAPTER 1

MALARIA

INTRODUCTION

Malaria, caused by the plasmodium parasite, is one of the most deadly infectious diseases in developing countries. According to world malaria report in 2012, there were an estimate of 219 million cases of malaria and about 600,000 fatalities worldwide of which, 90% were in Sub-Saharan Africa. The populations at high risk of severe infection are children under the age of five and pregnant women.[1] Four species of plasmodium parasites cause the infection in humans. These are *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium vivax*. Among these, *Plasmodium falciparum* is the most deadly species in humans because of the high prevalence of multi-drug resistance in this strain.[2] Therefore, new chemotherapeutic agents that are effective against resistant strains of the parasites are urgently needed to treat malaria.

Life cycle of Malaria Parasite

The transmission of the malaria parasite to humans takes place when the female Anopheles mosquito bites the human to feed on the blood and simultaneously injects saliva that contains sporozoites (Fig. 1.1), the infectious form of the parasite. Sporozoites travel in the human bloodstream and rapidly reach the liver cells where they divide into several thousands of merozoites. The erythrocytic stages of malaria are initiated when merozoites are released to the bloodstream and invade the

erythrocytes.[3] In this stage, merozoites change morphology from the ring form to mature trophozoites that feed on the erythrocytes hemoglobin and develop into mature schizonts.[4] In 2-3 days, rupturing of the mature schizonts releases the newly formed merozoites into the bloodstream, which subsequently invade the uninfected erythrocytes. During invasion, the dividing parasites degrade the hemoglobin into amino acids as a major source for nutrients.[5] In addition, hemoglobin degradation causes the release of free heme, which is toxic to the parasites. To avoid heme toxicity, the parasite polymerizes the free heme into insoluble non-toxic crystals called hemozoin.

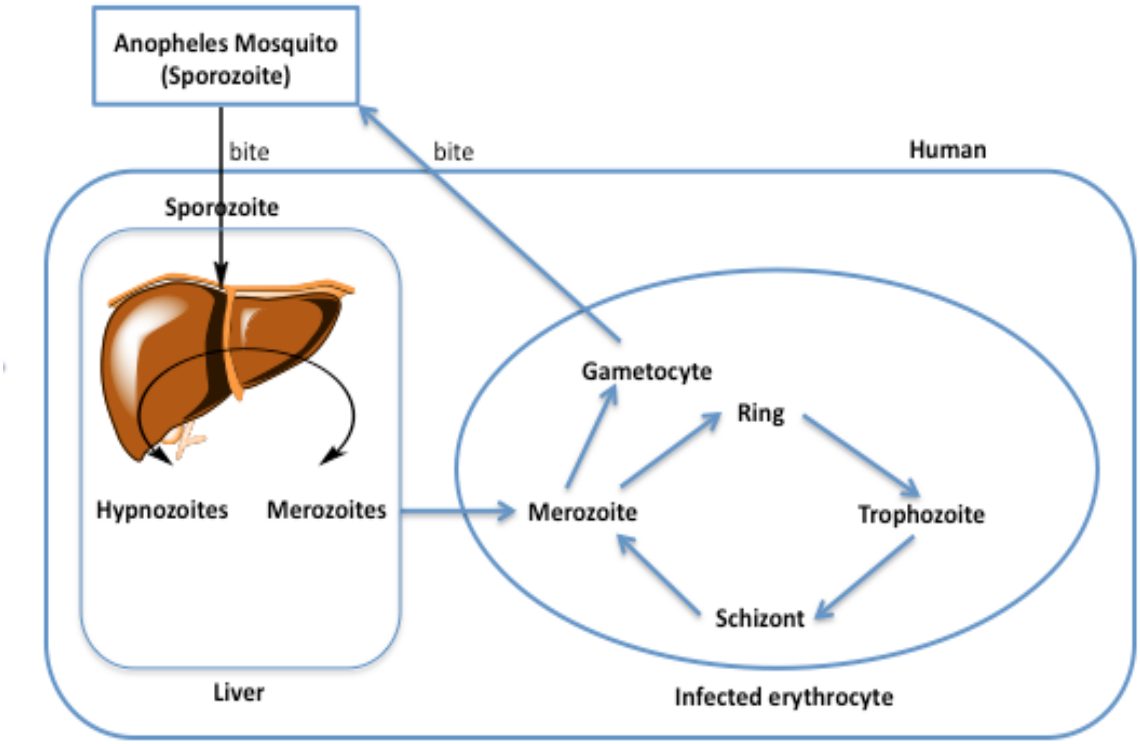


Fig. 1.1: Malaria life cycle

The lifecycle of the plasmodium parasite comes full circle when merozoites differentiate into sexual gametocytes and transmit back to the mosquito.[4] In the mosquito's mid-gut, gametocytes fertilize into infectious sporozoites, which travel to

the mosquito salivary gland. The cycle initiates again when the mosquito bites the host, transmitting the sporozoites into the bloodstream.

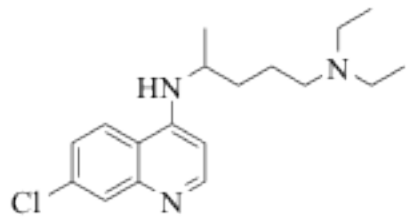
Current Malaria Treatment

Currently, the antimalarial drugs that have been approved as therapies and prophylaxes include the quinolines (e.g, chloroquine **1**), antifolates (e.g, sulphadoxine **2** /pyrimethamine **3**), artemisinin derivatives **4**, and naphthoquinones (e.g., atovaquone **5**) (Fig. 1.2). All standard treatments employ two of these four main mechanistic classes of antimalarials to prevent recrudescence and resistance development. Further, artemisinin is the current gold standard for malaria infections and is recommended by the World Health Organization as one of the drugs used in all uncomplicated treatments.

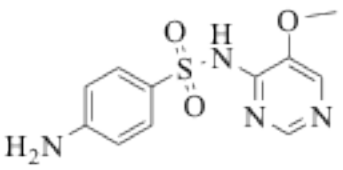
Chloroquine

Since it has been synthesized in 1934, chloroquine (CQ) has remained a mainstay in malaria treatment due to its excellent efficacy, affordability, low toxicity as well as ease of production.[6] Unfortunately, emergence of resistance to CQ was first observed in the 1950s in the East Asian countries, but expansion of drug-resistant strains since then has limited the use of CQ in treating malaria. Although multiple factors contribute to CQ resistance, *Plasmodium falciparum* CQ resistance transporter (*P*fcrt) gene plays the major role in resistance *in vitro* and treatment failure *in vivo*.[7]

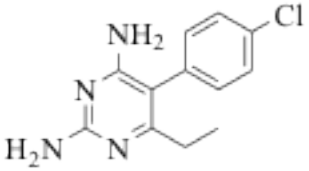
CQ is a diprotic weak base ($pKa_1 = 8.1$, and $pKa_2 = 10.2$) that moves down the pH gradient to accumulate in the acidic food vacuole (FV) of the intraerythrocytic parasite (pH of 4.5-5.0). Once inside the FV, the malarial parasite digests a large number of the host hemoglobin as a source of essential nutrients.[6, 8]



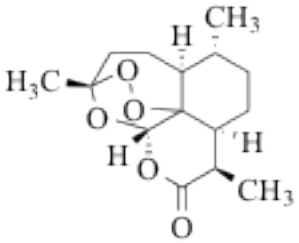
Chloroquine **1**



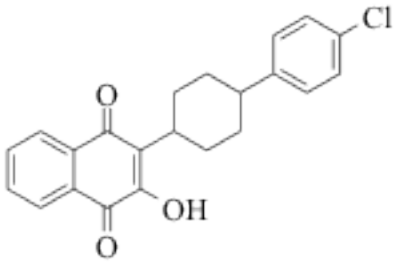
Sulfadoxine **2**



Pyrimethamine **3**



Artemisinin **4**



Atovaquone **5**

Fig. 1.2: Current malaria treatment structures

A major product of hemoglobin digestion is ferriprotoporphyrin IX (FPIX). FPIX is extremely toxic to cells and have been shown to cause cell lysis as well as the inhibition of essential enzymes in glycolysis.[9] The polymerization of FPIX into non-toxic hemozoin (malaria pigment) is believed to be the mechanism by which the parasite averts FPIX toxicity. CQ forms a complex with FPIX that leads to the disruption of the polymerization process and results in the buildup of free FPIX inside the food vacuole, which eventually kills the parasite.[10]

Antifolates

The success of antifolates such as methotrexate in the treatment of leukemia, a blood cancer characterized by a rapid division of immature white blood cells, prompted the use of this class of drugs in the treatment of other rapidly dividing cells such as bacteria and parasites. Two main classes of antifolates are used in the treatment of malaria infection: inhibitors of dihydropteroate synthase (DHPS), class I antifolates, and inhibitors of dihydrofolate reductase (DHFR), class II antifolates.[11] Sulfadoxine is a class I antifolate which mimics the *para*-aminobenzoic acid and blocks the formation of dihydropteroate from hydroxymethyldihydropterin by competing with the active site domain of DHPS (a bifunctional enzyme coupled with hydroxymethylpterin pyrophosphokinase (PPPK)). Pyrimethamine is a class II antifolate which mimics the pteridine ring of dihydrofolate (DHF) and competes for the active site pocket of DHFR (a bifunctional enzyme coupled with thymidylate synthetase (TS)).[12] DHFR inhibition by pyrimethamine therefore, prevents the reduction of DHF to tetrahydrofolate (THF), an essential cofactor for the biosynthesis of thymidylate, purine nucleotides and certain amino acids.

Fansidar™ (sulfadoxine/pyrimethamine) is a combination therapy that has been extensively used to replace CQ in most endemic areas. Since the parasites are capable of salvaging the folate from exogenous sources, sulfadoxine alone is inefficient in blocking the folate synthesis pathway (Fig. 1.3).[13] Therefore, addition of pyrimethamine provides an additional blockage of the downstream folate synthesis by inhibiting DHFR. Point mutations in the DHPS as well as in the DHFR result in the emergence of resistance for sulfadoxine and pyrimethamine, respectively.[11-12]

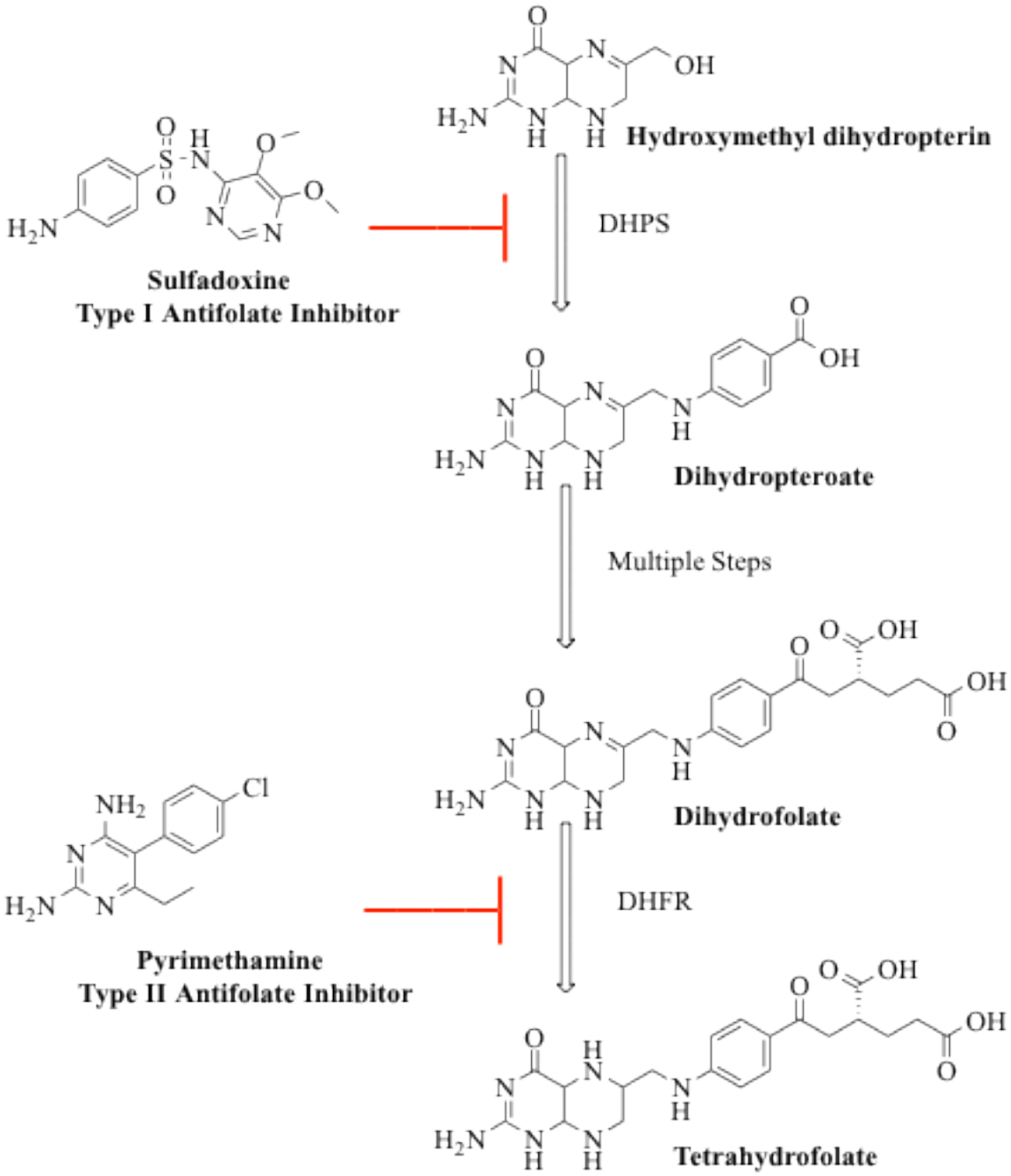


Fig. 1.3: Fansidar™ synergistic mechanism

Artemisinin Derivatives

Artemisinin, extracted from a *Qinghaosu* herb (i.e., sweet wormwood), has been used in traditional Chinese medicine for treatment of fever for over than 2000 years. In 1971, scientists at the Pharmaceutical Institute of the Academy of Traditional

Chinese Medicine succeeded in extracting the active ingredient of *Qinghaosu,* which is now known as artemisinin (**1**, Fig. 1.4).[14] The unique sesquiterpene peroxide lactone possesses a broad range of activity against most forms of the asexual intraerythrocytic parasite as well as young stages of gametocytes. Even though the artemisinin-based compounds have a short elimination half life, they act more rapidly in clearing the parasites in comparison to other antimalarial drugs.[15]

Although the exact mechanism of action of artemisinins against the malaria parasite is not clearly understood, two possible theories, which rely on the endoperoxide bridge activation to generate free radicals, have been proposed and become widely accepted among scientists.[16] The first theory suggests that the cleavage of the endoperoxide bridge by heme generates free radicals which alkylates some of the parasite proteins and ultimately causes its death.[16b, 17] On the other hand, the second proposed theory demonstrates the interference of artemisinins with sarcoplasmic/endoplasmic calcium ATPase (SERCA) of the plasmodium parasite. SERCA plays a critical role in maintaining calcium homeostasis, which is responsible for signaling, and post-translational processing of proteins. This proposed theory arises from the structure similarity of artemisinin to thapsigargin (**5**), a known inhibitor of SERCA. PfATP6, the only SERCA-type Ca^{2+} ATPase in *Plasmodium falciparum*, was selectively inhibited by artemisinin even at high concentrations.[18] Hydrophobic interaction of artemisinin with PfATP6 protein leaves the peroxide bond exposed as it has been shown by three-dimensional model. Fe^{2+} therefore, cleaves the peroxide bridge and generates carbon-centered radicals, which consequently lead to enzyme inactivation and parasite death.[19]

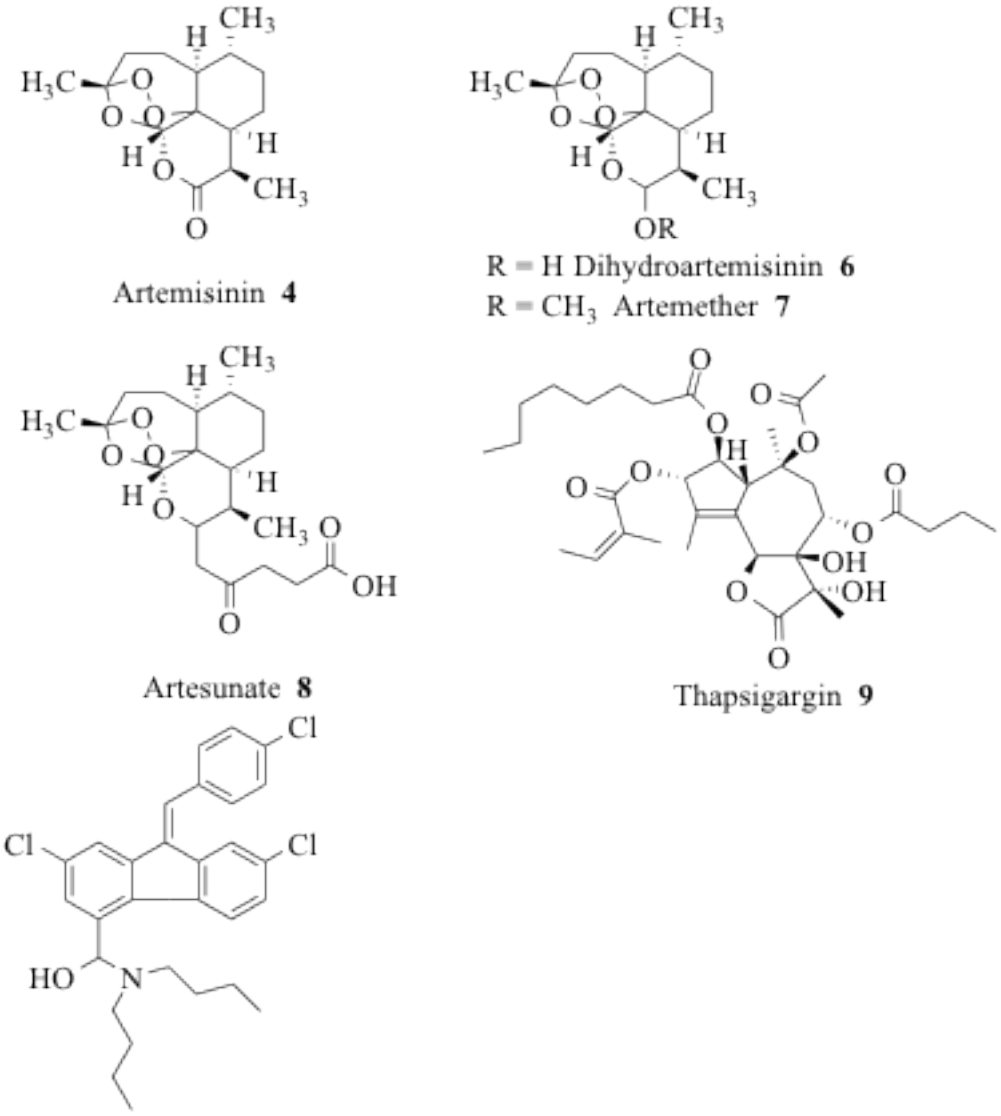


Fig. 1.4: Artemisinin derivatives structures

The WHO recommends the use of artemisinin combination therapy (ACT) instead of monotherapy in treating malaria to avoid the emergence of resistance.[20] The principle of combination therapy entails the use of rapid acting/long lasting agents for malaria treatment. CoArtem™ (artemether/lumefantrine), for example, is a combination of rapid acting artemether and long lasting lumefantrine in which the parasites that escape the rapid effect of artemether are killed afterward by the long lasting activity of lumefantrine. [21]

Naphthoquinones

Early investigations to target the plasmodium mitochondrial electron transport chain (mtETC) using naphthoquinones has led to the discovery of atovaquone.[22] Atovaquone (**5**, Fig. 1.5), a hydroxy 1,4-naphthoquinone analogue, has a broad-spectrum activity against multiple *Plasmodium* species and *P. carinii*, *Babesia* spp., and *Toxoplasma gondii*.[10b, 23] It is structurally similar to ubiquinone **11** (coenzyme Q, CoQ), an inner mitochondrial membrane cofactor, which acts as an electron carrier in the mitochondrial electron transport chain mtETC.

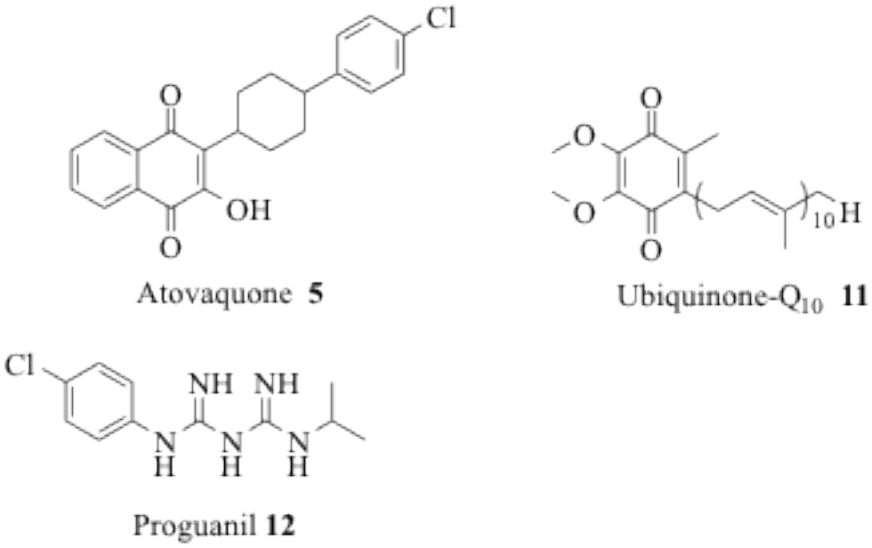


Fig. 1.5: Atovaquone, ubiquinone, and proquanil structures

Atovaquone has been found to strongly compete with CoQ for binding to cytochrome bc_1 (complex III) and leads to its inhibition.[23] As a result, collapse of the mitochondrial membrane potential takes place and multiple enzymes linked to the mtETC are inhibited as well.[24] Most importantly is the inhibition of dihydroorotate dehydrogenase (DHOD), the enzyme responsible for *de novo* pyrimidine biosynthesis. In clinical trials, atovaquone monotherapy was accompanied by a rapid emergence of

resistance and hence failure of treatment.[24b, 25] Therefore, synergistic combinations have been investigated and found that proguanil **12** significantly enhances the success of treatment with minimal occurrence of resistance.[25-26] Since then, Malarone™ (atovaquone/proguanil) has been approved for treatment and prophylaxis of malaria.

Mitochondria as a potential target for Malaria treatment

Mitochondria are discrete organelles with a cristae appearance found in most eukaryotic cells and considered the essential source of energy in the form of ATP. It consists of four compartments that carry out multiple functions: the outer mitochondrial membrane (OMM); the intermembrane space (IMS); the inner mitochondrial membrane (IMM); and the mitochondrial matrix (Fig. 1.6).[27] Electrons generated from oxidation of substrates in the tricarboxylic acid cycle (TCA) and from degradation of fatty acids by beta-oxidation accumulate as NADH. As a result, NADH oxidizes to NAD^+ at mitochondrial complex I (NADH:ubiquinone oxidoreductase) and the electrons transferred to the CoQ pool. The energy released at complex I pumps protons across the IMM. Similarly, mitochondrial complex II (succinate:ubiquinone oxidoreductase) accepts electrons from the TCA cycle and donates them to CoQ. Electrons from the CoQ pool are then transferred to complex III (ubiquinol:cytochrome c oxidoreductase) and finally are used to reduce oxygen at complex IV (cytochrome c oxidase). The energy generated at complex III and complex IV is also used to pump protons across the IMM. Complex V (F_0F_1-ATP synthase) is the final complex in the process of ATP synthesis. Protons motive force across the IMM generates a membrane potential of about -160 mV which is used to make ATP

from ADP and phosphate. ATP finally will be exported out of mitochondria to the cytoplasm.[28]

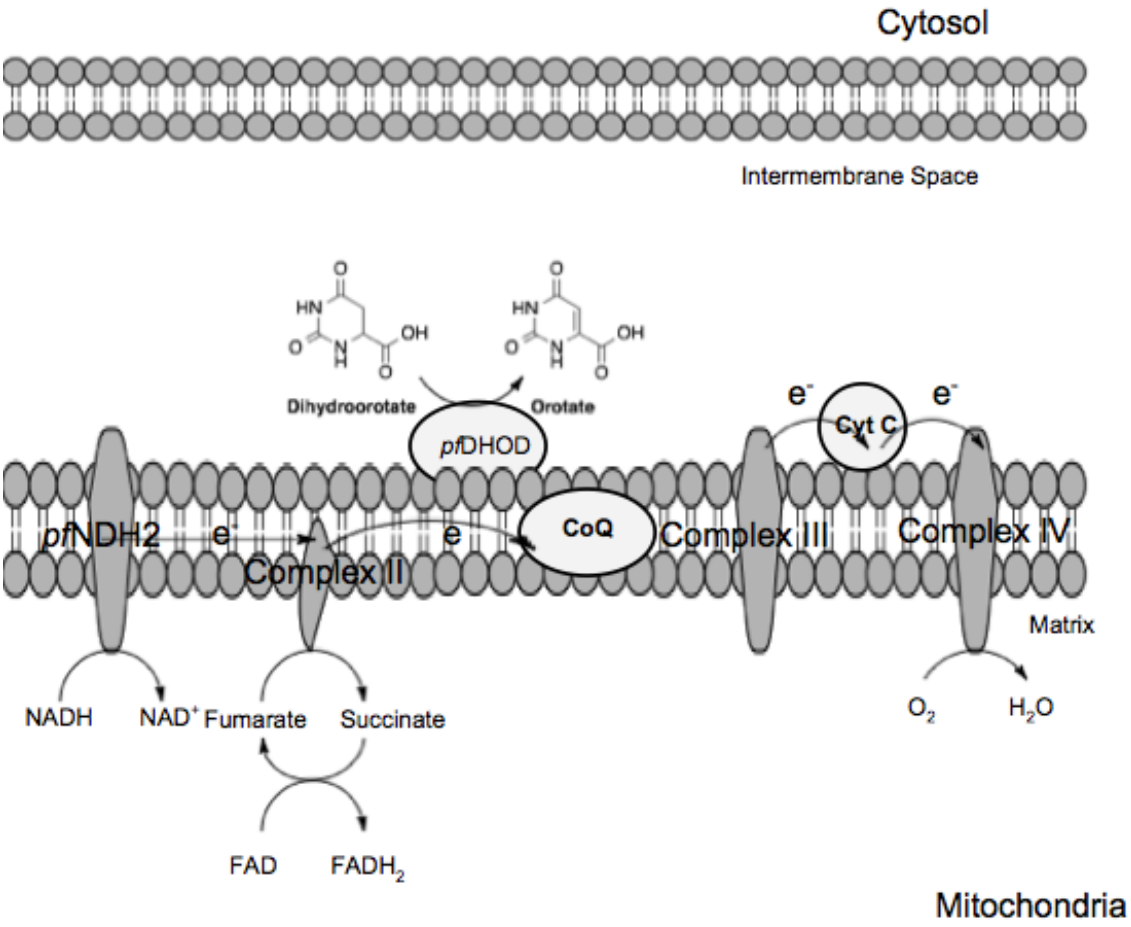


Fig. 1.6: Electron transport chain in plasmodium parasite

Mitochondrial acetyl-CoA, a key metabolite in oxidative phosphorylation, can be obtained in three ways: conversion of pyruvate generated via glycolysis by pyruvate dehydrogenase, degradation of fatty acids through beta-oxidation, and catabolism of branched-chain amino acids via transaminases.[28a, 29] Unlike mammals, a study in plasmodium parasites has shown that erythrocytic stages consume over 100-fold more glucose than uninfected red blood cells (RBCs).[30] However, ^{14}C-labeled glucose fed to malaria parasites was not converted into $^{14}CO_2$, but instead it was

converted mostly into lactate and thus deprived the TCA of pyruvate. Furthermore, recent investigations have shown that pyruvate dehydrogenase is exclusively located in the apicoplast rather than the mitochondrion.[31] The second source of acetyl-CoA is the decomposition of fatty acids by beta-oxidation. However, the malaria genome project failed to identify genes encoding enzymes for this pathway. The third source of acetyl-CoA is the degradation of branched amino acids via transaminases. The transaminases lead to the production of glutamate and alpha-keto acids by transferring the amino group to alpha-ketoglutarate. Several steps afterward lead to the formation of acetyl-CoA. However, genome analysis of plasmodium parasites has not identified any of the enzymes involved in the degradation of branched-chain amino acids. The aforementioned evidence therefore, indicate the apparent absence of oxidative phosphorylation by the TCA cycle in erythrocytic stages of malaria and consequently low activity of the mtETC.[28a, 29]

In most eukaryotes, the mtETC is composed of four integral membrane enzyme complexes embedded in the IMM. These include NADH:ubiquinone oxidoreductase (complex I), succinate:ubiquinone oxidoreductase (complex II), ubiquinol:cytochrome c oxidoreductase (complex III, or cytochrome bc_1), and cytochrome c oxidase (complex IV).[28a, 32] Although mtETC has low activity in plasmodium parasites, it is essential for the parasite survival and growth.[32] For example, atovaquone has been shown to inhibit the cytochrome bc_1 complex (complex III) of the mtETC and leads to parasite death. Moreover, the parasite mitochondrial genome encodes at least five mitochondrial dehydrogenases: NADH dehydrogenase, glycerol-3-phosphate dehydrogenase, succinate dehydrogenase, malate:quinone

oxidoreductase, and dihydroorotate dehydrogenase (DHOD). DHOD is an essential enzyme for the pyrimidine biosynthesis that catalyses the dihydroorotate oxidation to orotate, and result in the generation of electrons. CoQ provided by active mtETC links the mtETC to pyrimidine biosynthesis and acts as electrons acceptor for this pathway.[33] Additionally, malaria genome sequencing has revealed that malaria parasites depend completely on *de novo* pyrimidine biosynthesis, which is vital for the formation of nucleic acids, glycoproteins, and phospholipids.[34] These observations indicate that the active mtETC contributes significantly to *de novo* pyrimidine biosynthesis. Therefore, antimalarials that inhibit mtETC functions have the ability to interrupt essential metabolic pathways within the parasites and results in parasite death.

Phosphonium lipocations as mitochondrial-targeted molecules

As discussed earlier, mitochondria have an outer and an inner membrane composed of phospholipid bilayers that offer a barrier for delivery of mtETC inhibitors into mitochondria.[35] Therefore, certain attributes in mtETC inhibitors have to be attained to deliver them into mtETC complexes. The success of atovaquone and related inhibitors of cytochrome bc_1 complex (complex III), such as 4(1*H*)-quinolones (Fig. 1.7), are believed to be resulted from having hydrophobic side chains. These hydrophobic chains enhance their membrane permeability to the mitochondrial matrix where mtETC complexes are embedded and eventually lead to their inhibition.

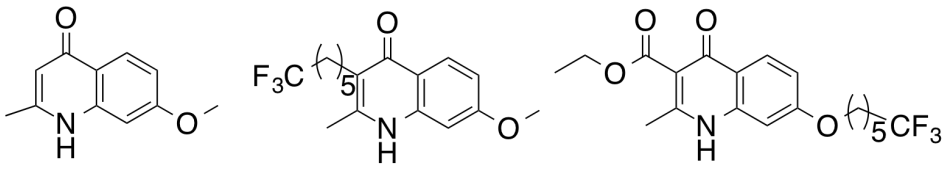


Fig. 1.7: 4(1*H*)-quinolones structures, and their Antiplasmodial activities against Dd2

In addition, lipophilic cations have been demonstrated to accumulate in mitochondria and are now widely employed for measuring the membrane potential of mitochondria (Fig. 1.8). Rhodamine, a fluorescent lipophilic cation, is used to visualize mitochondria under the microscope. Furthermore, triphenylphosphonium cations (TPP) attached to drugs have been shown to accumulate hundred-fold in mitochondria compared to the parent drugs without TPP moiety.[36] Accumulation of TPP inside mitochondria is believed to be mediated by electrostatic attraction between the large negatively-charged membrane potential across the IMM (-150-180 mV) and the positively-charged TPP moiety. In addition, the lipophilicity of TPP enhances their permeability across the lipid bilayer to reach the cytoplasm. Once inside, it will be further drawn into the negatively-charged mitochondria by electrostatic attraction. [36] Furthermore, antiparasitic activities of naphthoquinone-based phosphonium cations have been tested against CQ-resistant *P. falciparum* W2 strains. Some of the inhibitors have shown inhibitory activities (IC_{50}) in the sub-100 nanomolar range. It has been proposed that the inhibitory effect is conferred by their attachment to the TPP moiety which leads to several fold increases in their accumulation inside the mitochondria.[37]

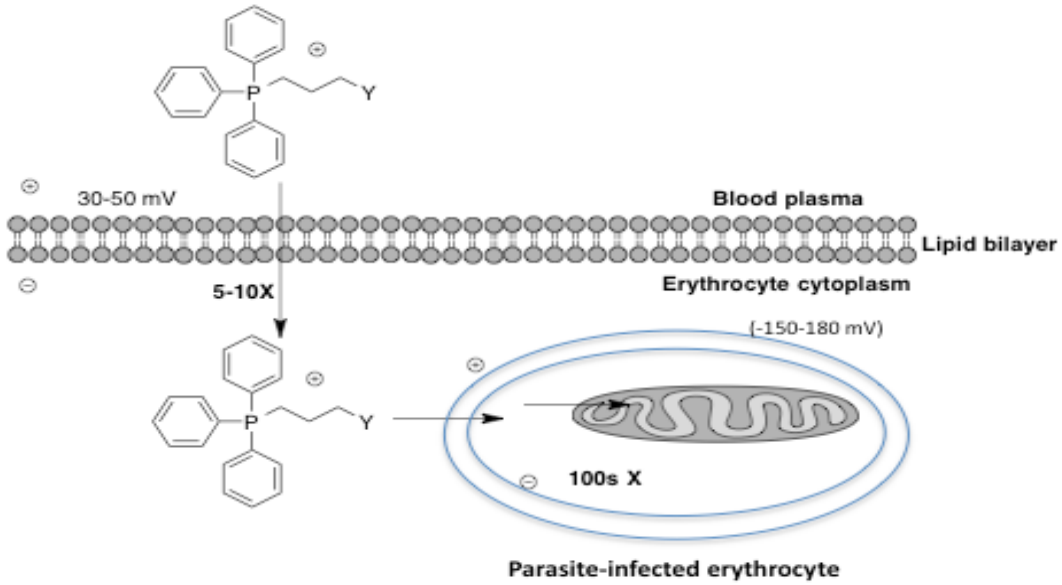


Fig. 1.8: Selective uptake of phosphonium cations by mitochondria

Antioxidants, such as ubiquinone and vitamin E, attached to TPP are by far the most studied class of molecules that have been developed based on this approach. These molecules have shown promising results in protecting mitochondria from the oxidative damage caused by the reactive oxygen species (ROS). For example, Mitoquinone ($MitoQ_{10}$, Fig 1.9), a synthetic analog of ubiquinone, has progressed to phase II trials in the U.S. for the management of fatty liver disease and Parkinson's disease. Furthermore, $MitoE_2$ is a synthetic analogue of vitamin E that has shown a protective effect against ROS in cells taken from Friedreich Ataxia patients, a disease condition caused by ROS damage to mitochondria due to a defect in the mitochondrial frataxin protein that leads to the formation of ROS.[36a, 38]

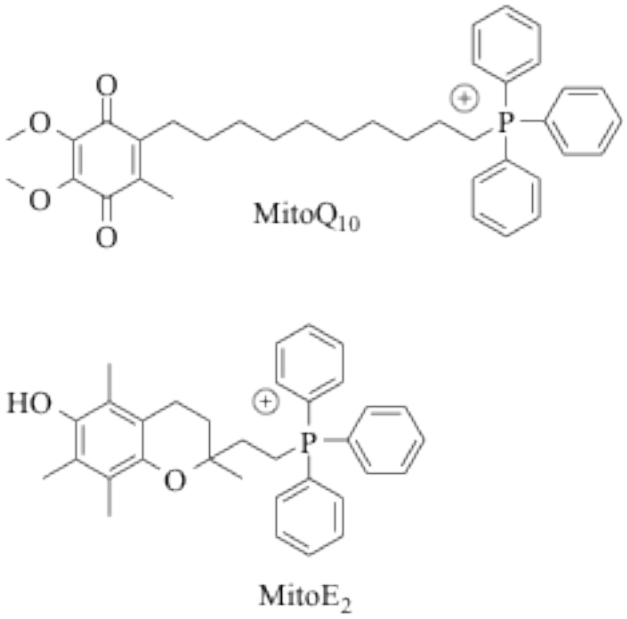


Fig. 1.9: Mitochondria-targeted phosphonium cations compounds

The significance of mtETC complexes for the survival of malaria parasites as well as their distinction from the mammalian mtETC complexes have attracted the attention of many laboratories and pharmaceutical companies for developing inhibitors of mtETC enzymes. However, constraints in the chemical structure of lead compounds, availability of only limited numbers of pharmacophore, and limitation in the mitochondria-targeted approaches render the task of developing mitochondriotropic antimalarials tedious.

In this project the use of phosphonium cations residues in the development of 4(1*H*)-pyridone and 4,7-dichloroquinoline-based antimalarials was explored. Development of compounds based on this design strategy we believe can reduce the of cost mitochondrion-acting antimalarials and improve the overall therapeutic efficacy resulting from their high accumulation in mitochondria.

REFERENCES

1. Greenwood, B. M.; Bojang, K.; Whitty, C. J.; Targett, G. A., Malaria. *Lancet* **2005,** *365* (9469), 1487-98.

2. (a) Greenwood, B., Anti-malarial drugs and the prevention of malaria in the population of malaria endemic areas. *Malaria Journal* **2010,** *9 Suppl 3*, S2; (b) Fidock, D. A., Drug discovery: Priming the antimalarial pipeline. *Nature* **2010,** *465* (7296), 297-8.

3. Aikawa, M., The fine structure of the erythrocytic stages of three avian malarial parasites, Plasmodium fallax, P. lophurae, and P. cathemerium. *The American Journal of Tropical Medicine and Hygiene* **1966,** *15* (4), 449-71.

4. Aikawa, M., Parasitological review. Plasmodium: the fine structure of malarial parasites. *Experimental Parasitology* **1971,** *30* (2), 284-320.

5. (a) Aikawa, M.; Hepler, P. K.; Huff, C. G.; Sprinz, H., The feeding mechanism of avian malarial parasites. *The Journal of Cell Biology* **1966,** *28* (2), 355-73; (b) Francis, S. E.; Sullivan, D. J., Jr.; Goldberg, D. E., Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. *Annual Review of Microbiology* **1997,** *51*, 97-123.

6. Foley, M.; Tilley, L., Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. *Pharmacology & Therapeutics* **1998,** *79* (1), 55-87.

7. Ecker, A.; Lehane, A. M.; Clain, J.; Fidock, D. A., PfCRT and its role in antimalarial drug resistance. *Trends in Parasitology* **2012,** *28* (11), 504-14.

8. Ginsburg, H.; Ward, S. A.; Bray, P. G., An integrated model of chloroquine action. *Parasitology Today* **1999,** *15* (9), 357-60.

9. (a) Orjih, A. U.; Banyal, H. S.; Chevli, R.; Fitch, C. D., Hemin lyses malaria parasites. *Science* **1981,** *214* (4521), 667-9; (b) Menting, J. G.; Tilley, L.; Deady, L. W.; Ng, K.; Simpson, R. J.; Cowman, A. F.; Foley, M., The antimalarial drug, chloroquine, interacts with lactate dehydrogenase from Plasmodium falciparum. *Molecular and Biochemical Parasitology* **1997,** *88* (1-2), 215-24.

10. (a) Hempelmann, E., Hemozoin biocrystallization in Plasmodium falciparum and the antimalarial activity of crystallization inhibitors. *Parasitology Research* **2007,**

100 (4), 671-6; (b) Foote, S. J.; Cowman, A. F., The mode of action and the mechanism of resistance to antimalarial drugs. *Acta Tropica* **1994,** *56* (2-3), 157-71.

11. Olliaro, P., Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology & Therapeutics* **2001,** *89* (2), 207-19.

12. Nzila, A., The past, present and future of antifolates in the treatment of Plasmodium falciparum infection. *The Journal of Antimicrobial Chemotherapy* **2006,** *57* (6), 1043-54.

13. Salcedo-Sora, J. E.; Ochong, E.; Beveridge, S.; Johnson, D.; Nzila, A.; Biagini, G. A.; Stocks, P. A.; O'Neill, P. M.; Krishna, S.; Bray, P. G.; Ward, S. A., The molecular basis of folate salvage in Plasmodium falciparum: characterization of two folate transporters. *The Journal of Biological Chemistry* **2011,** *286* (52), 44659-68.

14. Krishna, S.; Uhlemann, A. C.; Haynes, R. K., Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates : Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy* **2004,** *7* (4-5), 233-44.

15. Meshnick, S. R.; Taylor, T. E.; Kamchonwongpaisan, S., Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiological Reviews* **1996,** *60* (2), 301-15.

16. (a) Olliaro, P. L.; Haynes, R. K.; Meunier, B.; Yuthavong, Y., Possible modes of action of the artemisinin-type compounds. *Trends in parasitology* **2001,** *17* (3), 122-6; (b) Pandey, A. V.; Tekwani, B. L.; Singh, R. L.; Chauhan, V. S., Artemisinin, an endoperoxide antimalarial, disrupts the hemoglobin catabolism and heme detoxification systems in malarial parasite. *The Journal of Biological Chemistry* **1999,** *274* (27), 19383-8.

17. Meshnick, S. R.; Yang, Y. Z.; Lima, V.; Kuypers, F.; Kamchonwongpaisan, S.; Yuthavong, Y., Iron-dependent free radical generation from the antimalarial agent artemisinin (qinghaosu). *Antimicrobial Agents and Chemotherapy* **1993,** *37* (5), 1108-14.

18. (a) Krishna, S.; Pulcini, S.; Fatih, F.; Staines, H., Artemisinins and the biological basis for the PfATP6/SERCA hypothesis. *Trends in parasitology* **2010,** *26* (11), 517-23; (b) Golenser, J.; Waknine, J. H.; Krugliak, M.; Hunt, N. H.; Grau, G. E., Current perspectives on the mechanism of action of artemisinins. *International Journal for Parasitology* **2006,** *36* (14), 1427-41.

19. Jung, M.; Kim, H.; Nam, K. Y.; No, K. T., Three-dimensional structure of Plasmodium falciparum Ca2+ -ATPase(PfATP6) and docking of artemisinin derivatives to PfATP6. *Bioorganic & Medicinal Chemistry Letters* **2005,** *15* (12), 2994-7.

20. (a) White, N. J., Delaying antimalarial drug resistance with combination chemotherapy. *Parassitologia* **1999,** *41* (1-3), 301-8; (b) White, N. J.; Olliaro, P. L., Strategies for the prevention of antimalarial drug resistance: rationale for combination chemotherapy for malaria. *Parasitology Today* **1996,** *12* (10), 399-401.

21. Sinclair, D.; Zani, B.; Donegan, S.; Olliaro, P.; Garner, P., Artemisinin-based combination therapy for treating uncomplicated malaria. *Cochrane Database of Systematic Reviews* **2009,** (3), CD007483.

22. Hudson, A. T., Atovaquone - a novel broad-spectrum anti-infective drug. *Parasitology Today* **1993,** *9* (2), 66-8.

23. Baggish, A. L.; Hill, D. R., Antiparasitic agent atovaquone. *Antimicrobial Agents and Chemotherapy* **2002,** *46* (5), 1163-73.

24. (a) Srivastava, I. K.; Rottenberg, H.; Vaidya, A. B., Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *The Journal of Biological Chemistry* **1997,** *272* (7), 3961-6; (b) Looareesuwan, S.; Chulay, J. D.; Canfield, C. J.; Hutchinson, D. B., Malarone (atovaquone and proguanil hydrochloride): a review of its clinical development for treatment of malaria. Malarone Clinical Trials Study Group. *The American Journal of Tropical Medicine and Hygiene* **1999,** *60* (4), 533-41.

25. Looareesuwan, S.; Viravan, C.; Webster, H. K.; Kyle, D. E.; Hutchinson, D. B.; Canfield, C. J., Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. *The American Journal of Tropical Medicine and Hygiene* **1996,** *54* (1), 62-6.

26. Srivastava, I. K.; Vaidya, A. B., A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrobial Agents and Chemotherapy* **1999,** *43* (6), 1334-9.

27. Frantz, M. C.; Wipf, P., Mitochondria as a target in treatment. *Environmental and Molecular Mutagenesis* **2010,** *51* (5), 462-75.

28. (a) Vaidya, A. B.; Mather, M. W., Mitochondrial evolution and functions in malaria parasites. *Annual Review of Microbiology* **2009,** *63*, 249-67; (b) Smith, R. A.; Hartley, R. C.; Cocheme, H. M.; Murphy, M. P., Mitochondrial pharmacology. *Trends in Pharmacological Sciences* **2012,** *33* (6), 341-52.

29. van Dooren, G. G.; Stimmler, L. M.; McFadden, G. I., Metabolic maps and functions of the Plasmodium mitochondrion. *FEMS Microbiology Reviews* **2006,** *30* (4), 596-630.

30. Bryant, C.; Voller, A.; Smith, M. J., The Incorporation of Radioactivity from (14c)Glucose into the Soluble Metabolic Intermediates of Malaria Parasites. *The American Journal of Tropical Medicine and Hygiene* **1964,** *13*, 515-9.

31. Vaidya, A. B.; Mather, M. W., A post-genomic view of the mitochondrion in malaria parasites. *Current Topics in Microbiology and Immunology* **2005,** *295*, 233-50.

32. Krungkrai, J., The multiple roles of the mitochondrion of the malarial parasite. *Parasitology* **2004,** *129* (Pt 5), 511-24.

33. Mather, M. W.; Vaidya, A. B., Mitochondria in malaria and related parasites: ancient, diverse and streamlined. *Journal of Bioenergetics and Biomembranes* **2008,** *40* (5), 425-33.

34. (a) Mather, M. W.; Henry, K. W.; Vaidya, A. B., Mitochondrial drug targets in apicomplexan parasites. *Current Drug Targets* **2007,** *8* (1), 49-60; (b) Painter, H. J.; Morrisey, J. M.; Mather, M. W.; Vaidya, A. B., Specific role of mitochondrial electron transport in blood-stage Plasmodium falciparum. *Nature* **2007,** *446* (7131), 88-91.

35. Murphy, M. P.; Smith, R. A., Drug delivery to mitochondria: the key to mitochondrial medicine. *Advanced Drug Delivery Reviews* **2000,** *41* (2), 235-50.

36. (a) Mukhopadhyay, A.; Weiner, H., Delivery of drugs and macromolecules to mitochondria. *Advanced Drug Delivery Reviews* **2007,** *59* (8), 729-38; (b) Ross, M. F.; Kelso, G. F.; Blaikie, F. H.; James, A. M.; Cocheme, H. M.; Filipovska, A.; Da Ros, T.; Hurd, T. R.; Smith, R. A.; Murphy, M. P., Lipophilic triphenylphosphonium cations as tools in mitochondrial bioenergetics and free radical biology. *Biochemistry. Biokhimiia* **2005,** *70* (2), 222-30; (c) Asin-Cayuela, J.; Manas, A. R.; James, A. M.; Smith, R. A.; Murphy, M. P., Fine-tuning the hydrophobicity of a mitochondria-targeted antioxidant. *FEBS Letters* **2004,** *571* (1-3), 9-16; (d) Smith, R. A.; Porteous, C. M.; Gane, A. M.; Murphy, M. P., Delivery of bioactive molecules to mitochondria in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **2003,** *100* (9), 5407-12.

37. Long, T. E.; Lu, X.; Galizzi, M.; Docampo, R.; Gut, J.; Rosenthal, P. J., Phosphonium lipocations as antiparasitic agents. *Bioorganic & Medicinal Chemistry Letters* **2012,** *22* (8), 2976-9.

38. (a) Murphy, M. P.; Smith, R. A., Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annual Review of Pharmacology and Toxicology* **2007,** *47*, 629-56; (b) Murphy, M. P., Targeting lipophilic cations to mitochondria. *Biochimica et Biophysica Acta* **2008,** *1777* (7-8), 1028-31.

CHAPTER 2

4(1*H*)-pyridone-based phosphonium cations

BACKGROUND

In chapter II, 4(1*H*)-pyridinone **1** (Fig. 1.1) was introduced as a potential low cost inhibitor of mitochondrial electron transport chain (mtETC). Clopidol (**2)** was the first 4(1*H*)-pyridone used as an antiparasitic agent in the treatment of coccidiosis, an intestinal infection caused by a single cell parasite of birds and wild animals.[1] In the 1960s, clopidol was shown by scientists at the Walter Reed Army Institute of Research to have activity against *Plasmodium sp.*, including chloroquine-resistant *P. falciparum.*[1b, 2] This observation prompted subsequent efforts to improve the antiparasitic activity of clopidol, but the results were not adequate for further development. However, the interest in clopidol did not stop at this point, and pharmacological studies afterward indicated that the nicotinamide analog was an antagonist of mtETC. Additionally, clopidol was shown to potentiate the antimalarial activity of hydroxynaphthoquinones *in vitro* and *in vivo*.[3] Most importantly, the simple structure of clopidol gave rise to many attempts to manipulate its structure in order to improve the antiparasitic activity.

The first attempt to improve the antimalarial activity of clopidol was to introduce the lipophilic side chain of atovaquone **3** (see Fig. 2.1) at C_3 of clopidol. Likewise, this modification was expected to enhance the membrane permeability of clopidol across the inner mitochondrial phospholipid bilayers where the mtETC

complexes are embedded thereby increasing inhibitor concentrations at the drug target site.

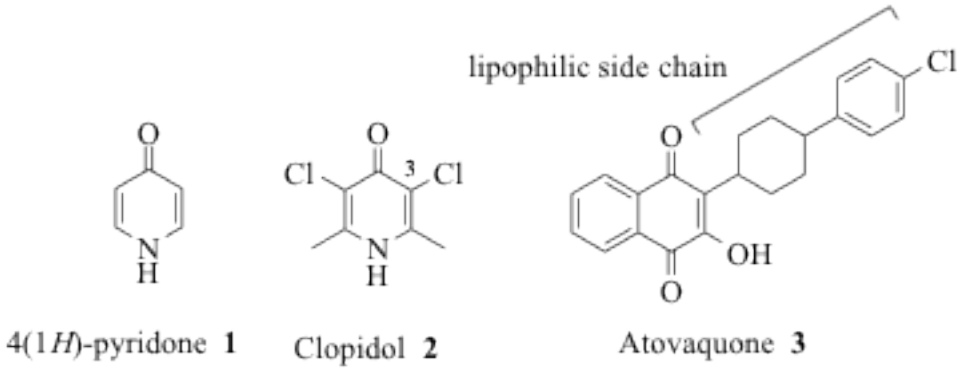


Fig. 2.1: 4(1*H*)-pyridones **1**, clopidol **2**, and atovaquone **3** structures

Accordingly, substitution with phenoxyaryl side chain increases the activity of clopidol and generates a promising candidate (GW844520, Fig. 2.2), which has a significant activity against atovaquone-resistant strains and high degree of selectivity for *Plasmodium* bc_1. Unfortunately, the development of GW844520 **4** was abolished because of its cardiotoxicity.[2, 4]

Another candidate from GlaxoSmithKline (GSK932121) was investigated afterward and showed a potent activity against resistant strains of *P. falciparum.* However, Phase 1 clinical trials have shown many toxicity issues and the development was stopped. Despite the failure of the 4(1*H*)-pyridinones as drug candidates, the drug-like properties of this series, potent activity against chloroquine-resistant *Plasmodium* spp., and their activity against the liver stage parasite encourages further investigation of this series.[2, 5]

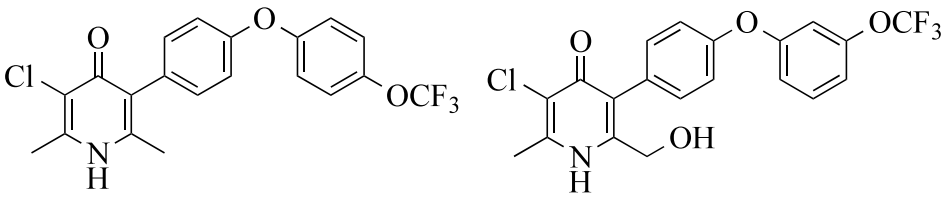
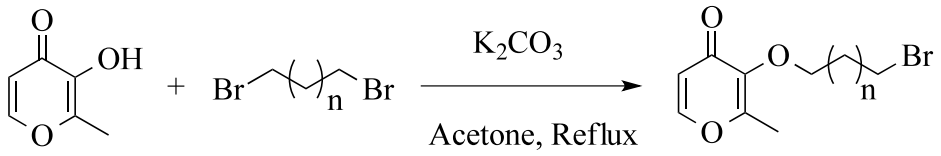


Fig. 2.2: Clopidol- antiplasmodial drug candidate

Synthesis 4(1*H*)-pyridinone-based phosphonium cations

With the advantage of attaching a phosphonium group in delivering biomolecules to mitochondria as discussed in Chapter 1, an investigation to enhance the distribution of 4(1H)-pyridones into mitochondria by attaching lipophilic cationic side chains was initiated. The synthetic strategy to prepare 4(1*H*)-pyridone-based phosphonium cations was to employ maltol **6** (Fig. 2.3) as a starting material. In the first step, introduction of an *n*-bromo alkyl side chain (hydrophobic linker) to the hydroxyl group of maltol at C_3 was achieved by simple alkylation under basic condition to give the corresponding 3-bromoalkoxy pyran-4-one **7** (scheme 2.1)**.** In attempt to improve the yield, combination of stronger bases such as cesium carbonate (Cs_2CO_3) and potassium *tert*-butoxide (*t*-BuO^-K^+) with polar aprotic solvents such as tetrahydrofuran (THF) and dimethylformamide (DMF) were tested. However, only slight increase in the yield was obtained. For economical purposes, potassium carbonate (K_2CO_3) under acetone reflux became the conditions used in the *O*-alkylation reaction (Scheme 2.1).

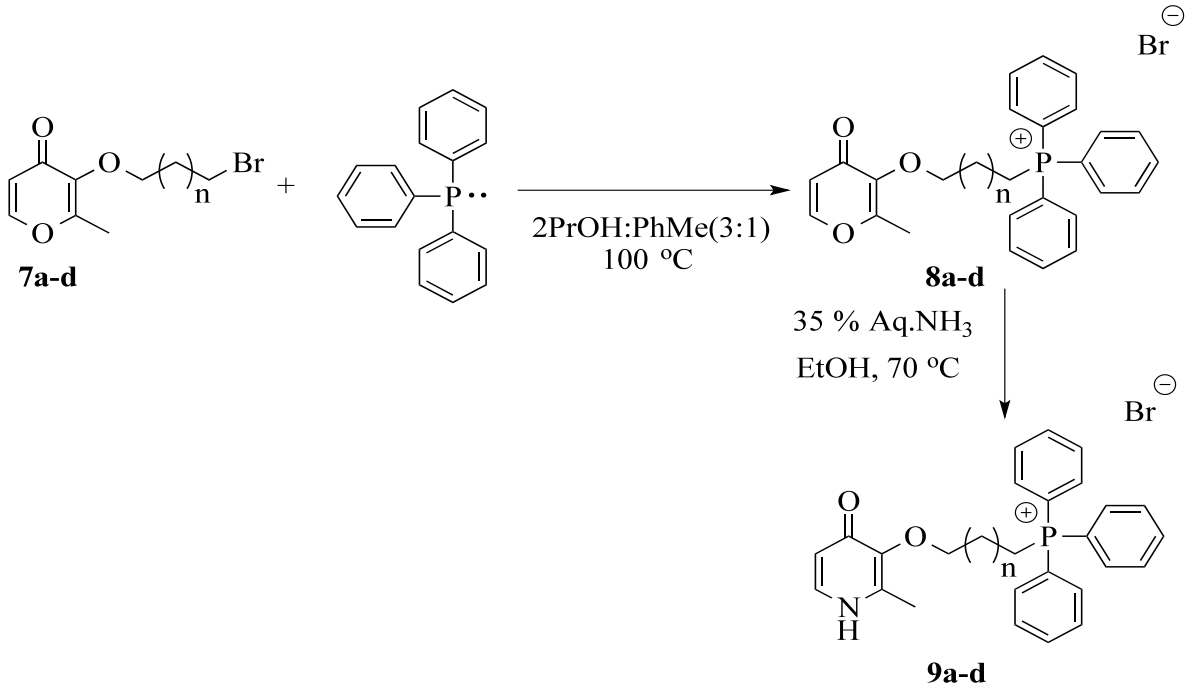
Scheme 2.1: Synthesis of 3-bromoalkoxy pyran-4-one



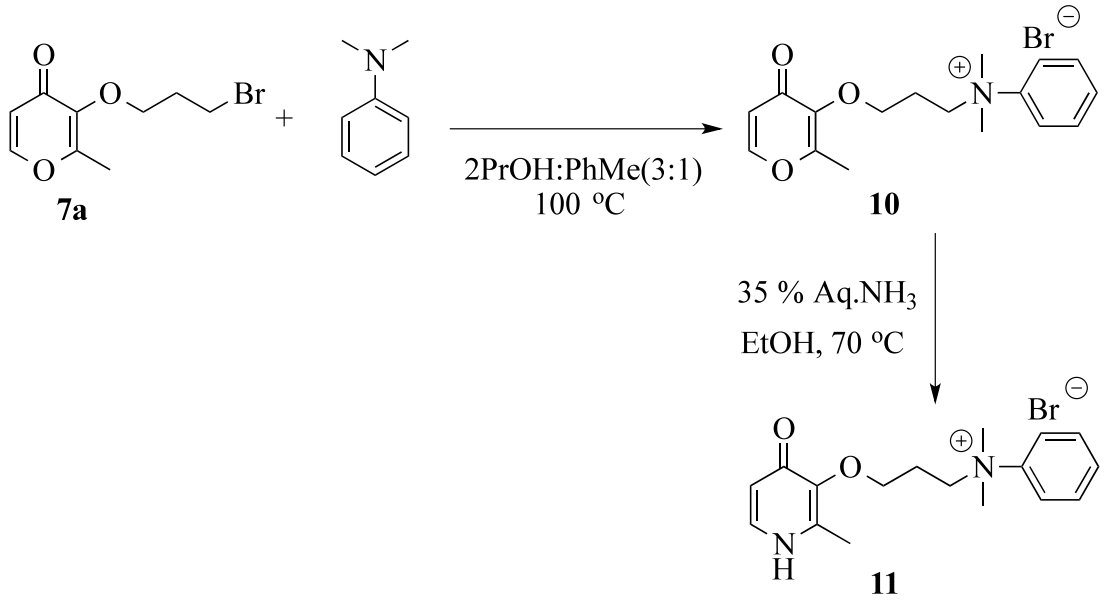
Maltol **6** Compound **7**

Coupling of phosphonium moiety with the 3-bromoalkoxy pyran-4-one **7** of various hydrocarbon lengths was successfully obtained by heating a mixture of **7** and a selected dibromoalkane in 3:1 2-PrOH:PhMe solvent at 100 °C to produce the phosphonium cations **8a-d** (Scheme 2.2). Similarly, an ammonium cation was obtained using the same reaction conditions (Scheme 2.3). In the final step, 4(1*H*)-pyridone-based phosphonium cations **9 a-d** and 4(1*H*)-pyridinone-based ammonium cation **11** were obtained by heating the corresponding 4*H*-pyran-4-one **7** in 35% aqueous ammonia (Scheme 2.2, 2.3).

Scheme 2.2: Synthesis of 4(1*H*)-pyridinone -based phosphonium cations



Scheme 2.3: Synthesis of 4(1*H*)-pyridinone -based ammonium cations



Conversion of pyran-4-one to 4(1*H*)-pyridinone **1** initially involves Michael addition at the α,β-unsaturated function of the pyran-4-one followed by ring-opening and ring-closure (Fig. 2.3).

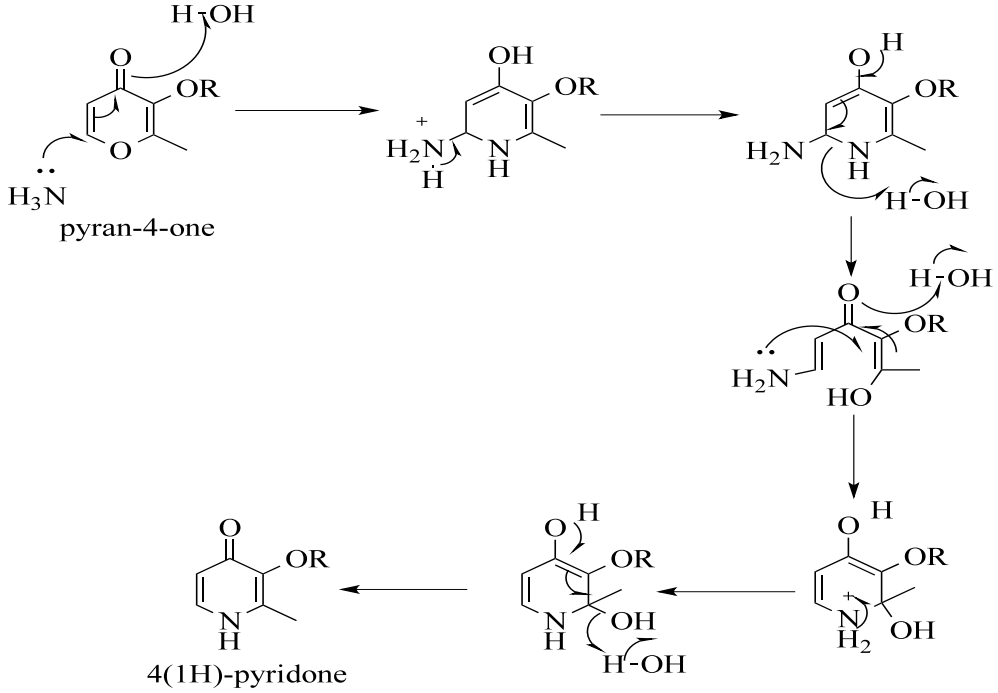


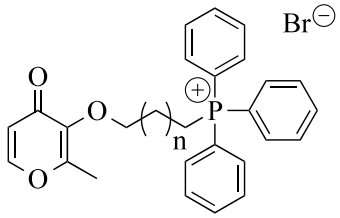
Fig. 2.3: Mechanism of pyran-4-one conversion to 4(1*H*)-pyridinone.[6]

Results and Discussion

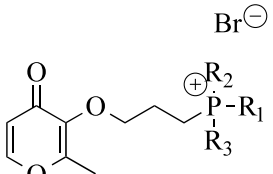
Philip J. Rosenthal's laboratory at the University of California, San Francisco General Hospital, performed the antiparasitic activity of **8a-d** and **9a-d**. In this assay, serial dilutions of test compounds in DMSO were incubated for 48 h with *P. falciparum* strain W2 cultured in human erythrocytes. Minimum inhibitory concentrations (IC_{50}) were then determined from plots of percentage parasitemia compared to control (untreated parasites) over inhibitor concentration.[7]

The results ranged from 370 nM to > 6.5 μM, compared to 185 nM for chloroquine (Tables 1 and 2). Analyses of the structure activity relationship (SAR) of the pyra-4-one analogs (Table 1) indicate that the inhibitory capacities are conferred by the phosphonium moiety and that the presence of pyra-4-one diminishes the antiparasitic activity. Similar effect was previously seen with phthalamide-based phosphonium cations. However, the inhibitory effect of 3-(butoxy) pyran-4-one **12** (IC_{50} 1300 nM) indicates that this compound may exhibit its effect by iron-chelation. Furthermore, introducing the phosphonium moiety **8b** and phosphonium substituents **8e-f** significantly decreased the inhibitory effect of **12,** probably because of the high lipophilicity of these substituents. Additionally, variation in the hydrophobic side chain was also tested and further confirmed that the presence of pyra-4-one decreased the antiparasitic activity. Since 4(1*H*)-pyridinone ring has been shown to act as an inhibitor of mtETC system, 4(1*H*)-pyridinone-based phosphonium cations as well as ammonium cations were investigated.[8 9] Surprisingly, abolishing of the activity of the pyran-4-one-based phosphonium cations was observed when converted to the corresponding 4(1*H*)-pyridinone-based phosphonium cations (Table 2.2).

Table 2.1: Comparison of IC_{50} of 3-alkylated pyran-4-one-based phosphonium cations for *P. falciparum* growth

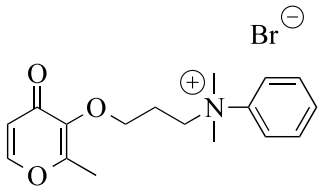


8a-d

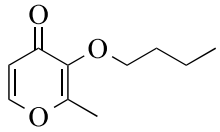


8e $R_1 = R_2 = Ph$
$R_3 = Me$
$IC_{50} > 10{,}000$ nM

8f $R_1 = R_2 = Me$
$R_3 = Ph$
$IC_{50} > 10{,}000$ nM



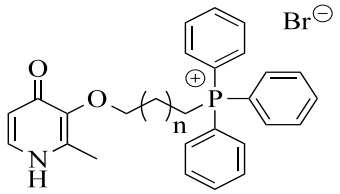
10
$IC_{50} > 10{,}000$ nM



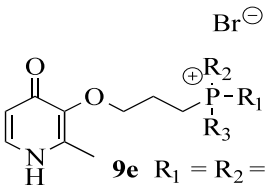
12
$IC_{50} = 1300$ nM

Compound	n	R	IC_{50} (nM)
8a	1	Ph	>10,000
8b	2	Ph	6500
8c	4	Ph	3450
8d	8	Ph	370
CQ			185

Table 2.2: Comparison of IC_{50} of 4(1*H*)-pyridinone -based phosphonium cations for *P. falciparum* growth

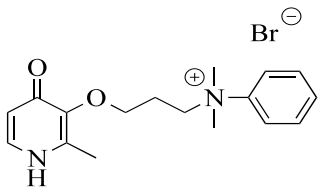


9a-d

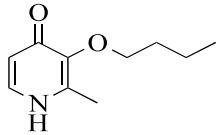


9e $R_1 = R_2 = Ph$
$R_3 = Me$
$IC_{50} > 10{,}000$ nM

9f $R_1 = R_2 = Me$
$R_3 = Ph$
$IC_{50} > 10{,}000$ nM



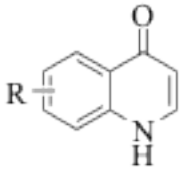
11
$IC_{50} > 10{,}000$ nM



13
$IC_{50} > 10{,}000$ nM

Compound	N	R	IC_{50} (nM)
9a	1	Ph	>10,000
9b	2	Ph	>10,000
9c	4	Ph	>10,000
9d	8	Ph	4720
CQ			185

Overall, antiparasitic activity examination and analysis of SAR indicate that neither pyran-4-one-based phosphonium cations nor 4(1*H*)-pyridinone-based phosphonium cations are acting as inhibitors of mtETC enzymes. The unexpected outcome of these compounds, particularly the 4(1*H*)-pyridinone-based phosphonium cations, led us to turn our interest to examining the antiparasitic activity of 4(1*H*)-quinolones **14**, which have been found in many of the mtETC enzymes inhibitors.[9]

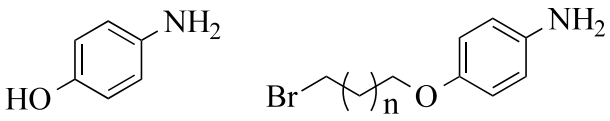


4(1*H*)-quinolone **14**

Fig. 2.4: 4 (1*H*)-quinolone structure

Synthesis 4 (1*H*)-quinolone-based phosphonium cations

The original synthesis plan was to prepare the substituted aniline intermediate **15** to introduce into Gould-Jacobs reaction.[10] The commercially available 4-aminophenol **15** was used as a starting material to generate **16** (Fig. 2.5).

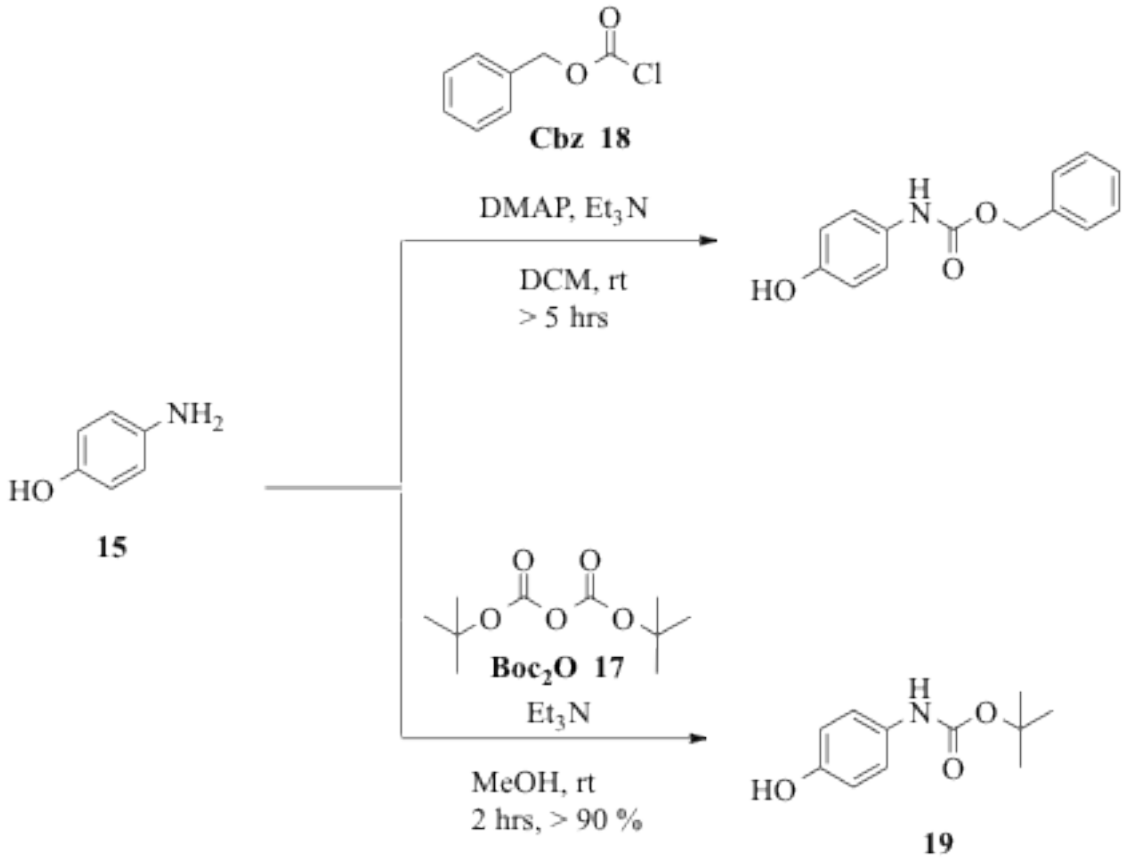


15 **16**

Fig. 2.5: 4-Aminophenol and its intermediate structures

O-alkylation of the para hydroxyl group of **15** was successfully accomplished after the selective protection of the primary amine. For the purpose of protection, di-tert-butyl dicarbonate (Boc_2O) **17** and carboxybenzyl chloride (Cbz) **18** were tested. Both methods were successful, however Boc_2O protection was faster and no purification was required to produce **19** (Scheme 2.4).

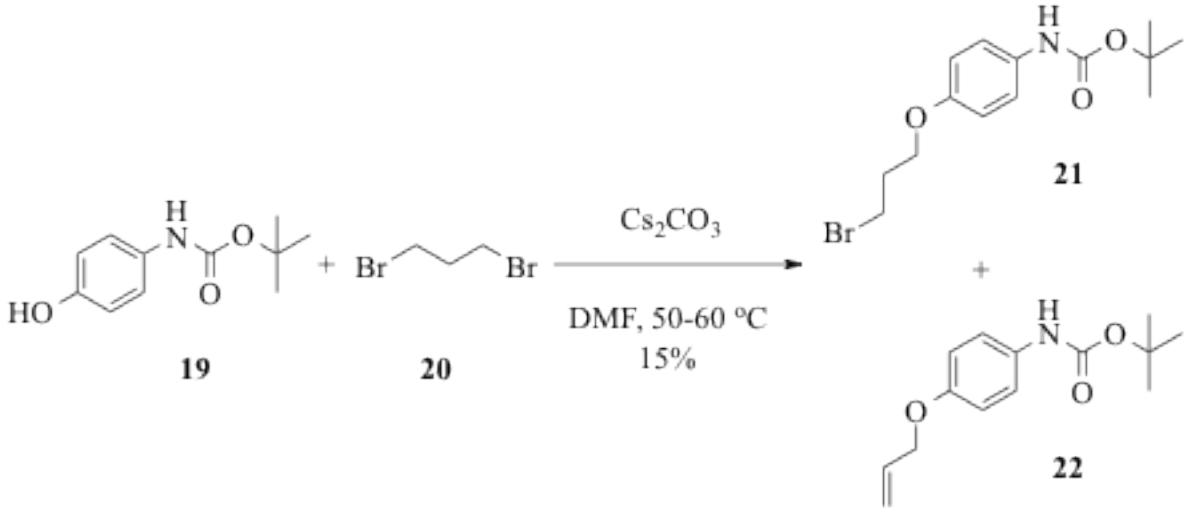
Scheme 2.4: Selective protection of 4-aminophenol **15**



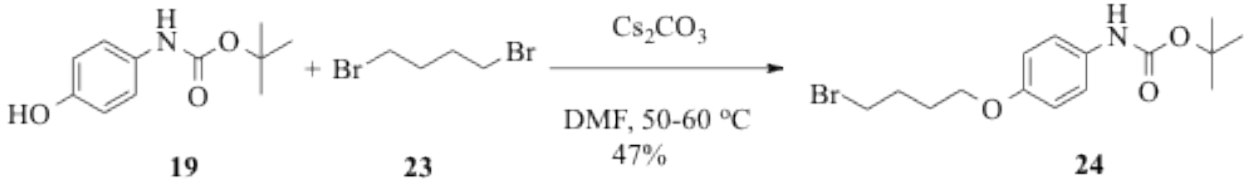
Unlike the *O*-alkylation of maltol **6,** a mixture of K_2CO_3 in acetone was not useful in the *O*-alkylation of phenol **19.** However, Cs_2CO_3 in DMF produced the desired product **19** in a moderate yield at room temperature due to the cesium effect.[11] In order to enhance the yield, the reaction was conducted at 50-60 ^oC, and resulted in about 10% increase in the final product acquired (Scheme 2.5). Noticeably, low yield

was obtained when the reaction was performed using 1,3-dibromopropane **20**. Monitoring the TLC showed two newly formed spots that were very close. After purification, the less polar spot corresponded to the desired product **21,** while the more polar spot was identical to the elimination product **22**. In contrast to the *O*-alkylation with 1,3-dibromopropane, 1,4-dibromobutane **23** was used and only one new spot on TLC was observed which corresponded to the desired product **24**. Because the yield of precursor **24** was superior to that of **21**, O-alkylation with 1,4- dibromobutane was selected in order to get sufficient amount of starting material to proceed for the next reaction (Scheme 2.6).

Scheme 2.5: O-alkylation of N-Boc protected phenol **19** with 1,3-dibromopropane **20**

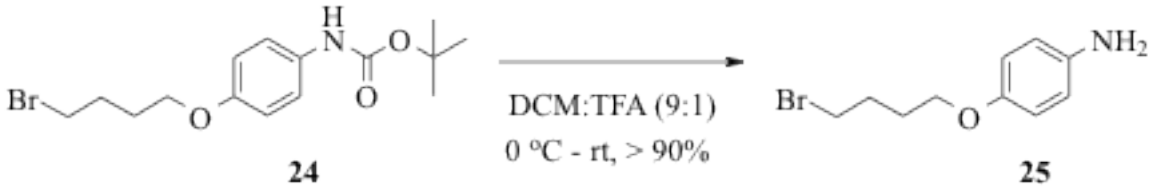


Scheme 2.6: O-alkylation of N-Boc protected phenol **19** with 1,4-dibromobutane **23**



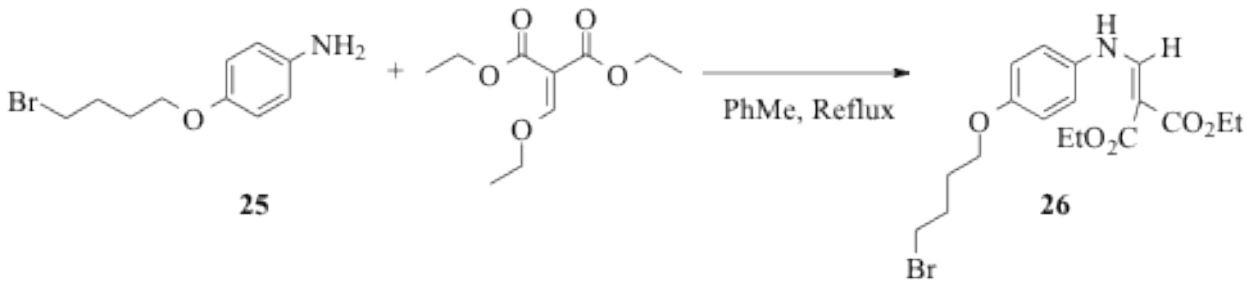
Following deprotection of the N-Boc amine **24** using trifluoroacetic acid (TFA) in dichloromethane (DCM) in 1:9 (TFA:DCM) proportions (Scheme 2.7). The next step in the formation of quinolone **14** was to employ the Gould-Jacobs reaction. In the first step, aniline derivative **25** was reacted with malonic acid derivative diethyl ethoxymethylenemalonate (DEMM) to provide 4-(4-bromobutoxy)anilino methylene malonate **26** (Scheme 2.8). Refluxing at 90-100 ^{o}C for about 16 hrs generated two spots as observed on TLC. Purification of the mixture indicated that the less polar spot (R_f = 0.47, 2:1 Hex:EtOAc) corresponded to the desired product **26.**

Scheme 2.7: Deprotection of N-Boc aniline **24**



24 25

Scheme 2.8: Reaction of aniline derivative **25** with malonic acid derivative DEMM

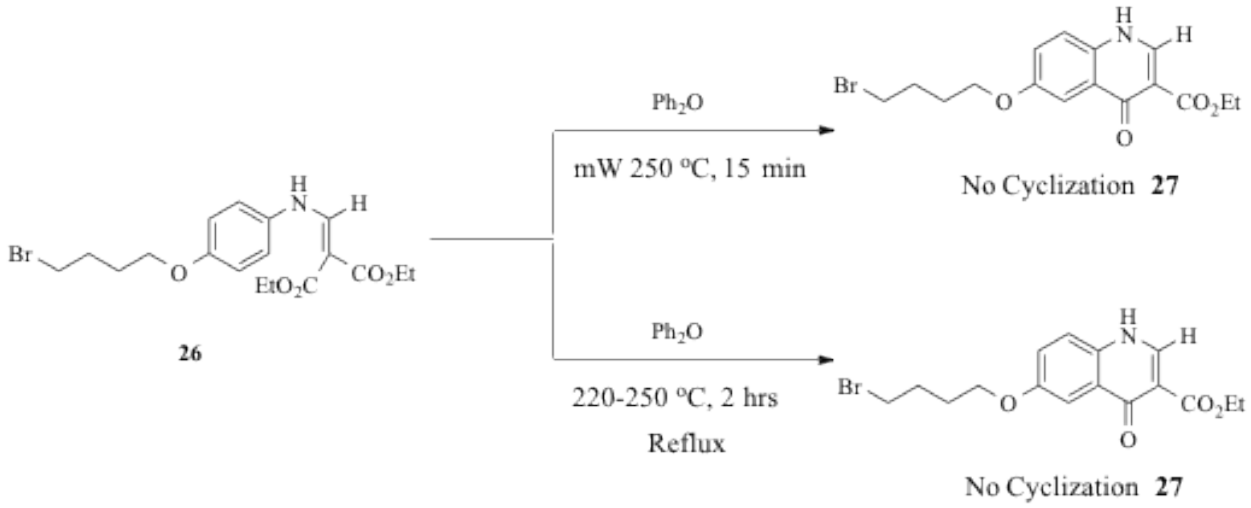


25 26

Two methods were then utilized to accomplish the cyclization products **26.** The first method was to employ the microwave-assisted Gould-Jacobs reaction**.** Heating aniline **26** at 250 ^{o}C for a time-interval ranging from 5-15 min was not successful in generating the desired product **27** (Scheme 2.9). A black precipitate was formed after heating at 250 °C for 15 min, but the TLC of the precipitate showed many closely-spaced spots that were hard for purification. The second method to obtain the

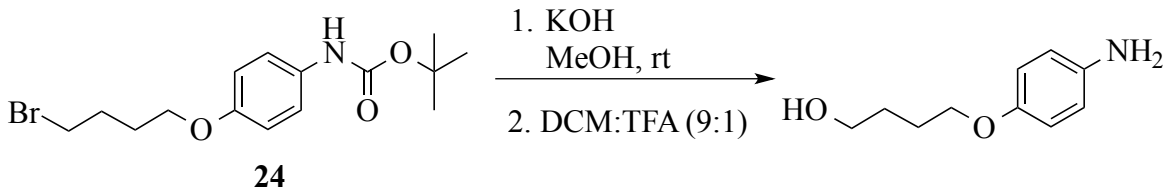
cyclization product **27** was to reflux the intermediate **26** in diphenyl ether (Ph_2O) at extremely high temperature of 220-250 °C. Unfortunately, no precipitated product was formed which was believed to be due to decomposition of the bromide side chain.

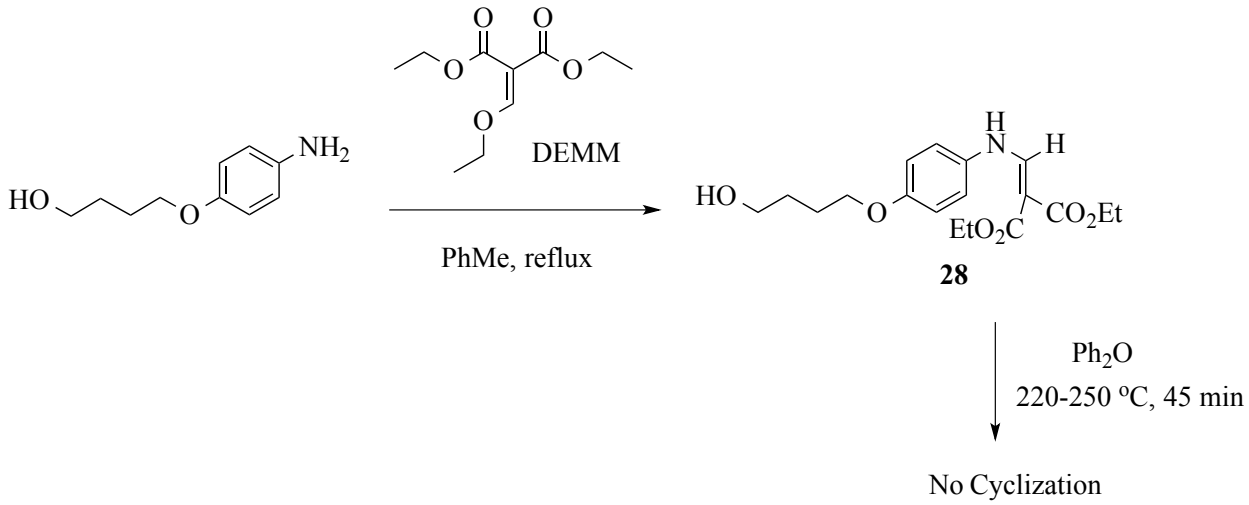
Scheme 2.9: Gould-Jacobs reaction of **26**



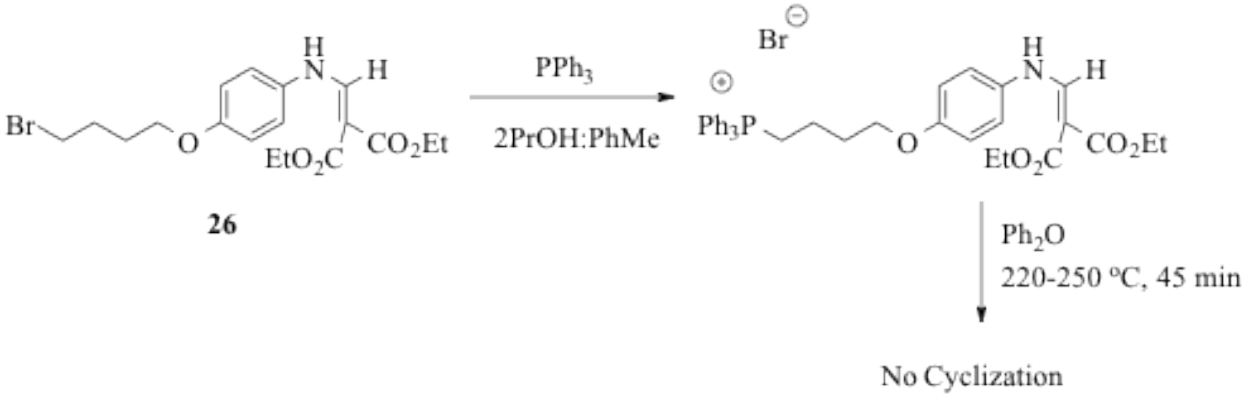
Methanolysis reaction was then used to convert the bromide of N-Boc protected amine **23** into a stable hydroxyl, and followed by the Gould-Jacobs cyclization of ester **28** (Scheme 2.10). Alternatively, TPP was attached to bromide **26** and Gould-Jacobs reaction was attempted to provide quinolone-based phosphonium (Scheme 2.11). However, both reactions failed and no solids formed after 45 minutes refluxing at 220-250 °C in Ph_2O.

Scheme 2.10: Methanolysis and Gould-Jacob reactions of **24**





Scheme 2.11: Gould-Jacob reaction of phosphonium-attached intermediate



Failure in obtaining the cyclization product by employing Gould-Jacob reaction prompted us also to examine Conrad Limpach reaction. In this reaction, thermal condensation of aniline with β-ketoester leads to the formation of quinolone (Fig. 2.4).[12]

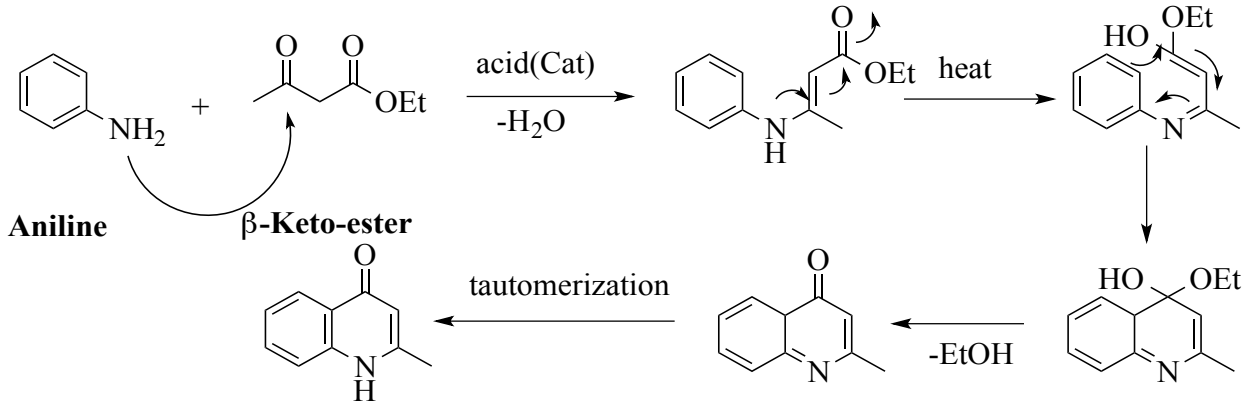
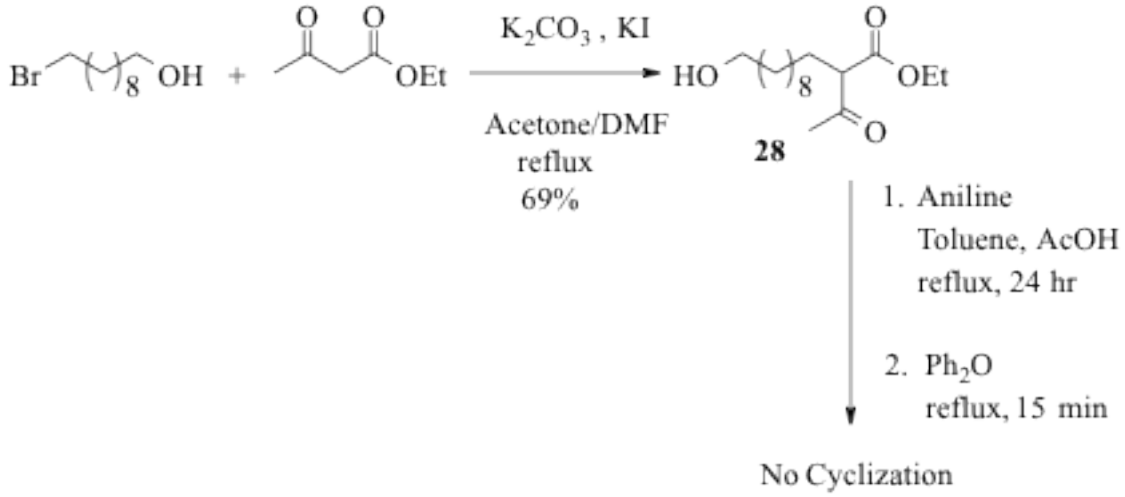


Fig. 2.6: Conrad Limpach reaction mechanism

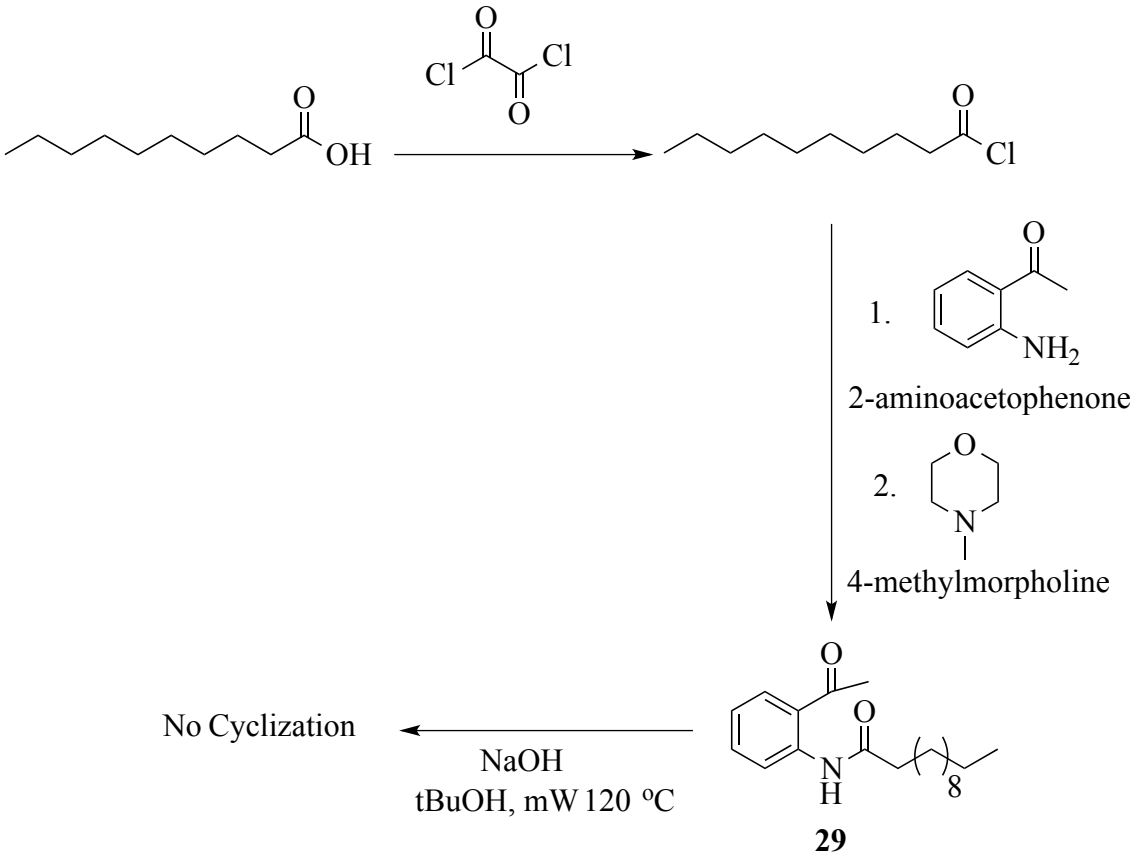
Reaction of 10-bromo-1-decanol with β-ketoester 28 generated α-Substituted β-ketoester **28**. The resultant product was then reacted with aniline following Conrad Limpach reaction procedure (Scheme 2.12). However, this method was not successful in producing the 2-substituted quinolone, probably because of inability to collect H_2O produced in this reaction.

Scheme 2.12: Conrad Limpach reaction of α-substituted β-keto ester with aniline



The previous cyclization methods employed harsh condition such as high temperature (>200 °C), which leads to the decomposition of the final products and formation of its isomers. Since the reaction conditions are relatively mild in Camps cyclization, this reaction was employed to generate the 2-substitiuted quinolones.[13] In this reaction, cyclocondensation of 2-amidoacetophenone carried out under basic condition and relatively low temperature. 2-Aminoacetophenone was reacted decanoyl chloride to generate the substituted 2-amidoacetophenone **29,** which then proceeded to the Camps cyclization (Scheme 2.13).[14] Unfortunately, this method was not successful probably because of poor regioselectivity that leads to the formation of 2- and 4-quinolones.

Scheme 2.13: Camps Cyclization reaction of intermediate **29**



Conclusion

4(1*H*)-pyridone-based phosphonium cations were successfully synthesized and tested against the chloroquine-resistant *P. falciparum* strain W2. The results showed minimal antiparasitic activity in sub-micromolar range and indicated that 4(1*H*) pyridone was not acting as antagonists of mtETC complexes. Based on this outcome, we shifted the interest to examine quinolone-based derivatives that have been known to inhibit mtETC complexes. However, efforts to synthesize 7-substituted and 2-substituted quinolone-based phosphonium cations were not accomplished by utilizing three different methods. Therefore, the progress of these quinolone-based phosphonium cations were impeded. Since quinolone compounds can also be existed

in the enol form, this alerted our attention to investigate quinoline-based phosphonium cations as potential inhibitors of mtETC complexes. This subject will be addressed in detail in chapter III.

REFERENCES

1. (a) Markley, L. D.; Eckman, M. K., Synthesis and anticoccidial activity of 3-fluoro-5-chloro- and -bromo-2,6-dimethyl-4-pyridinol. *Journal of Medicinal Chemistry* **1973,** *16* (3), 297-8; (b) Markley, L. D.; Van Heertum, J. C.; Doorenbos, H. E., Antimalarial activity of clopidol, 3,5-dichloro-2,6-dimethyl-4-pyridinol, and its esters, carbonates, and sulfonates. *Journal of Medicinal Chemistry* **1972,** *15* (11), 1188-9.

2. Yeates, C. L.; Batchelor, J. F.; Capon, E. C.; Cheesman, N. J.; Fry, M.; Hudson, A. T.; Pudney, M.; Trimming, H.; Woolven, J.; Bueno, J. M.; Chicharro, J.; Fernandez, E.; Fiandor, J. M.; Gargallo-Viola, D.; Gomez de las Heras, F.; Herreros, E.; Leon, M. L., Synthesis and structure-activity relationships of 4-pyridones as potential antimalarials. *Journal of Medicinal Chemistry* **2008,** *51* (9), 2845-52.

3. Fry, M.; Williams, R. B., Effects of decoquinate and clopidol on electron transport in mitochondria of Eimeria tenella (Apicomplexa: Coccidia). *Biochemical Pharmacology* **1984,** *33* (2), 229-40.

4. Xiang, H.; McSurdy-Freed, J.; Moorthy, G. S.; Hugger, E.; Bambal, R.; Han, C.; Ferrer, S.; Gargallo, D.; Davis, C. B., Preclinical drug metabolism and pharmacokinetic evaluation of GW844520, a novel anti-malarial mitochondrial electron transport inhibitor. *Journal of Pharmaceutical Sciences* **2006,** *95* (12), 2657-72.

5. Bathurst, I.; Hentschel, C., Medicines for Malaria Venture: sustaining antimalarial drug development. *Trends in Parasitology* **2006,** *22* (7), 301-7.

6. L. Saghaie, M. M. S. a. A. N., Synthesis, analysis and determination of partition coefficients of N-arylhydroxypyridinone derivatives as iron chelators. *Reasearch in Pharmaceutical Sciences* **2006,** *1*, 40-48.

7. Coteron, J. M.; Catterick, D.; Castro, J.; Chaparro, M. J.; Diaz, B.; Fernandez, E.; Ferrer, S.; Gamo, F. J.; Gordo, M.; Gut, J.; de las Heras, L.; Legac, J.; Marco, M.; Miguel, J.; Munoz, V.; Porras, E.; de la Rosa, J. C.; Ruiz, J. R.; Sandoval, E.; Ventosa, P.; Rosenthal, P. J.; Fiandor, J. M., Falcipain inhibitors: optimization studies of the 2-pyrimidinecarbonitrile lead series. *Journal of Medicinal Chemistry* **2010,** *53* (16), 6129-52.

8. Barton, V.; Fisher, N.; Biagini, G. A.; Ward, S. A.; O'Neill, P. M., Inhibiting Plasmodium cytochrome bc1: a complex issue. *Current Opinion in Chemical Biology* **2010,** *14* (4), 440-6.

9. Rodrigues, T.; Lopes, F.; Moreira, R., Inhibitors of the mitochondrial electron transport chain and de novo pyrimidine biosynthesis as antimalarials: The present status. *Current Medicinal Chemistry* **2010,** *17* (10), 929-56.

10. Gordonggouldj, R.; Walter, J., The Synthesis of Certain Sabstituted Quinolines and S,6-Benzoquinolines. *Journal of the American Chemical Society* **1939,** *61*, 2890-2895.

11. Salvatore, R. N.; Nagle, A. S.; Jung, K. W., Cesium effect: high chemoselectivity in direct N-alkylation of amines. *The Journal of Organic Chemistry* **2002,** *67* (3), 674-83.

12. Li, J. J., *Name Reactions*. Springer: 2009.

13. Derong Ding, X. L., Xin Wang, Yongli Du and Jingkang Shen, Microwave-assisted rapid and straightforward synthesis of 2-aryl-4-quinolones from acylated 20-aminoacetophenones. *Tetrahedron Letters* **2006,** *47*, 6997-6999.

14. Jones, C. P.; Anderson, K. W.; Buchwald, S. L., Sequential Cu-catalyzed amidation-base-mediated camps cyclization: a two-step synthesis of 2-Aryl-4-quinolones from o-halophenones. *The Journal of Organic Chemistry* **2007,** *72* (21), 7968-73.

CHAPTER 3

7-Chloro-4-amino quinoline-based phosphonium cations

BACKGROUND

For decades, quinoline antimalarials have been the mainstay and the most successful class of drugs for malaria treatment. This class contains a quinoline ring as the basic pharmacophore, which is essential for antiparasitic activity. Further, substituents bound to the quinoline ring, sub-divides the class into three families of quinolone antimalarials: 8-aminoquinolines (primaquine **1**, PQ); quinolines methanol (mefloquine **2**, MQ); and 4-aminoquinolines (Chloroquine **3**, CQ) (Fig. 3.1).

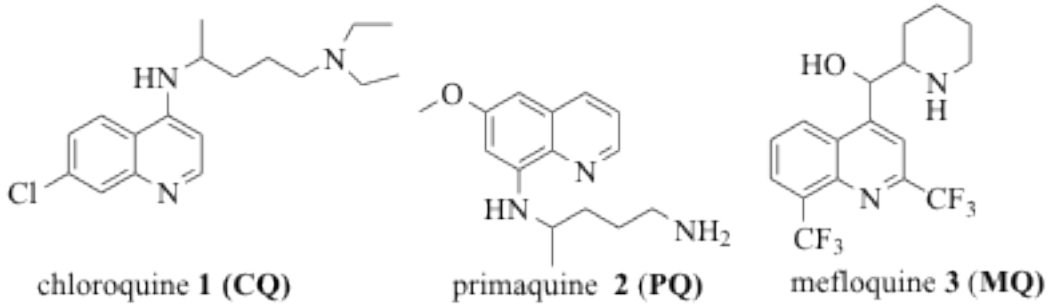


Fig 3.1: Families of quinoline antimalarials

4-Aminoquinolines, in particular CQ **3**, have been widely used as a first line choice for treatment of malaria in endemic areas.[1] However, sporadic distribution of resistance for CQ has limited the usefulness of this drug. In attempts to develop more efficacious antimalarial against CQ sensitive and CQ resistant strains of plasmodium, modifications on the quinoline pharmacophore have been investigated.[2] Altering the side chain length of CQ has resulted in AQ-13 **4** (Fig. 3.2), a promising candidate that progressed to Phase II trials in Mali.[2c, 3] Amodiaquine **5** (AQ), a phenyl-substituted

analogue of CQ, was discovered in 1946 to be active against non-human malaria, and has been used in malaria treatment thereafter. However, clinical use of AQ has restricted use due to hepatotoxicity and agranulocytosis resulting from the formation of the toxic quinoneimine metabolite **8** by Cyp450-mediated metabolism (Fig. 3.3).[4]

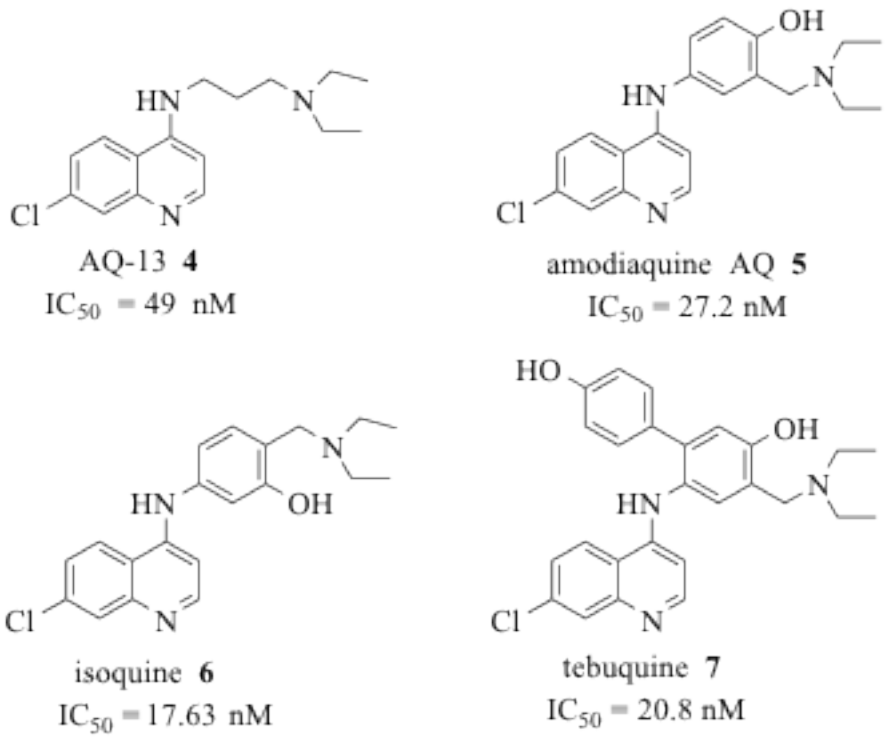


Fig. 3.2: Chloroquine analogue AQ-13 and amodiaquine analogues activity against CQ-resistant (K1) strain

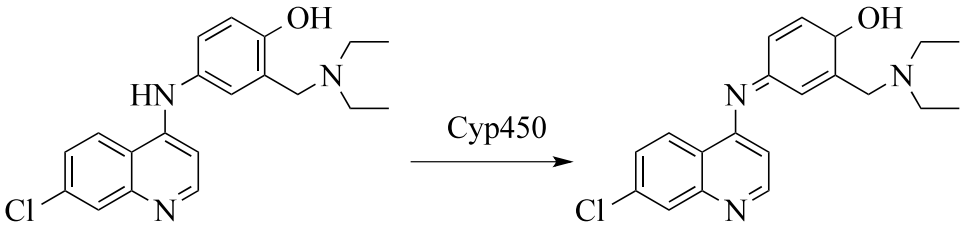


Fig. 3.3: Cyp450-mediated metabolism of AQ

Since amodiaquine (AQ) has shown a remarkable clinical efficacy in endemic areas with CQ-resistant strains, attempts to overcome AQ toxicity have revealed promising candidates against CQ-resistance plasmodium such as isoquine **6** and tebuquine **7** (Fig. 3.2).[5] These results therefore, suggest that the resistance of CQ in endemic areas is highly specific to the CQ structure, and indicate that changes to the target of this class is not involved in CQ resistance. According to this, plenty of projects have continued to develop quinoline-based antimalarials, and have revealed several promising candidates that entered clinical trials to overcome CQ-resistant plasmodium.

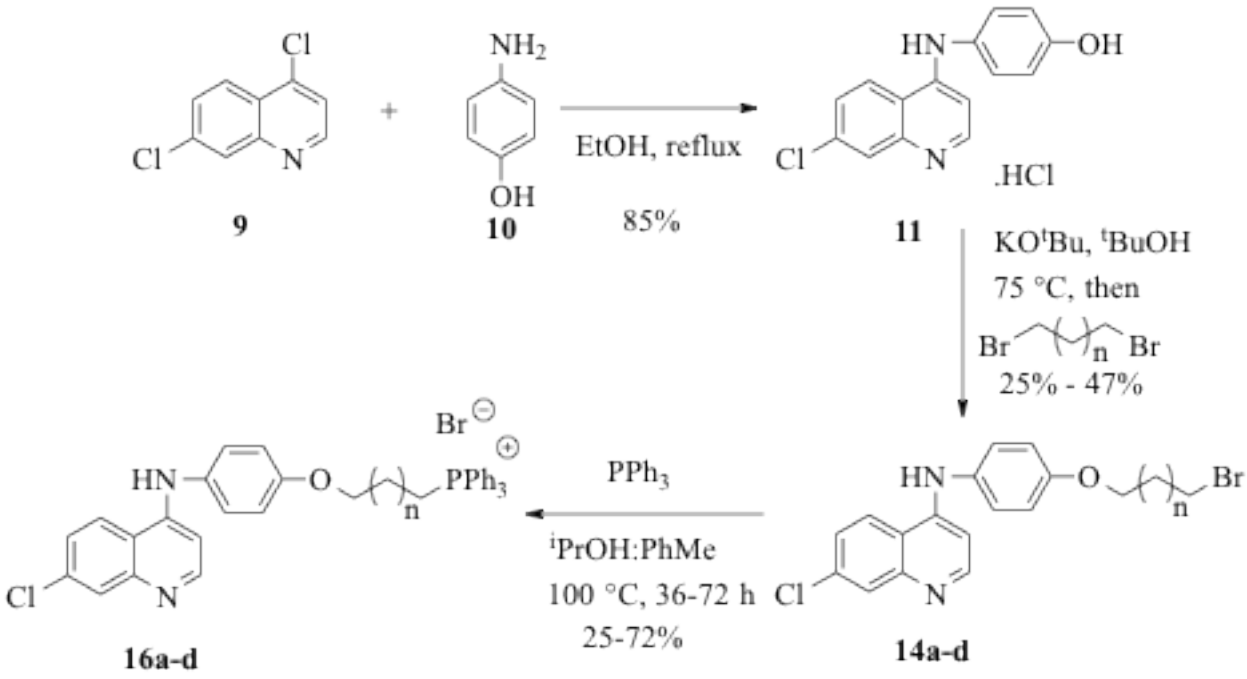
Although the heme detoxification pathway targeted by CQ is generally accepted as a major mode of action of CQ, multiple evidences have shown that CQ also affects many cellular enzymes, which may in part contribute to the antimalarial effect of CQ.[6] Among these evidences, a study has shown that mitochondrial NADH, succinate dehydrogenase (SDH), and cytochrome C oxidase activities are reduced following CQ treatment in rats.[7] Additionally, CQ has been shown to adversely affect mitochondrial energy transduction, and to inhibit the NADH-dehydrogenase CoQ system.[8] In addition to its effect on O_2 consumption in asexual and sexual blood stages of *Plasmodium falciparum*, CQ also induces morphological changes in mitochondria from *Plasmodium falciparum*.[9] A swollen mitochondrion is often observed after CQ treatment. Furthermore, 8-aminoquinoline-based antimalarials such as PQ **1** have been shown to interact with the bc_1 complex of mtETC system.[10] [8c] As a result, they are effective in prophylaxis against *P. falciparum.*

With these observations in mind, quinoline-based antimalarials show a strong possibility of interfering with mtETC system. Exploiting the advantage of attaching phosphonium moiety in delivering drugs to the mitochondria, the potential of using amodiaquine-based phosphonium cations as inhibitors of mitochondrial NADH dehydrogenase is presented in this chapter.

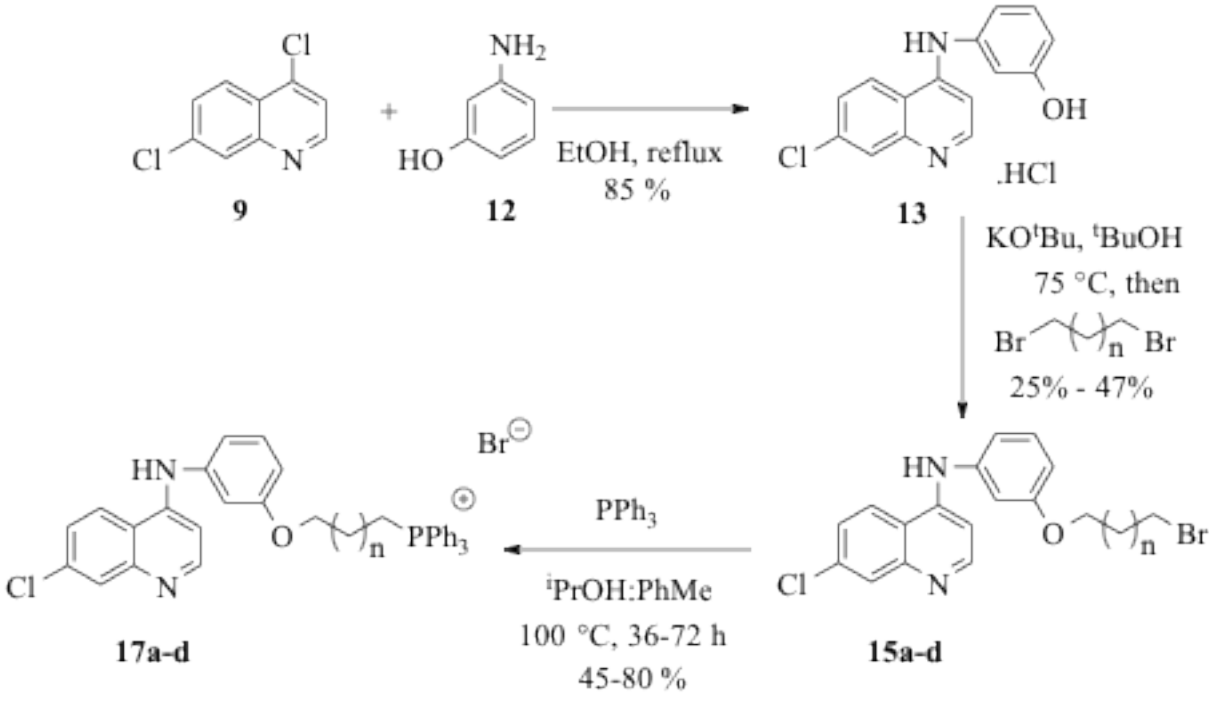
Synthesis of amodiaquine-based phosphonium cations

Synthesis of amodiaquine-based phosphonium cations was successfully accomplished in three steps. In the first step, a scalable route to prepare *para*-substituted amodiaquine intermediate **11** and *meta*-substituted amodiaquine intermediate **13** were employed. In this reaction, 4,7-dichloroquinoline **9** and *para*-aminophenol **10** were refluxed in EtOH for 18 hrs. Yellow solid started to precipitate in the first two hours of the reaction and was collected by filtration after the reaction was complete (Scheme 3.1). Similarly, *meta*-aminophenol **12** was refluxed with **9** under the same condition to prepare the *meta*-substituted amodiaquine intermediate **13** (Scheme 3.2)**.** In the second step, *O*-alkylation of **11** and **13** to introduce an n-bromoalkyl chain (hydrophobic linker) was accomplished under a relatively strong basic condition (Scheme 3.1 and 3.2). The final step involved the coupling of the phosphonium moiety with the n-bromoalkoxy amodiaquine intermediate **14** and **15** using the same condition discussed in chapter II (Scheme 3.1 and 3.2).

Scheme 3.1: Synthesis of *para*-substituted amodiaquine intermediate **11, 14,** and amodiaquine-based phosphonium cations **16**

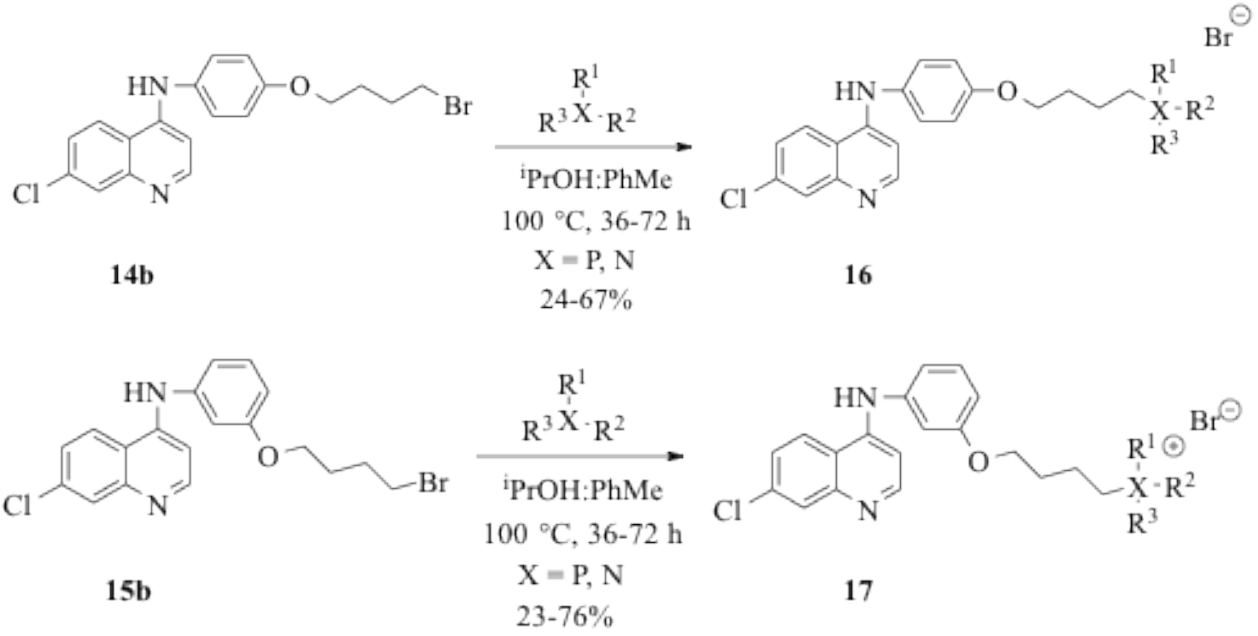


Scheme 3.2: Synthesis of *meta*-substituted amodiaquine intermediate **13, 15,** and amodiaquine-based phosphonium cations **17**

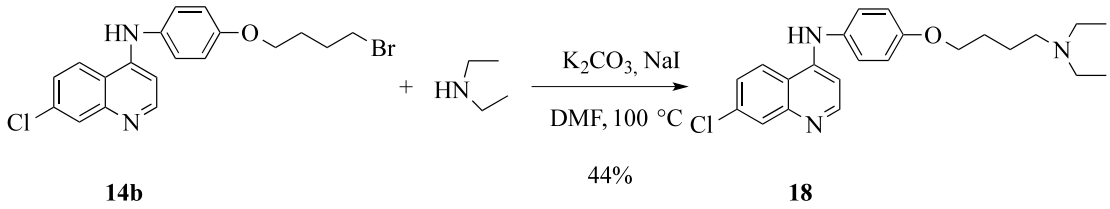


Similarly, amodiaquine-based ammonium cations as well as substituent of phosphonium moiety (R = Me) were prepared using the same reaction conditions (Scheme 3.3). For comparison, diethyl amine attached to amodiaquine intermediate **14b** was obtained under slightly basic condition (Scheme 3.4).

Scheme 3.3: Synthesis of *para*- and *meta*-substituted amodiaquine-based onium cations **16** and **17**



Scheme 3.4: Attachment of diethyl amine to amodiaquine intermediate **14b**



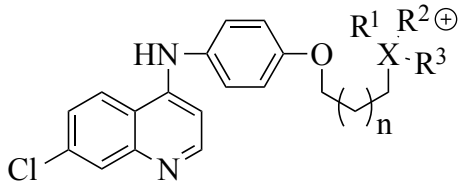
Results and Discussion

Compounds **16-18** were synthesized to test the hypothesis that amodiaquine-based phosphonium cations act as inhibitors of mitochondrial NADH dehydrogenase (Complex I). The rationale behind this hypothesis is that attachment of phosphonium

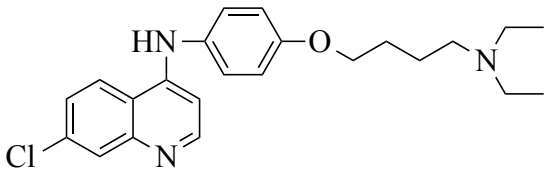
moiety will enhance the distribution of amodiaquine derivatives into mitochondria. Once inside, the 7-chloro-4-aminoquinoline pharmacophore may act as a competitive inhibitor of nicotinamide-containing substrates (e.g., NADH)-, and potentially serve as an antagonist of the mtETC system. Additionally, substituents on the phosphonium moiety, and analogs that possess ammonium cation moieties were tested to investigate the relative activity of these derivatives in comparison to the phosphonium-based compounds.

Antiplasmodial activity for these compounds against CQ-resistant *P. falciparum* strain W2 was determined according to a previously described method in chapter II. The minimum inhibitory concentrations for these compounds (IC_{50}s) are presented in Table 1 and 2. Analysis of IC_{50} values of these compounds indicated that *para*-substituted amodiaquine-based phosphonium cations **16a-d** are generally more active than the corresponding *meta*-substituted amodiaquine-based phosphonium cations **17a-d.** A possible explanation for the discrepancy in activities observed with *para*-substituted and *meta*-substituted analogues may be attributed to the difference in drug-substrate specificity of these analogues to NADH dehydrogenase (Complex I).

Table 3.1: Comparison of *para*-substituted amodiaquine IC_{50}s for *P. falciparum* growth



16a-f

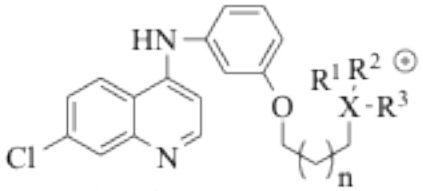


18

IC_{50} = 45.21 nM

Compd	n	X	R^1	R^2	R^3	IC_{50} (nM)
16a	1	P	Ph	Ph	Ph	292.6
16b	2	P	Ph	Ph	Ph	254.6
16c	4	P	Ph	Ph	Ph	273.4
16d	8	P	Ph	Ph	Ph	417.6
16e	2	P	Ph	Ph	Me	270.05
16f	2	P	Ph	Me	Me	299
16g	2	P	^{n}Bu	^{n}Bu	^{n}Bu	274.25
16h	2	N	Et	Et	Et	2154
19	2	C	H	H	H	1357
CQ						185

Table 3.2: Comparison of *meta*-substituted amodiaquine IC_{50}s for *P. falciparum* growth



17a-f

Compd	n	X	R^1	R^2	R^3	IC_{50} (nM)
17a	1	P	Ph	Ph	Ph	1146
17b	2	P	Ph	Ph	Ph	1143.7
17c	4	P	Ph	Ph	Ph	593
17d	8	P	Ph	Ph	Ph	335.4
17e	2	P	Ph	Ph	Me	684.7
17f	2	P	Ph	Me	Me	439
17g	2	P	^{n}Bu	^{n}Bu	^{n}Bu	371
17h	2	N	Et	Et	Et	764.35
20	2	C	H	H	H	2979
CQ						185

Additionally, the results showed that *para*-substituted amodiaquine-based phosphonium cations with shorter hydrophobic linker **16a** and **16b** (Table 3.1) are slightly more active than longer hydrophobic linker **16c** and **16d** (n= 6 and n= 10). On the other hand, the reverse is observed with *meta*-substituted amodiaquine-based phosphonium cations **17a-17d** (Table 3.2). Effect of steric hindrance possibly explains the relative low activity of *meta*-substituted analogues. However, elimination of steric hindrances with longer chain hydrophobic linker (**17c** and **17d** IC_{50} = 539 nM, 335.4 nM, respectively). Since compound **16b** showed the greatest activity (IC_{50} = 254 nM) in comparison to other derivatives, phosphonium substituents (R = Me) and ammonium cations analogues were tested to examine the effect of charge distribution over the phosphonium moiety.[11] It is noteworthy that comparable activities were observed, and IC_{50}s of 270 nM, 299 nM, and 274 nM were recorded for **16e-16g**, respectively. Therefore, phosphonium substituents with lower molecular weight can be exploited for further development of these analogues to be consistent with Lipinski's rule. However, abolishment of antiparasitic activity was observed when an ammonium cation was tethered to **14b** to give **16h** (IC_{50} = 2154 nM). The lack of activity of **16h** might result from the decrease in lipophilicity, which resulted in less permeability across the mitochondrial membrane.

Interestingly, restoration of the activity was observed when **15b** (IC_{50} = 1143.7 nM) was attached to alkyl-containing phosphonium substituents **17e-17g** (IC_{50} = 371 - 684.7 nM). As discussed previously, less steric-Hindered phosphonium substituents possible improve the drug-substrate binding of these analogues. Interestingly, compound **18** showed the greatest antimalarial activity (IC_{50} = 45.2 nM) among AQ

analogs. This observation was not uncommon due to the diprotic weak base attribute of AQ. Therefore, compound **18** will be drawn to the parasite digestive vacuole (DV) following the pH gradient (DV pH = 5.0-5.2) in a similar mechanism to CQ.[12] However, introduction of a positive charge on the terminal amine (i.e. quaternary amine) significantly diminished the antiparastic activity **16h** (IC_{50} = 2154) and **17h** (764.36 nM). This outcome provides strong evidence that AQ-based phosphonium cations are not acting on parasite DV, probably because of their high lipophilicity and their positive charge, which hindered their penetration across parasite DV membrane.

Based on these results, the inhibitory capacities of cationic AQ analogues are likely conferred by their attachment to the phosphonium moiety, which directed them into mitochondria. Once delivered to the mitochondria, AQ analogues will be accumulated in the mitochondria hydrophobic core where they compete with NADH for binding to Complex I. As a result, parasites respiration will be disrupted, consequently leading to death of *Plasmodium* parasite.

Conclusion

The study presented in this chapter examined AQ analogues with side chain modifications that direct them to mitochondria. The results indicated that slight changes in AQ structure lead to significant changes in *in vitro* activities as well as the ability to accumulate in the parasite DV. Furthermore, this study presents a novel approach to design mitochondrion-acting antimalarials. This approach could speed up numerous possibilities for development of inexpensive non-artemisinin therapy for treatment and prophylaxis against highly resistant strains of *Plasmodium.*

REFERENCES

1. Jensen, M.; Mehlhorn, H., Seventy-five years of Resochin in the fight against malaria. *Parasitology Research* **2009,** *105* (3), 609-27.

2. (a) Hocart, S. J.; Liu, H.; Deng, H.; De, D.; Krogstad, F. M.; Krogstad, D. J., 4-aminoquinolines active against chloroquine-resistant Plasmodium falciparum: basis of antiparasite activity and quantitative structure-activity relationship analyses. *Antimicrobial Agents and Chemotherapy* **2011,** *55* (5), 2233-44; (b) Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, E.; Monti, D., Structure-activity relationships in 4-aminoquinoline antiplasmodials. The role of the group at the 7-position. *Journal of Medicinal Chemistry* **2002,** *45* (16), 3531-9; (c) Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dorn, A.; Masciadri, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M. A.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W., 4-aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant Plasmodium falciparum. *Antimicrobial Agents and Chemotherapy* **1996,** *40* (8), 1846-54; (d) De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J., Structure-activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines. *Journal of Medicinal Chemistry* **1998,** *41* (25), 4918-26.

3. Mzayek, F.; Deng, H.; Mather, F. J.; Wasilevich, E. C.; Liu, H.; Hadi, C. M.; Chansolme, D. H.; Murphy, H. A.; Melek, B. H.; Tenaglia, A. N.; Mushatt, D. M.; Dreisbach, A. W.; Lertora, J. J.; Krogstad, D. J., Randomized dose-ranging controlled trial of AQ-13, a candidate antimalarial, and chloroquine in healthy volunteers. *PLoS Clinical Trials* **2007,** *2* (1), e6.

4. (a) Neftel, K. A.; Woodtly, W.; Schmid, M.; Frick, P. G.; Fehr, J., Amodiaquine induced agranulocytosis and liver damage. *British medical journal* **1986,** *292* (6522), 721-3; (b) O'Neill, P. M.; Bray, P. G.; Hawley, S. R.; Ward, S. A.; Park, B. K., 4-Aminoquinolines--past, present, and future: a chemical perspective. *Pharmacology & Therapeutics* **1998,** *77* (1), 29-58.

5. (a) Biagini, G. A.; O'Neill, P. M.; Bray, P. G.; Ward, S. A., Current drug development portfolio for antimalarial therapies. *Current Opinion in Pharmacology* **2005,** *5* (5), 473-8; (b) O'Neill, P. M.; Mukhtar, A.; Stocks, P. A.; Randle, L. E.; Hindley, S.; Ward, S. A.; Storr, R. C.; Bickley, J. F.; O'Neil, I. A.; Maggs, J. L.; Hughes, R. H.; Winstanley, P. A.; Bray, P. G.; Park, B. K., Isoquine and related amodiaquine analogues: a new generation of improved 4-aminoquinoline antimalarials. *Journal of Medicinal Chemistry* **2003,** *46* (23), 4933-45; (c) O'Neill, P. M.; Willock, D. J.; Hawley, S. R.; Bray, P. G.; Storr, R. C.; Ward, S. A.; Park, B. K.,

Synthesis, antimalarial activity, and molecular modeling of tebuquine analogues. *Journal of Medicinal Chemistry* **1997,** *40* (4), 437-48.

6. Cooper, R. G.; Magwere, T., Chloroquine: novel uses & manifestations. *The Indian Journal of Medical Research* **2008,** *127* (4), 305-16.

7. Deepalakshmi, P. D.; Parasakthy, K.; Shanthi, S.; Devaraj, N. S., Effect of chloroquine on rat liver mitochondria. *Indian Journal of Experimental Biology* **1994,** *32* (11), 797-9.

8. (a) Katewa, S. D.; Katyare, S. S., Treatment with antimalarials adversely affects the oxidative energy metabolism in rat liver mitochondria. *Drug and Chemical Toxicology* **2004,** *27* (1), 41-53; (b) Porter, T. H.; Folkers, K., Antimetabolites of coenzyme Q. Their potential application as antimalarials. *Angewandte Chemie* **1974,** *13* (9), 559-69; (c) Skelton, F. S.; Pardini, R. S.; Heidker, J. C.; Folkers, K., Inhibition of coenzyme Q systems by chloroquine and other antimalarials. *Journal of the American Chemical Society* **1968,** *90* (19), 5334-6.

9. (a) Krungkrai, J.; Burat, D.; Kudan, S.; Krungkrai, S.; Prapunwattana, P., Mitochondrial oxygen consumption in asexual and sexual blood stages of the human malarial parasite, Plasmodium falciparum. *The Southeast Asian Journal of Tropical Medicine and Public Health* **1999,** *30* (4), 636-42; (b) Jacobs, G. H.; Oduola, A. M.; Kyle, D. E.; Milhous, W. K.; Martin, S. K.; Aikawa, M., Ultrastructural study of the effects of chloroquine and verapamil on Plasmodium falciparum. *The American Journal of Tropical Medicine and Hygiene* **1988,** *39* (1), 15-20; (c) Aikawa, M.; Beaudoin, R. L., Effects of chloroquine on the morphology of the erythrocytic stages of Plasmodium gallinaceum. *The American Journal of Tropical Medicine and Hygiene* **1969,** *18* (2), 166-81.

10. (a) Vaidya, A. B.; Lashgari, M. S.; Pologe, L. G.; Morrisey, J., Structural features of Plasmodium cytochrome b that may underlie susceptibility to 8-aminoquinolines and hydroxynaphthoquinones. *Molecular and Biochemical Parasitology* **1993,** *58* (1), 33-42; (b) Vale, N.; Moreira, R.; Gomes, P., Primaquine revisited six decades after its discovery. *European Journal of Medicinal Chemistry* **2009,** *44* (3), 937-53.

11. (a) Ross, M. F.; Kelso, G. F.; Blaikie, F. H.; James, A. M.; Cocheme, H. M.; Filipovska, A.; Da Ros, T.; Hurd, T. R.; Smith, R. A.; Murphy, M. P., Lipophilic triphenylphosphonium cations as tools in mitochondrial bioenergetics and free radical biology. *Biochemistry. Biokhimiia* **2005,** *70* (2), 222-30; (b) Murphy, M. P.; Smith, R. A., Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annual Review of Pharmacology and Toxicology* **2007,** *47*, 629-56.

12. Foley, M.; Tilley, L., Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. *Pharmacology & Therapeutics* **1998,** *79* (1), 55-87.

CHAPTER 4

MATERIALS AND METHODS

Section-I

This section illustrates the materials and methods that were used in Chapter II schemes

Chemicals were purchased from commercial sources and used as received. Products purifications were performed by flash chromatography on 60-100-mesh silica and visualized by UV on TLC plates (silica gel 60 F254). ^{1}H and ^{13}C NMRs were recorded on a 500 MHz NMR and referenced to residual CDCl3.

Preparation of 3-(bromoalkyloxy)-4*H*-pyran-4-one, general procedure: To a solution of maltol (196 mg, 1.55 mmol) in acetone (10 ml) was added the appropriate dibromoalkyl (1.705 mmol) followed by potassium carbonate (235 mg, 1.705 mmol). The solution was heated at 70 ºC with stirring for 12-16 h and cooled to rt. The mixture was then filtered and the solvent was evaporated. After evaporation, the mixture was dissolved in EtOAc (10 ml) and washed 2x with 5% NaOH (10 ml) and once with distilled H_2O (10 ml). The combined organic fractions were then dried over $MgSO_4$, filter and concentrated. The resulting residue was purified by silica gel chromatography using 1:1 hexanes:EtOAc.

3-(3-bromopropoxy)-2-methyl-4*H*-pyran-4-one (7a): Yield 43%; orange oil; TLC (SiO_2) R_f 0.38 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, 1H, *J* =

5.5 Hz), 6.35 (d,1H, *J* = 6 Hz), 4.17 (t, 2H, *J* = 6.0 Hz), 3.64 (t, 2H, *J* = 6.5 Hz), 2.35 (s, 3H), 2.30-2.25 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 159.2, 153.6, 144.6, 117.2, 69.5, 33.0, 30.2, 14.7 .

3-(4-bromobutoxy)-2-methyl-4*H*-pyran-4-one (7b): Yield 46%; yellow oil; TLC (SiO_2) R_f 0.29 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, 1H, *J* = 5 Hz), 7.46-7.44, 6.35 (d, 1H, *J* = 5.5 Hz), 4.07 (t, 2H, *J* = 6.5 Hz), 3.52 (d, 2H, *J* = 6.5 Hz), 2.33 (s, 3H), 2.09-2.04 (m, 2H), 1.91-1.85 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 164.2, 159.1, 153.6, 144.7, 117.1, 71.0, 33.6, 29.2, 28.6,14.8 .

3-((6-bromohexyl)oxy)-2-methyl-4*H*-pyran-4-one (7c): Yield 44%; yellow oil; TLC (SiO_2) R_f 0.27 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, 1H, *J* = 6 Hz), 6.34 (d,1H, *J* = 5.5 Hz), 4.05 (t, 2H, *J* = 7.0 Hz), 3.42 (t, 2H, *J* = 7.0 Hz), 2.33 (s, 3H), 1.90-1.87(m, 2H), 1.75-1.72 (m, 2H), 1.51-1.47 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 159.0, 153.5, 144.8, 117.2, 72.0, 33.8, 32.7, 29.8, 27.9, 25.0, 14.7 .

3-((10-bromodecyl)oxy)-2-methyl-4*H*-pyran-4-one (7d): Yield 35%; orange oil; TLC (SiO_2) R_f 0.27 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 1H, *J* = 5.5 Hz), 6.34 (d, 1H, *J* = 5.5 Hz), 4.04 (t, 2H, *J* = 6.5 Hz), 2.32 (s, 3H), 1.85 (qnt, 2H, *J* = 7.0 Hz), 1.71 (qnt, 2H, *J* = 7.0 Hz), 1.42-1.41 (m, 4H), 1.30 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 159.0, 153.4, 144.9, 117.2, 72.2, 34.0, 32.8, 30.0, 29.4, 29.3, 28.7, 28.1, 25.8, 14.7 .

3-butoxy-2-methyl-4*H*-pyran-4-one (12): Yield 39%; yellow oil; TLC (SiO_2) R_f 0.49 (1:1 hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, 1H, *J* = 6.0 Hz), 6.47 (d, 1H, *J* = 6.0 Hz), 4.07-4.04 (m, 2H), 2.32 (s, 3H), 1.73-1.67(m, 2H), 1.49-1.43

(m, 2H), 0.97-0.94 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 164.2, 159.0, 153.4, 144.9, 117.2, 72.0, 32.1, 19.1, 14.7, 13.8, 13.6 .

Preparation of (3-alkyloxy-4H-pyran-4-one) phosphonium bromide; general procedure: Triphenylphosphine (2 equiv) and 4H-pyran-4-one were combined in a 5-ml conical-shaped tube containing 3:1 2-PrOH:PhMe (2 ml). The tube was sealed and heated to 100 °C for 48-72 h. The solution was evaporated and the crude residue was purified by flash chromatography on silica gel using acetone to elute the nonpolar impurities followed by 9:1 DCM:MeOH.

(3-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)propyl)triphenylphosphonium bromide (8a): Yield 88%; orange oil; TLC (SiO_2) R_f 0.47 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85-7.81 (m, 10H), 7.76-7.72 (m, 8H), 6.33 (d, 1H, *J* = 5.5 Hz), 4.22 (t, 2H, *J* = 5.5 Hz), 4.06-4.00 (m, 2H), 2.39 (s, 3H), 2.20-2.15 (m, 2H).

(4-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)butyl)triphenylphosphonium bromide (8b): Yield 72%; yellow oil; TLC (SiO_2) R_f 0.27 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89-7.78 (m, 11H,), 7.74-7.71 (m, 6H), 6.33 (d, 1H, *J* = 5.5 Hz), 4.06 (t, 2H, *J* = 6.0 Hz), 3.94-3.88 (m, 2H), 2.28 (s, 3H), 2.15-2.11 (m, 2H), 1.96-1.91 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 165.6, 159.8, 154.1, 144.3, 135.0, 133.7, 133.6, 118.4, 117.7, 116.9, 116.8, 69.8, 29.4, 18.9,14.7

(6-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)hexyl)triphenylphosphonium bromide (8c): Yield 42%; yellow oil; TLC (SiO_2) R_f 0.49 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86-7.74 (m, 17H), 6.30 (d,1H, *J* = 5.5 Hz), 3.96 (t, 2H, *J* = 6.5 Hz), 3.69-3.64 (m, 2H), 2.31 (s, 3H), 1.72-1.64(m, 6H), 1.51-1.47 (m, 2H); ^{13}C NMR (125

MHz, $CDCl_3$) δ 175.2, 164.2, 159.5, 153.7, 144.7, 135.1, 133.6, 130.6, 118.5, 117.8, 117.1, 116.9, 71.9, 50.3, 30.1, 29.5, 25.3, 22.5, 14.8 .

(10-((4-oxo-4*H*-pyran-3-yl)oxy)decyl)triphenylphosphonium bromide (8d): Yield 63%; yellow oil; TLC (SiO_2) R_f 0.39 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84-7.80 (m, 9H), 7.75-7.71 (m, 6H), 7.65 (d, 1H, *J* = 5.0 Hz), 6.32 (d,1H, *J* = 5.5Hz), 4.00 (t, 2H, *J* = 7.0 Hz), 3.63-3.59 (m, 2H),2.32 (s, 3H), 1.69-1.63(m, 2H), 1.27-1.23 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 159.2, 153.8, 144.7, 135.1, 133.5, 133.4, 130.5, 118.4, 117.7, 117.0, 116.8, 72.2, 30.4, 30.2, 29.8, 29.2, 29.1, 29.0, 28.9, 25.6, 22.8, 22.4, 14.7

dimethyl(3-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)propyl)(phenyl)phosphonium bromide (8f): Yield 58%; yellow oil; TLC (SiO_2) R_f 0.17 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09-8.05 (m, 2H), 7.72-7.66 (m, 5H), 7.65 (d, 1H, *J* = 5.0 Hz), 6.31 (d,1H, *J* = 5.0 Hz), 4.00 (bs, 2H), 3.20-3.14 (m, 2H), 2.57-2.55 (m, 6H), 2.30 (s, 3H), 2.04 (bm, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 160.0, 154.1, 154.0, 144.4, 134.3, 131.6, 131.5, 130.0, 120.6, 119.79, 117.0, 116.9, 71.2, 22.7, 21.0, 20.5, 15.0 .

***N*,*N*-dimethyl-*N*-(3-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)propyl)benzenaminium bromide (10):** Yield 43%; yellow oil ; TLC (SiO_2) R_f 0.37 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62-7.60 (m, 1H), 7.24-7.21 (m, 2H), 6.74-6.67 (m, 3H), 6.35-6.33 (m, 1H), 4.13-4.11 (m, 2H), 3.58-3.52 (m, 2H), 2.50 (s, 3H), 2.32 (s, 3H), 2.02-2.00 (m, 2H), 1.16-1.15 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 159.0, 153.4, 149.3, 144.9, 129.3, 129.2, 117.3, 117.2, 116.2, 112.3, 70.2, 49.6, 38.5, 25.7, 22.2, 14.8 .

3-butoxy-2-methyl-4*H*-pyran-4-one (12): Yield 39%; white solid; m.p. 142-144; TLC (SiO_2) R_f 0.47 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 1H, *J* = 5.0 Hz), 6.34 (d, 1H, *J* = 10.0 Hz), 4.06 (m, 2H), 2.50 (s, 3H), 1.73-1.67 (m, 2H), 1.49-1.43 (m, 2H), 0.97-0.94 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 164.2, 159.0, 153.4, 144.9, 117.2, 72.0, 32.1, 19.1, 14.7, 13.8 .

Preparation of (3-(alkoxy)-2-methylpyridin-4(1H)-one) phosphonium bromide; general procedure: To a solution of (3-alkyloxy-4H-pyran-4-one) phosphonium bromide in ethanol (1.5 ml) was added 35% aqueous ammonium hydroxide (2ml) and heated for 6 h at 80 ºC. The solvent was then evaporated and the product was purified by flash chromatography on silica gel using 5:1 DCM:2-PrOH.

(3-((2-methyl-4-oxo-1,4-dihydropyridin-3-yl)oxy)propyl)triphenylphosphonium bromide (9a): Yield 30%; yellow oil; TLC (SiO_2) R_f 0.20 (9:1 DCM:MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.87-7.71 (m, 16H), 7.43 (d, 1H, *J* = 7.5 Hz), 6.31 (d, 1H, *J* = 7.0 Hz), 4.11 (m, 2H), 4.02 (m, 2H), 2.36 (s, 3H), 2.08-2.07 (m, 2H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 145.1, 141.0, 135.3, 133.5, 133.4, 130.7, 130.6, 118.4, 117.7, 116.3, 70.3, 53.5, 23.6, 14.0 .

(4-((4-oxo-1,4-dihydropyridin-3-yl)oxy)butyl)triphenylphosphonium bromide (9b): Yield 50%; yellow oil; TLC (SiO_2) R_f 0.37 (9:1 DCM:MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.84-7.79 (m, 9H,), 7.71-7.68 (m, 6H), 7.45 (d,1H, *J* = 7.0 Hz), 6.33 (d, 1H, *J* = 6.5 Hz), 4.08 (t, 2H, *J* = 5.0 Hz), 4.00-3.94 (m, 2H), 2.37 (s, 3H), 1.99-1.96(m, 2H), 1.95-1.91 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 145.0, 140.8, 135.1, 133.6, 130.6, 118.6, 117.9, 116.4, 67.8, 29.1, 19.1, 13.8 .

(6-((2-methyl-4-oxo-1,4-dihydropyridin-3-yl)oxy)hexyl)triphenylphosphonium bromide (9c): Yield 50%; yellow oil; TLC (SiO_2) R_f 0.49 (5:1 DCM:MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.84-7.81 (m, 3H), 7.76-7.71 (m, 12H), 7.40 (d, 1H, *J* = 7.5 Hz), 6.27 (d, 1H, *J* = 7.0 Hz), 3.96 (t, 2H, *J* = 6.5 Hz), 3.94 (t, 2H, *J* = *6.5*), 3.51 (m, 2H),2.32 (s, 3H), 1.65-1.57(m, 6H), 1.48-1.47 (m, 2H) .

(10-((4-oxo-1,4-dihydropyridin-3-yl)oxy)decyl)triphenylphosphonium bromide (9d): Yield 58%; yellow oil ; TLC (SiO_2) R_f 0.28 (9:1 DCM:MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.93-7.80 (m, 16H), 7.62 (d, 1H, *J* = 7.0 Hz), 6.47 (d,1H, *J* = 7.0 Hz), 4.00 (t, 2H, *J* = 7 Hz), 3.51-3.45 (m, 2H), 2.38 (s, 3H), 1.78-1.69(m, 4H), 1.63-1.58 (m, 2H), 1.46-1.45 (m, 2H), 1.38-1.32 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 145.8, 141.4, 134.9, 134.8, 133.5, 133.4, 130.2, 130.1, 119.0, 118.3, 115.9, 71.7, 29.8, 29.1, 28.9, 28.5, 25.7, 22.2, 21.5, 12.8 .

Section-II

This section describes the materials and synthetic methods that were used in chapter III schemes.

Preparation of 4-((7-chloroquinolin-4-yl)amino)phenol, general procedure: 4,7-Dichloroquinoline **9** (1.0 equiv) and aminophenol (1.0 equiv) were refluxed in absolute ethanol for 5 h. The precipitated product was isolated by vacuum filtration and washed with cold ethanol. The filtered ethanol was then refluxed for 18 h and the precipitated product **11** was isolated by filtration and used without further purification.

Preparation of N-(4-(4-bromoalkoxy)phenyl)-7-chloroquinolin-4-amine (14), general procedure: Quinoline.HCl **11** (300 mg, 1.07 equiv) was suspended in 10 ml of *tert*-butanol. Diisopropyl ethylamine (1.1 equiv) was added and the mixture was stirred at 70 °C for 10 min. Potassium *tert*-butoxide (2.2 equiv) was then added and the solution stirred for additional 15 mins at 70 °C. Dibromide (2.0 equiv) was added and the heterogenous mixture stirred for 18 h at 70 °C. After cooling to rt, the heterogeneous solution was filtered with methylene chloride and concentrated. Flash silica gel chromatography with 9:1 followed by 2:1 hexanes:EtOAc gave the product as a pale oil.

N-(4-(4-bromobutoxy)phenyl)-7-chloroquinolin-4-amine (14b): Yield 47%; yellow oil; TLC (SiO_2) R_f 0.21 (2:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, DMSO d_6) δ 8.99 (s, 1H), 8.45-8.40 (m,2H), 7.89 (s, 1H), 7.57-7.55 (m, 2H), 7.29-7.28 (m, 2H), 7.05-7.03 (m, 2H), 6.65-6.64 (m, 2H), 4.05 (t, 2H, J = 6.5 Hz), 4.05 (t, 2H, J = 7.0 Hz), 2.03-2.00 (m, 2H), 1.90-1.86 (m, 2H); ^{13}C NMR (125 MHz, DMSO d_6) δ 156.4, 150.0, 149.7, 134.3, 132.9, 128.1, 126.3, 126.2, 126.1, 125.1, 124.8, 118.3, 115.8, 115.7, 101.1, 67.3, 35.4, 29.6, 27.9 .

N-(4-(4-bromohexoxy)phenyl)-7-chloroquinolin-4-amine (14c): Yield 38%; yellow oil; TLC (SiO_2) R_f 0.26 (2:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, 1H, J = 5.0 Hz), 7.97 (s,1H), 7.91 (d, 1H, J = 9.5 Hz), 7.37 (d, 2H, J = 10.0 Hz), 7.21-7.20 (m, 2H), 6.94-6.93 (m, 2H), 6.65-6.64 (m, 2H), 6.66 (d, 1H, J = 5.0 Hz), 3.98 (t, 2H, J = 5.0 Hz), 3.43 (t, 2H, J = 5.0 Hz), 1.91-1.89 (m, 2H), 1.83-1.81 (m, 2H), 1.52 (bm, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.1, 152.0, 149.6, 149.3, 135.2,

131.8, 128.8, 126.2, 126.1, 125.1, 121.5, 117.6, 115.6, 115.5, 101.5, 68.1, 33.9, 32.7, 29.1, 27.9, 25.3 .

N-(4-(4-bromodecyloxy)phenyl)-7-chloroquinolin-4-amine (14d): Yield 43%; yellow oil; TLC (SiO_2) R_f 0.17 (4:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, 1H, *J* = 5.0 Hz), 7.95 (s,1H), 7.91 (d, 1H, *J* = 5.0 Hz), 7.35 (d, 2H, *J* = 10.0 Hz), 7.18 (d, 2H, *J* = 10.0 Hz), 6.93 (d, 2H, *J* = 10.0 Hz), 6.65(d, 2H, *J* = 5.0 Hz), 3.96 (t, 2H, *J* = 5.0 Hz), 3.40 (t, 2H, *J* = 5.0 Hz), 1.86-1.78 (m, 4H), 1.48-1.41 (m, 4H), 1.31 (bm, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 152.0, 149.6, 149.4, 135.1, 131.7, 128.7, 126.2, 126.1, 125.7, 125.6, 121.6, 117.7, 115.6, 115.5, 101.4, 68.4, 34.1, 32.8, 29.5, 29.4, 29.3, 28.8, 28.2,26.1 .

Preparation of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)alkyl) triphenylphosphonium bromide (16 and 17), general procedure: To a threaded 1.5 dram vial containing 1 ml of 3:1 2-PrOH:PhMe was added quinoline (0.1 mmol) and phosphine (1.1-1.3 equiv). The vial was tightly capped and placed on a heat block set to 100 °C. After 36 hr, the solution was cooled to rt and poured onto a 3-5 cm silica gel column with 100% acetone as the elution solvent. After removal of the non-polar impurities, the phosphonium product was isolated employing a 9:1 to 8:2 DCM:MeOH mixture.

(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)propyl)triphenylphosphonium bromide (16a): Yield 25%; yellow oil; TLC (SiO_2) R_f 0.20 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (d, 1H, *J* = 10.0 Hz), 8.26-8.25 (m,1H), 7.89 (s, 1H) 7.79-7.75 (m, 10H), 7.68-7.66 (m, 6H), 7.28-7.20 (m, 4H), 6.71-6.69 (m, 2H), 6.57-6.56 (m, 1H), 4.13 (bm, 2H), 3.84 (bm, 2H), 2.10 (bm, 2H) .

(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy) butyl)triphenylphosphonium bromide (16b): Yield 64%; yellow oil; TLC (SiO_2) R_f 0.23 (9:1 DCM:MeOH); ^1H NMR (500 MHz, CD_3OD) δ 8.41 (d, 1H, *J* = 10.0 Hz), 8.32 (d, 1H, *J* = 5.0 Hz), 7.94-7.85 (m, 10H). 7.81-7.77 (m, 6H), 7.55 (d, 1H, *J* = 10.0 Hz), 7.32 (d, 2H, *J* = 10.0 Hz), 7.01 (d, 2H, *J* = 10.0 Hz), 6.70 (d, 1H, *J* = 10.0 Hz), 4.13 (t, 2H, *J* = 5.0 Hz), 3.61-3.57 (m, 2H), 2.12-2.09 (m, 2H), 1.97-1.93 (m, 2H) .

(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)hexyl)triphenylphosphonium bromide (16c): Yield 47%; yellow oil; TLC (SiO_2) R_f 0.29 (9:1 DCM:MeOH); ^1H NMR (500 MHz, CD_3OD) δ 8.40 (d, 1H, *J* = 10.0 Hz), 8.32 (d,1H, *J* = 5.0 Hz), 7.93-7.84 (m, 10H). 7.81-7.79 (m, 6H), 7.57-755 (m, 1H), 7.31 (d, 2H, *J* = 10.0 Hz), 7.01 (d, 2H, *J* = 5.0 Hz), 6.69 (d, 1H, *J* = 5.0 Hz), 4.01 (t, 2H, *J* = 5.0 Hz), 3.53-3.48 (m, 2H), 1.80-1.75 (m, 4H), 1.72-1.68 (m, 2H), 1.60-1.56 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 152.4, 145.9, 136.6, 135.0, 134.9, 133.5, 133.4, 133.1, 133.0, 131.1,130.2, 130.1, 130.0, 126.3, 126.2, 118.9, 118.3, 117.2, 115.3, 100.3, 67.7, 53.5, 28.6, 25.1, 21.6, 21.2 .

(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)decyl)triphenylphosphonium bromide (16d): Yield 72%; yellow oil; TLC (SiO_2) R_f 0.30 (9:1 DCM:MeOH); ^1H NMR (500 MHz, CD_3OD) δ 8.43 (d, 1H, *J* = 10.0 Hz), 8.30 (d, 1H, *J* = 5.0 Hz), 7.91-7.82 (m, 10H), 7.80-7.78 (m, 6H), 7.50 (d, 1H, *J* = 10.0 Hz), 7.29 (d, 2H, *J* = 10.0 Hz), 7.00 (d, 2H, *J* = 5.0 Hz), 6.68 (d, 1H, *J* = 5.0 Hz), 3.96 (t, 2H, *J* = 5.0 Hz), 3.51-3.45 (m, 2H), 1.77-1.73 (m, 2H), 1.71-1.66 (m, 2H), 1.62-1.57 (m, 2H), 1.46-1.43 (m, 2H), 1.35-1.29 (m, 8H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.5, 152.0, 149.0, 146.3, 136.4, 134.9, 134.8, 133.5, 133.4, 131.2, 130.3, 130.2, 130.1, 126.2, 126.1, 125.8,

125.7, 124.5, 124.1, 119.0, 118.3, 117.3, 115.3, 115.2, 100.3, 68.0, 30.3, 30.2, 29.2, 29.1, 29.0, 28.9, 28.6, 25.8, 22.3, 21.6, 21.2 .

(4-(4-((7-chloroquinolin-4- yl)amino) phenoxy) butyl) (methyl) diphenylphosphonium bromide (16e): Yield 24%; yellow oil; TLC (SiO_2) R_f 0.19 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.55 (d, 1H, *J* = 10.0 Hz), 8.37 (d, 1H, *J* = 5.0 Hz), 7.98-7.94 (m, 5H). 7.88-7.85 (m, 2H), 7.78-7.76 (m, 4H), 7.71-7.69 (m, 1H), 7.37 (d, 2H, *J* = 5.0 Hz), 7.07 (d, 2H, *J* = 5.0 Hz), 6.67 (d, 1H, *J* = 5.0 Hz), 4.13 (t, 2H, *J* = 5.0 Hz), 3.19 (bm, 2H), 2.09-2.06 (m, 2H), 1.88-1.85 .

(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy) butyl) dimethyl (phenyl) phosphonium bromide (16f): Yield 31%; yellow oil; TLC (SiO_2) R_f 0.37 (8:2 DCM:MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (d, 1H, *J* = 5.0 Hz), 8.37 (d, 1H, *J* = 5.0 Hz), 8.04-8.00 (m, 2H), 7.93 (s, 1H), 7.85-7.83 (m, 1H), 7.76 (bm, 2H), 7.68 (d, 1H, *J* = 5.0 Hz), 7.36 (d, 2H, *J* = 5.0 Hz), 7.07 (d, 1H, *J* = 5.0 Hz), 6.76 (d, 1H, *J* = 5.0 Hz), 4.13 (t, 2H, *J* = 5.0 Hz), 2.74 (bm, 2H), 2.35 (s, 3H), 2.32 (s, 3H), 2.01-1.99 (m, 2H), 1.81-1.79 (m, 2H) .

tributyl(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl) phosphonium bromide (16g): Yield 46%; yellow oil; TLC (SiO_2) R_f 0.13 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.53 (d, 1H, *J* = 10.0 Hz), 8.37 (d,1H, *J* = 10.0 Hz), 7.93 (s, 1H), 7.68 (d, 1H, *J* = 10.0 Hz), 7.38 (d, 1H, *J* = 10.0 Hz), 7.13 (d, 2H, *J* = 10.0 Hz), 6.76 (d, 1H, *J* = 10.0 Hz), 4.17 (t, 2H, *J* = 5.0 Hz), 2.46-2.40 (m, 2H), 2.35-2.29 (m, 6H), 2.07-2.04 (m, 2H), 1.90-1.86 (m, 2H), 1.67-1.53 (m, 12H), 1.03 (t, 9H, *J* = 5.0 Hz) .

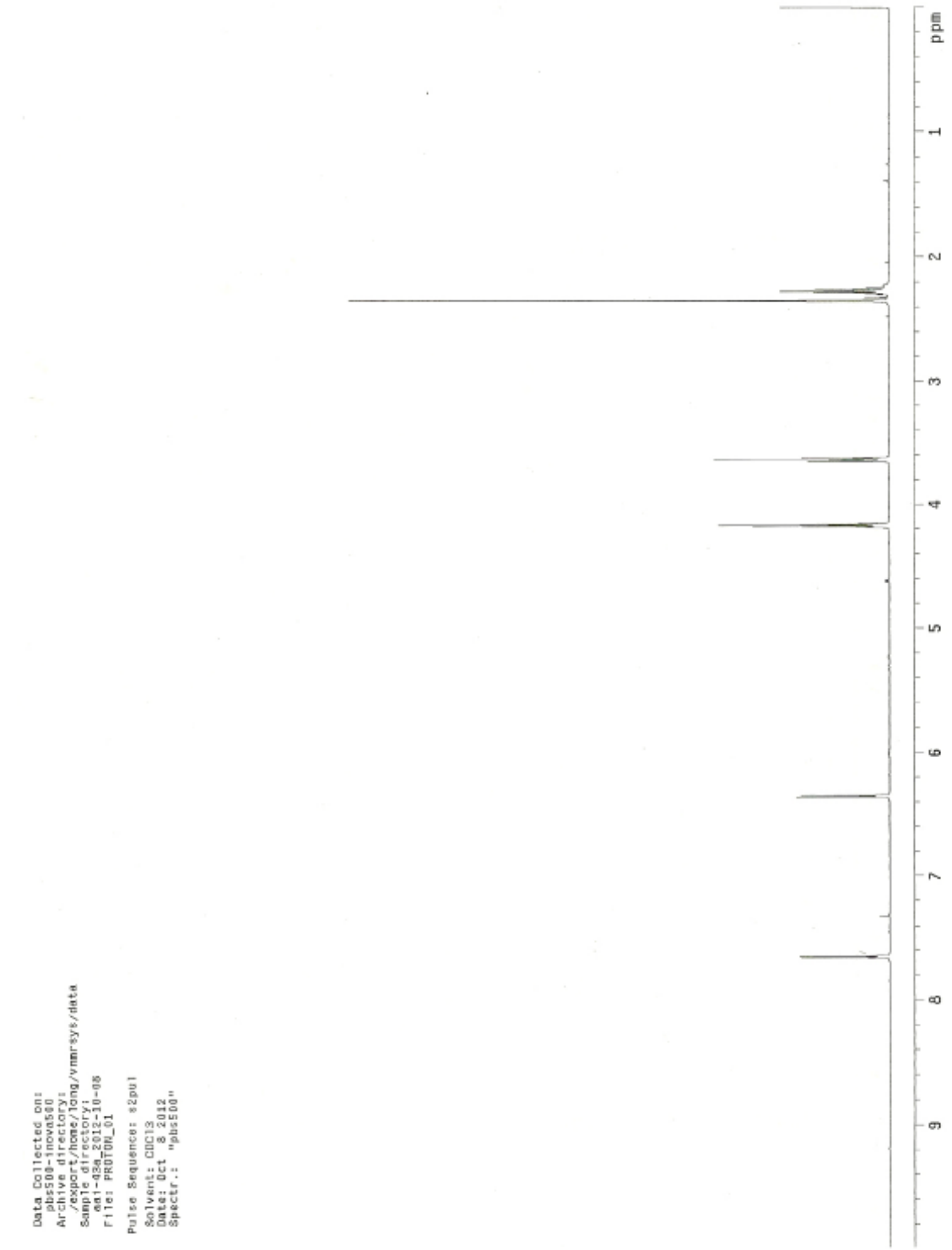
4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)-*N*,*N*,*N*-triethylbutan-1-aminium bromide (16h): Yield 25%; yellow oil; TLC (SiO_2) R_f 0.21 (8:2 DCM:MeOH); ^1H NMR (500 MHz, CD_3OD) δ 8.47 (d, 1H, *J* = 10.0 Hz), 8.37 (d, 1H, *J* = 10.0 Hz), 7.93 (s, 1H), 7.67 (d, 1H, *J* = 10.0 Hz), 7.38 (d, 1H, *J* = 10.0 Hz), 7.15 (d, 2H, *J* = 10.0 Hz), 6.76 (d, 1H, *J* = 5.0 Hz), 4.18 (t, 2H, *J* = 5.0 Hz), 3.43-2.39 (m, 8H), 1.98 (bm, 4H), 1.36 (t, 9H, *J* = 10.0 Hz) .

7-chloro-*N*-(4-(4-(diethylamino)butoxy)phenyl)quinolin-4-amine (18): Yield 44%; yellow oil; TLC (SiO_2) R_f 0.6 (8:2 DCM:MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, 1H, *J* = 5.0 Hz), 8.00 (s,1H), 7.93 (s, 1H), 7.88 (d, 1H, *J* = 10.0 Hz)), 7.39 (d, 1H, *J* = 5.0 Hz), 7.20 (d, 2H, *J* = 10.0 Hz), 6.95 (d, 1H, *J* = 10.0 Hz), 6.84 (s, 1H), 6.66 (d, 1H, *J* = 5.0 Hz), 4.00 (t, 2H, *J* = 6.5 Hz), 2.57-2.49 (m, 6H), 1.84-1.79 (m, 2H), 1.68-1.62 (m, 2H), 1.05-1.02 (m, 6H);^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 152.0, 149.6, 149.2, 135.2, 131.7, 128.9, 128.8, 126.2, 126.1, 121.3, 117.6, 115.6, 115.5, 101.5, 68.2, 52.6, 46.9, 27.4, 23.7, 11.7 .

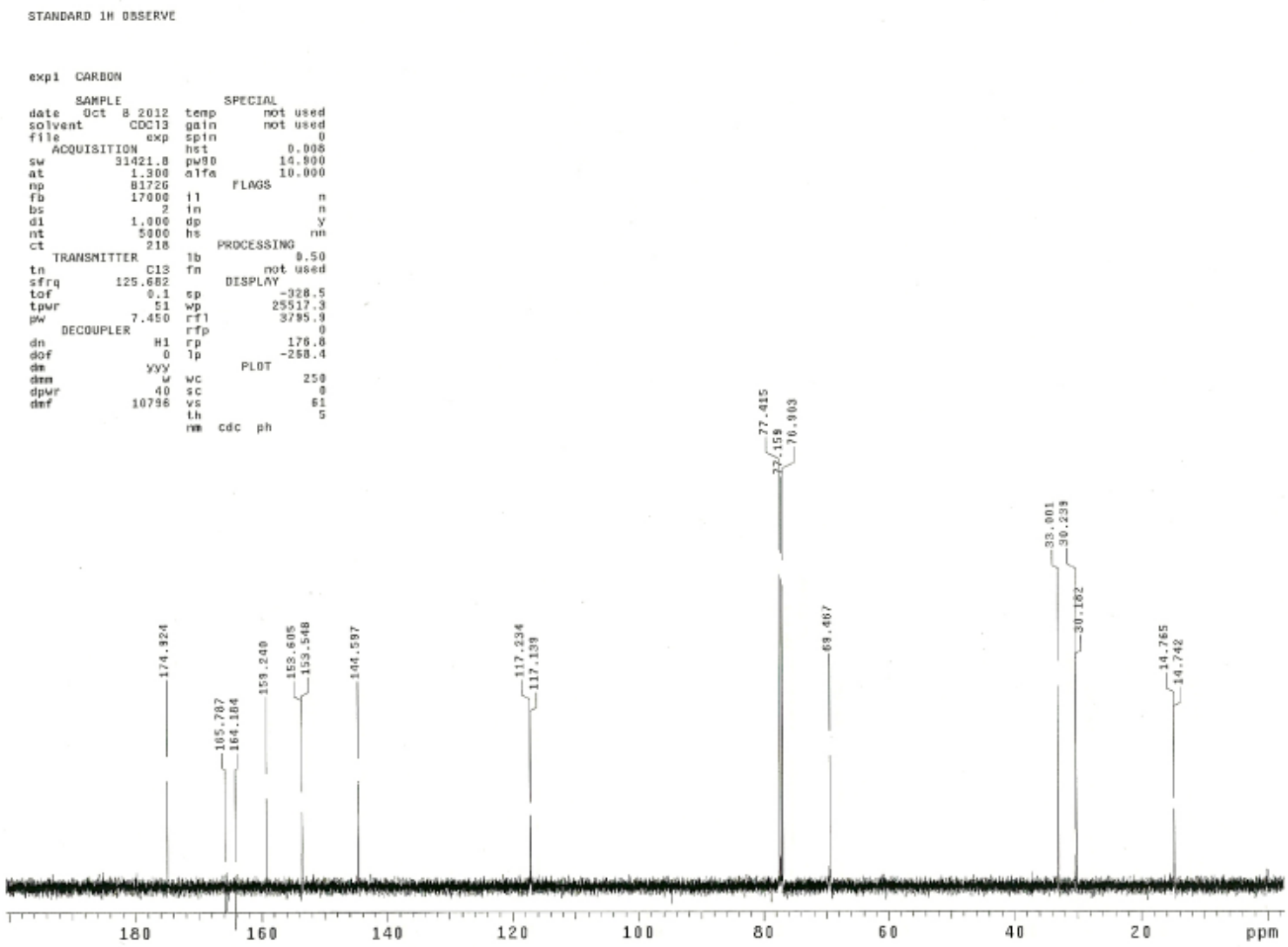
***N*-(4-butoxyphenyl)-7-chloroquinolin-4-amine (19):** Yield 67%; white solid; m.p. 212-214; TLC (SiO_2) R_f 0.32 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, CD_3OD) δ 8.39-8.37 (m, 2H), 7.93 (s, 1H), 7.57 (d, 1H, *J* = 10.0 Hz), 7.32 (d, 2H, *J* = 10.0 Hz), 7.05 (d, 2H, *J* = 10.0 Hz), 6.69 (d, 1H, *J* = 10.0 Hz), 4.20 (t, 2H, *J* = 5.0 Hz), 1.84-1.78 (m, 2H), 1-6-1.54 (m, 2H), 1.04 (t, 3H, *J* = 5.0 Hz) .

APPENDICES

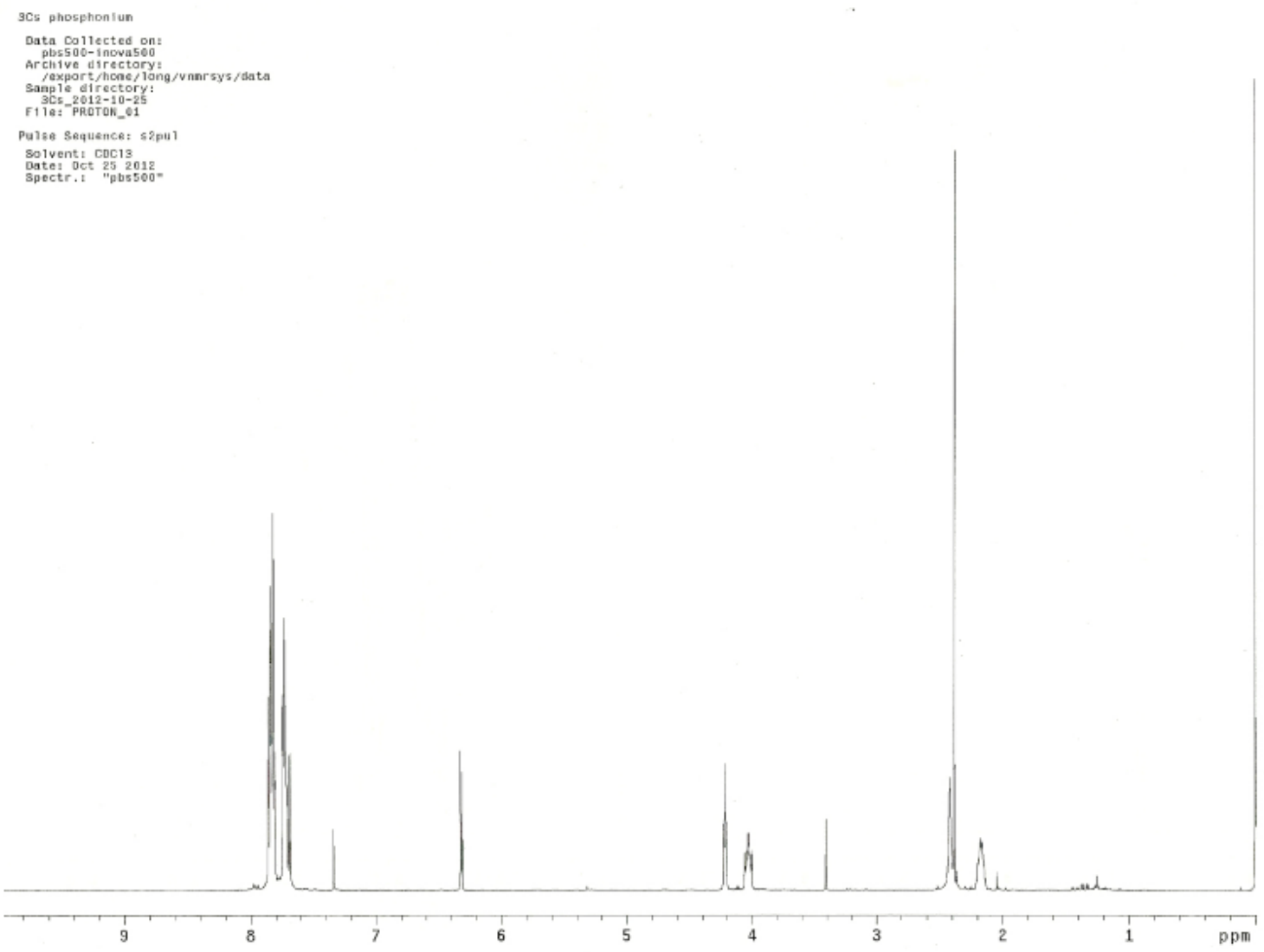
^{1}H of 3-(3-bromopropoxy)-2-methyl-4*H*-pyran-4-one (7a)



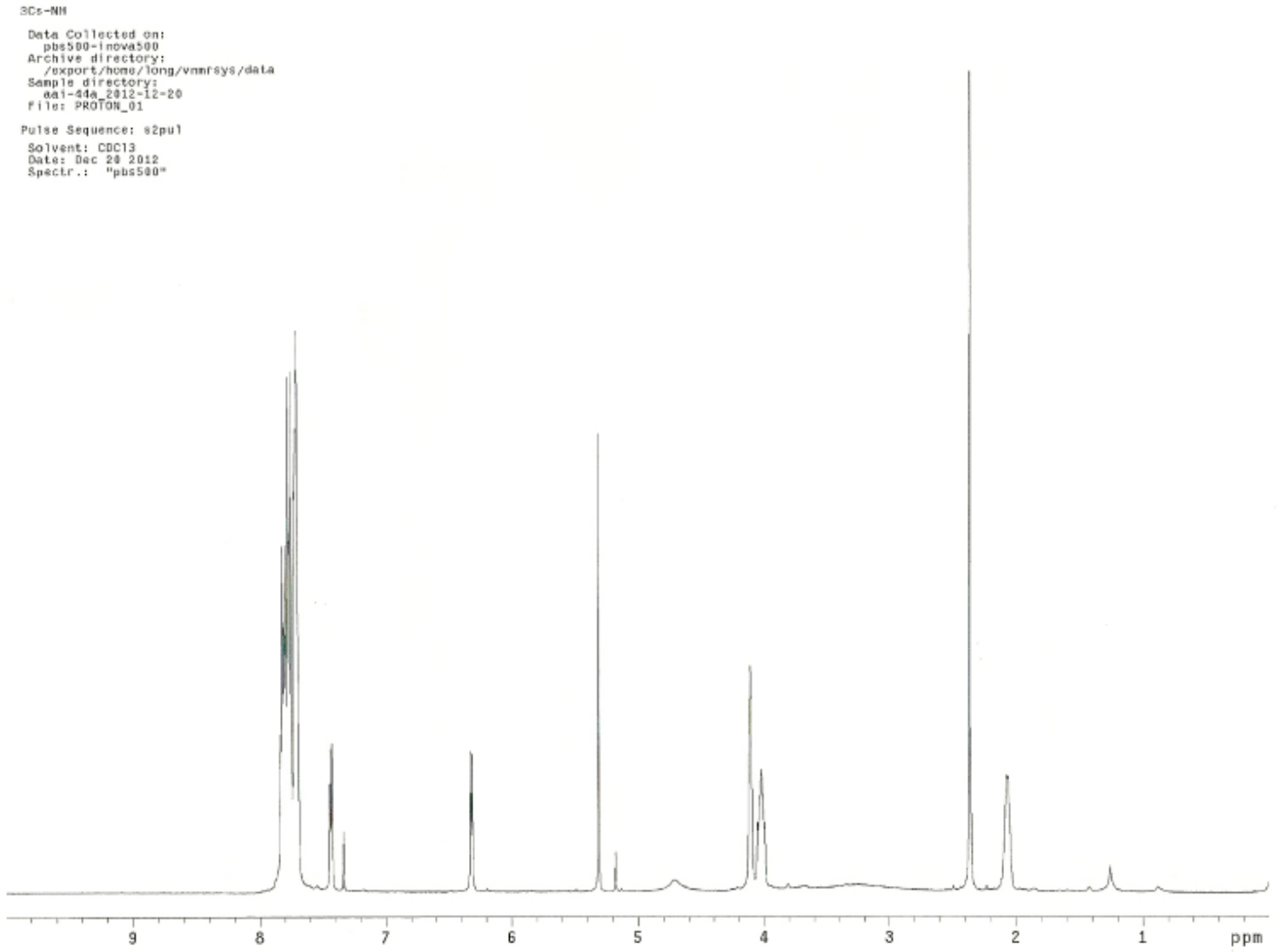
^{13}C of 3-(3-bromopropoxy)-2-methyl-4*H*-pyran-4-one (7a)



^{1}H of (3-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)propyl)triphenylphosphonium bromide (8a)



^{1}H of (3-((2-methyl-4-oxo-1,4-dihydropyridin-3-yl)oxy)propyl)triphenylphosphonium bromide (9a)



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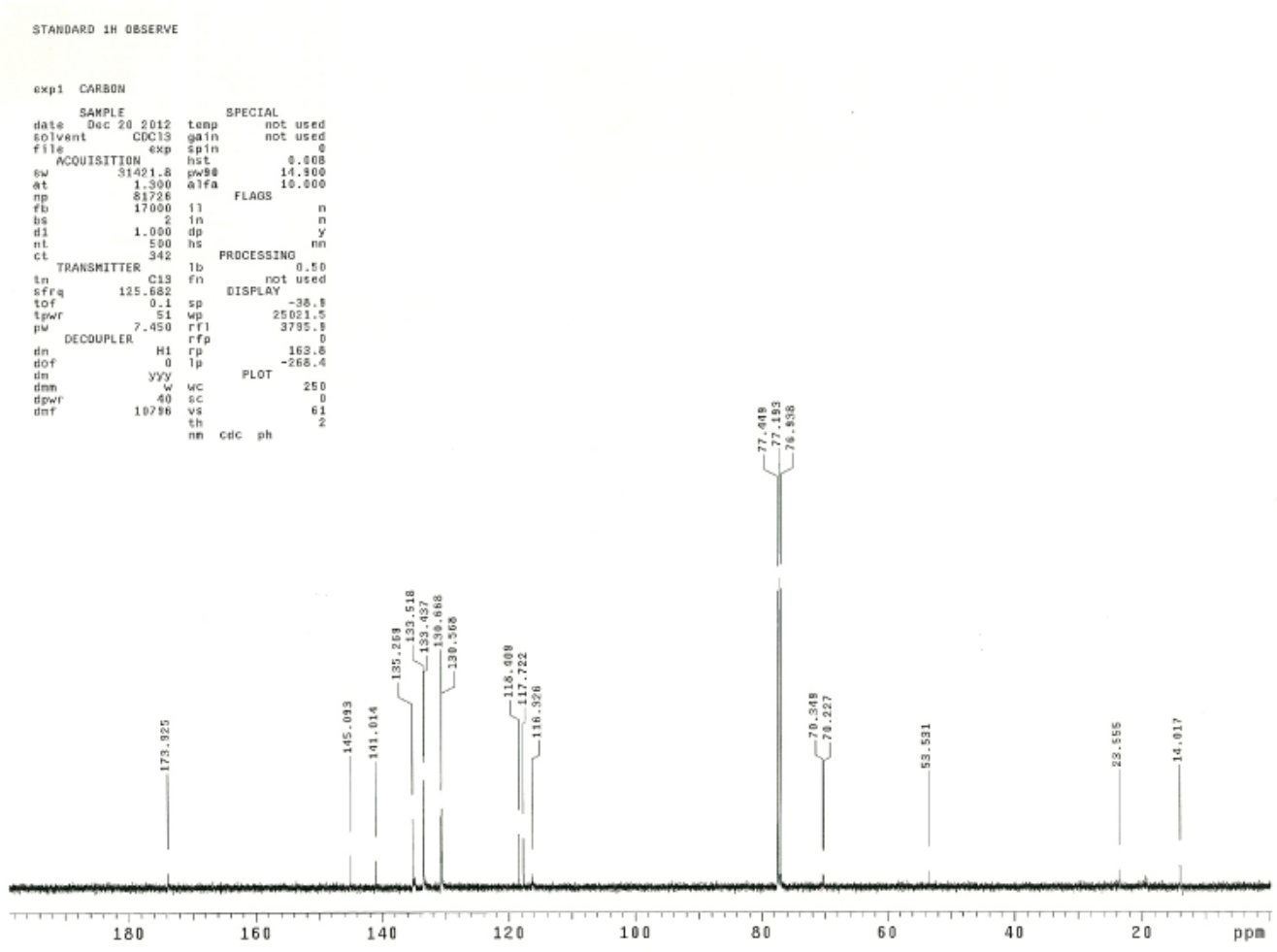
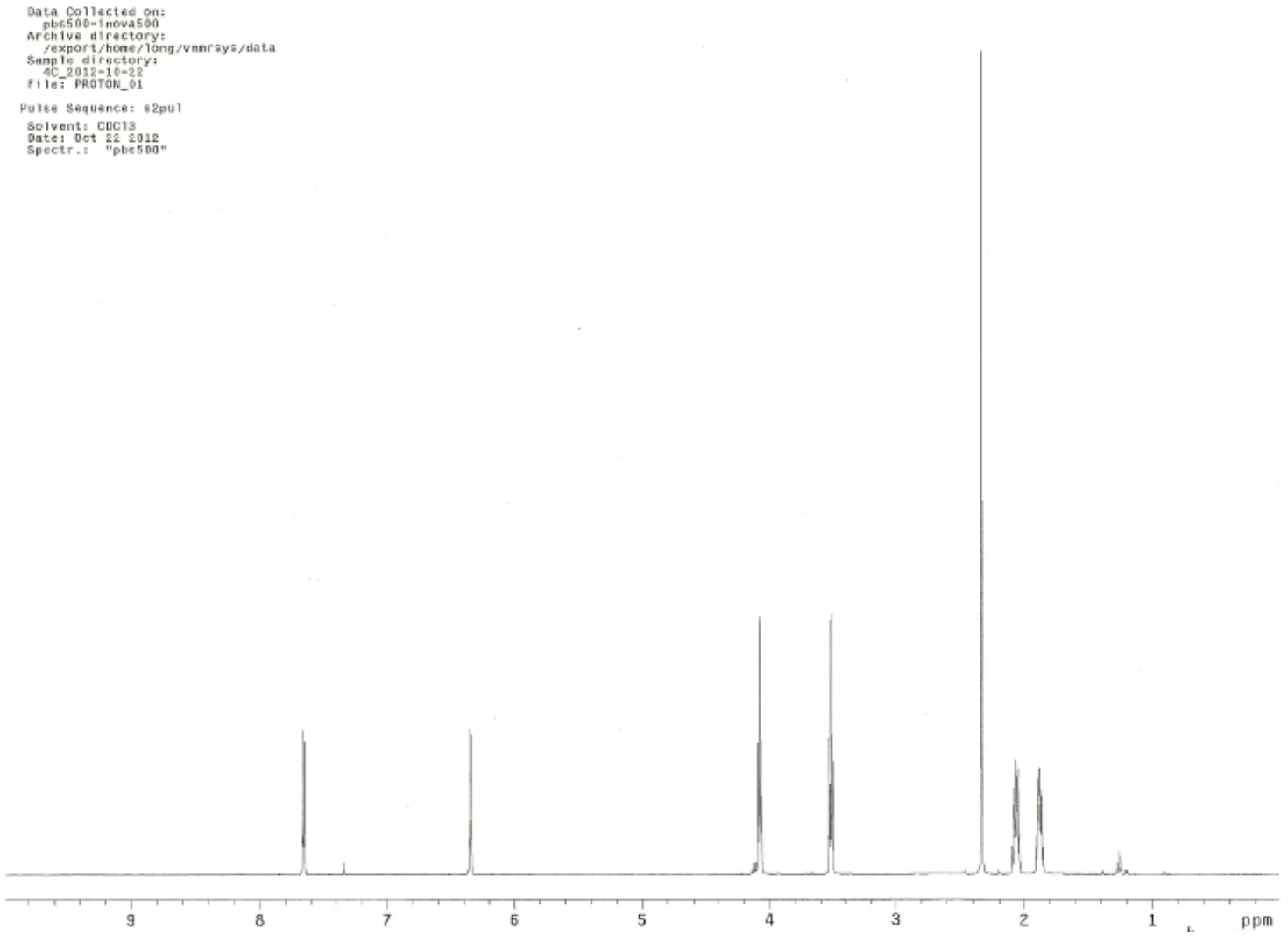


^{1}H of 3-(4-bromobutoxy)-2-methyl-4*H*-pyran-4-one (7b)



^{13}C of 3-(4-bromobutoxy)-2-methyl-4*H*-pyran-4-one (7b)

STANDARD 1H OBSERVE

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file	exp	spin	0
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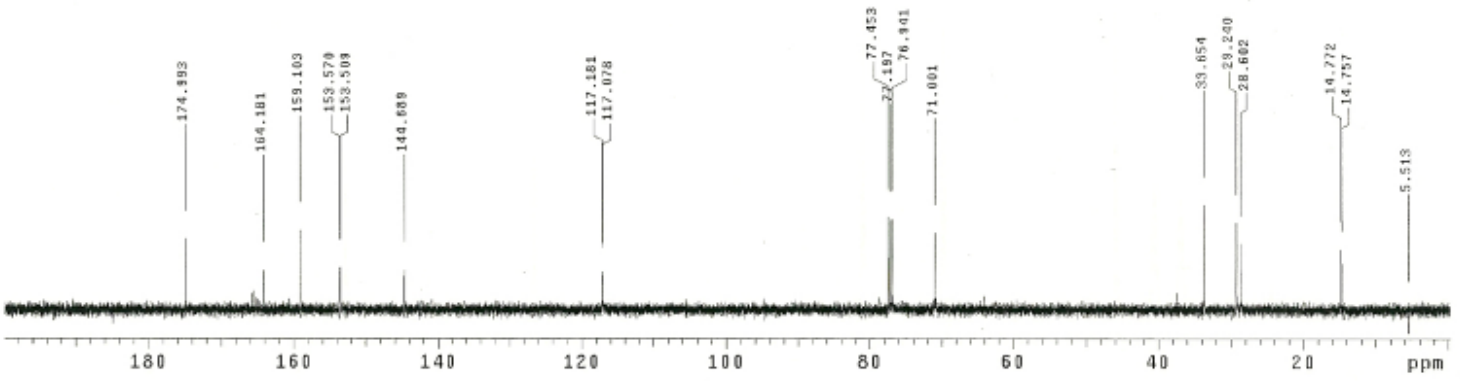
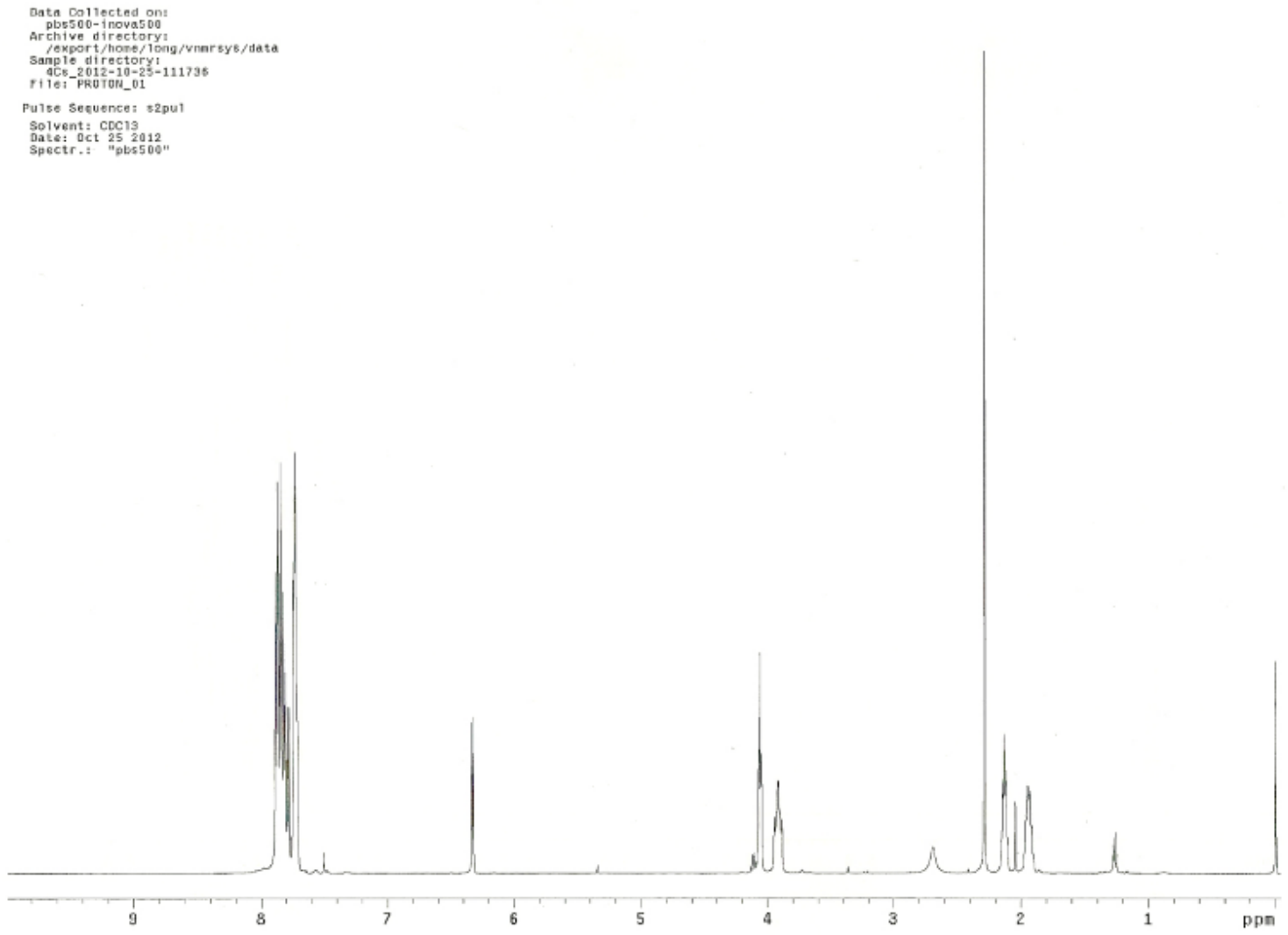
at	1.300	alfa	10.000
np	81726	FLAGS	
fb	17800	il	n
bs	2	in	n
dl	1.800	dp	y
nt	5800	hs	nn
ct	74	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.682	DISPLAY	
tof	0.1	sp	-38.4
tpwr	51	wp	25145.2
pw	7.450	rfl	3785.9
DECOUPLER		rfp	0
dn	H1	rp	157.6
dof	0	lp	-265.6
dm	yyy	PLOT	
dmm	w	wc	250
dpwr	40	sc	0
dmf	10796	vs	18
		th	3
		nm	cdc ph



^{1}H of (4-((2-methyl-4-oxo-4*H*-pyran-3-yl)oxy)butyl)triphenylphosphonium bromide (8b)



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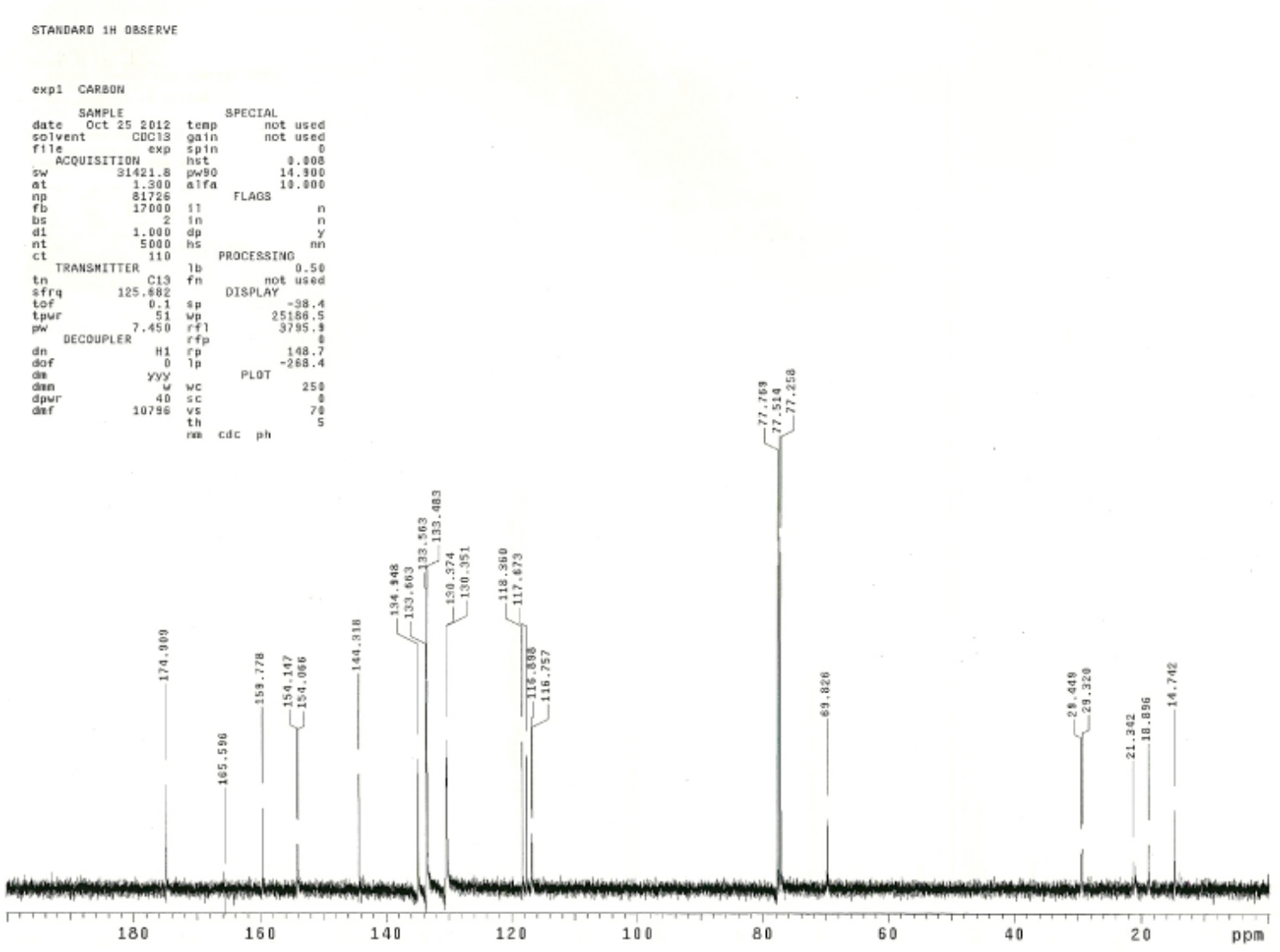
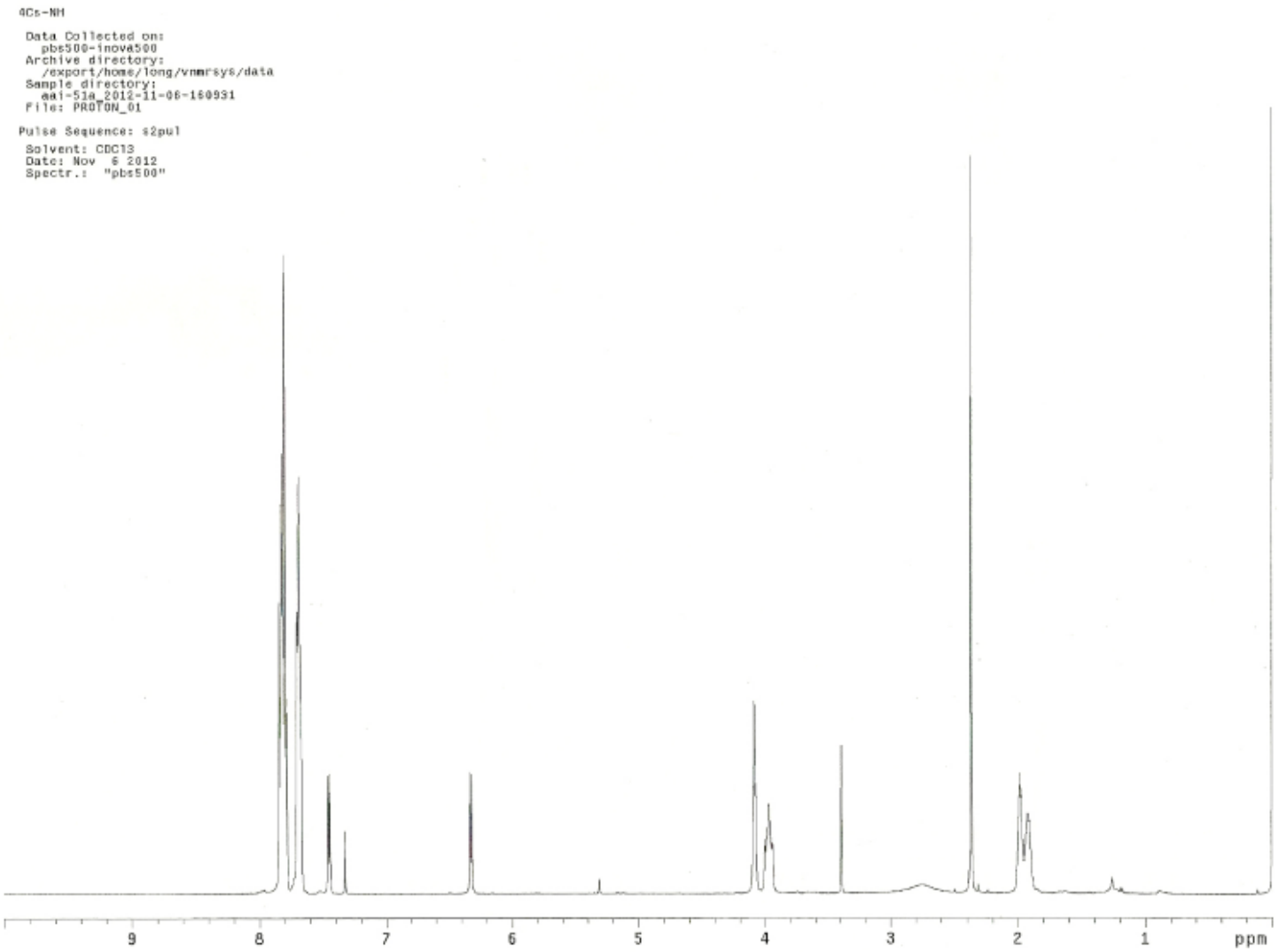
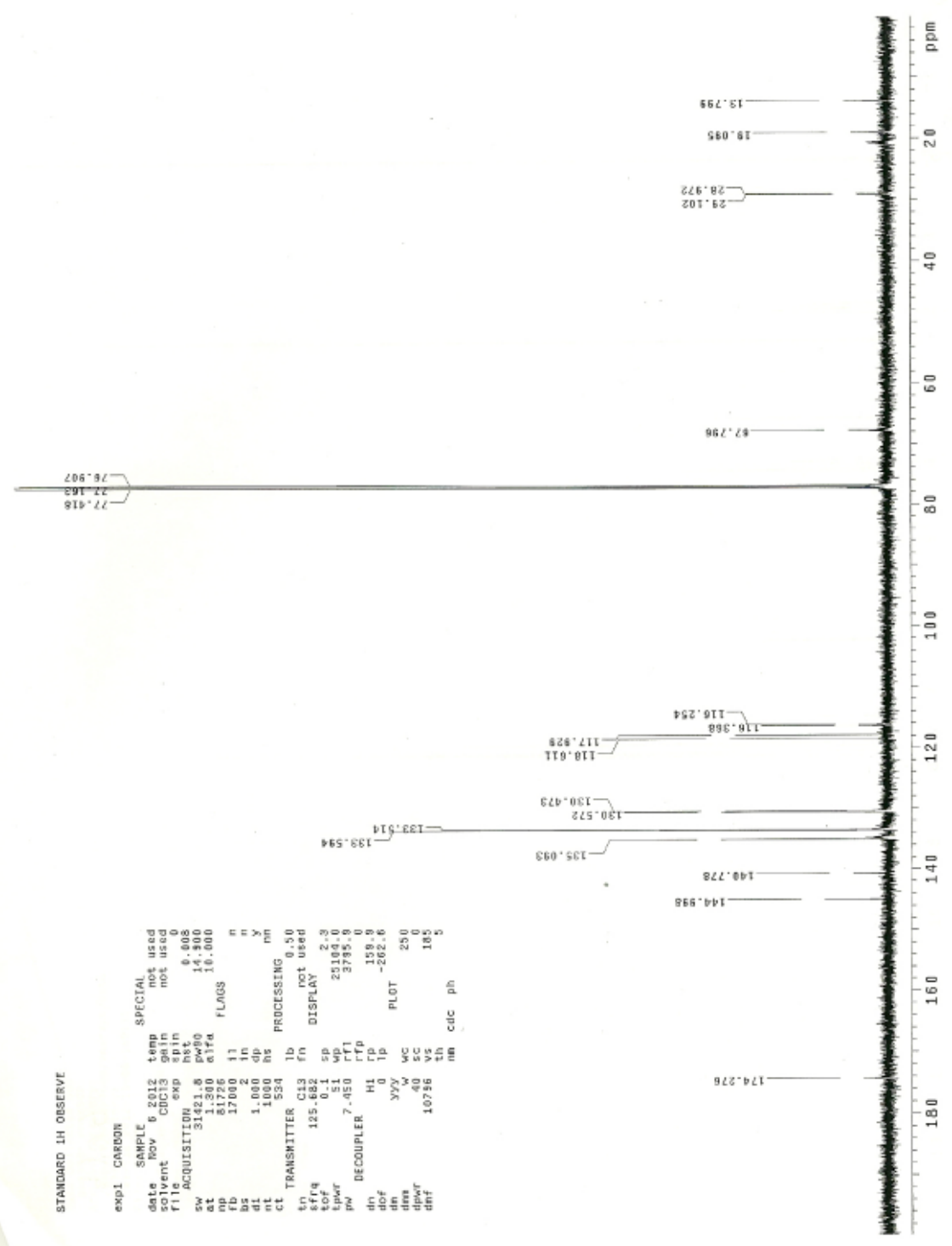


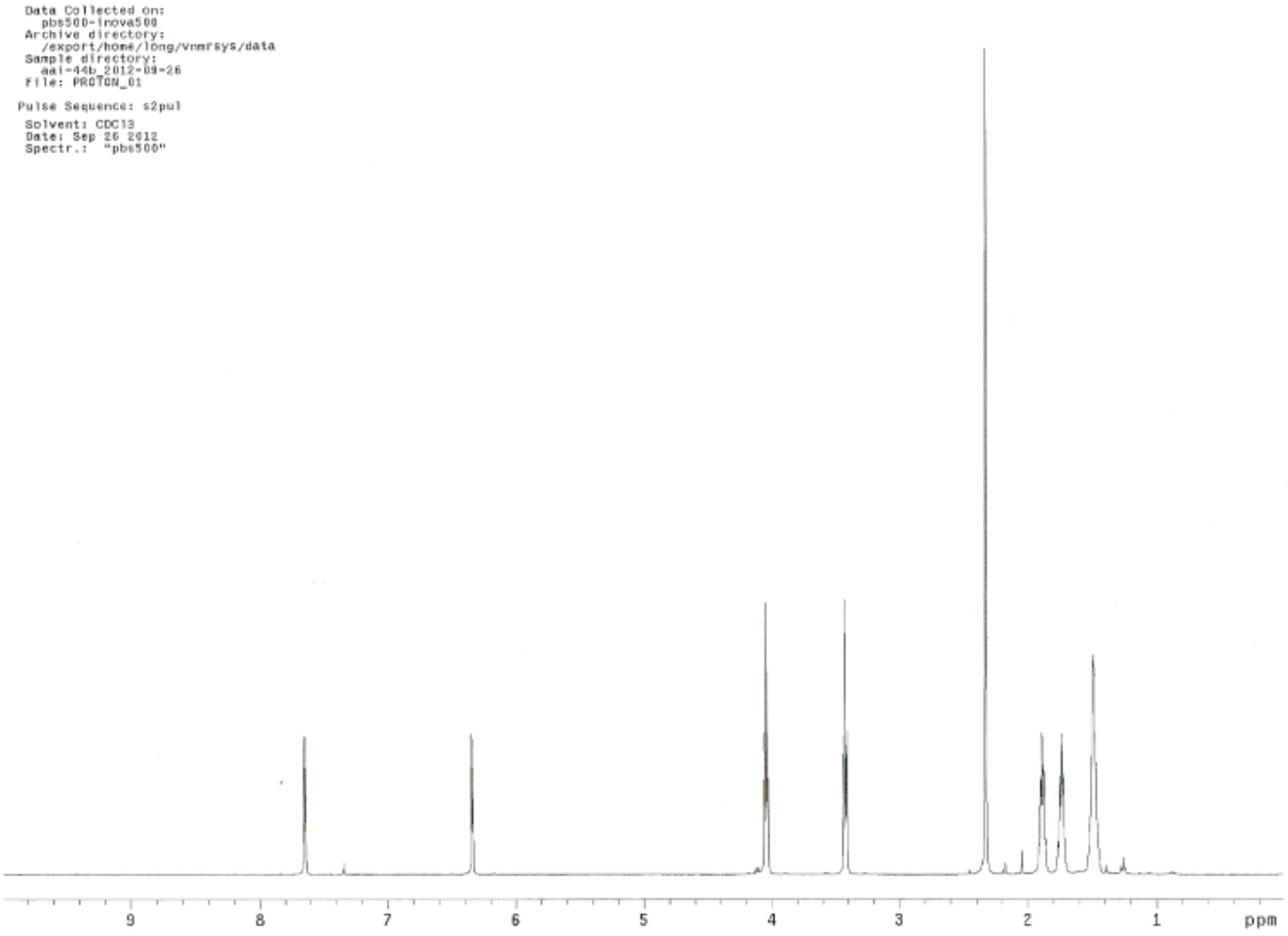
^{1}H of (4-((4-oxo-1,4-dihydropyridin-3-yl)oxy)butyl)triphenylphosphonium bromide (9b)



^{13}C of (4-((4-oxo-1,4-dihydropyridin-3-yl)oxy)butyl)triphenylphosphonium bromide (9b)



^{1}H of 3-((6-bromohexyl)oxy)-2-methyl-4*H*-pyran-4-one (7c)



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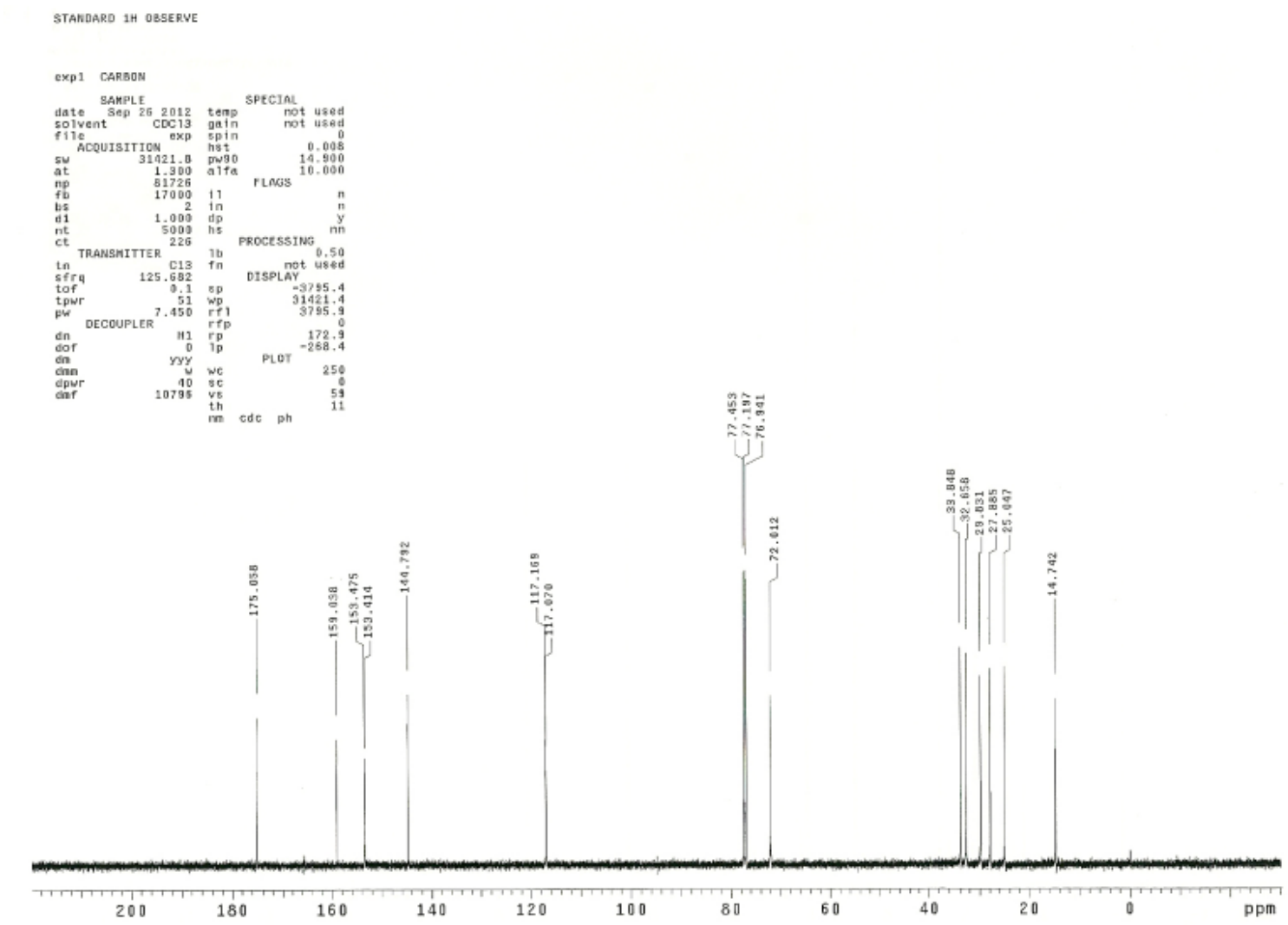
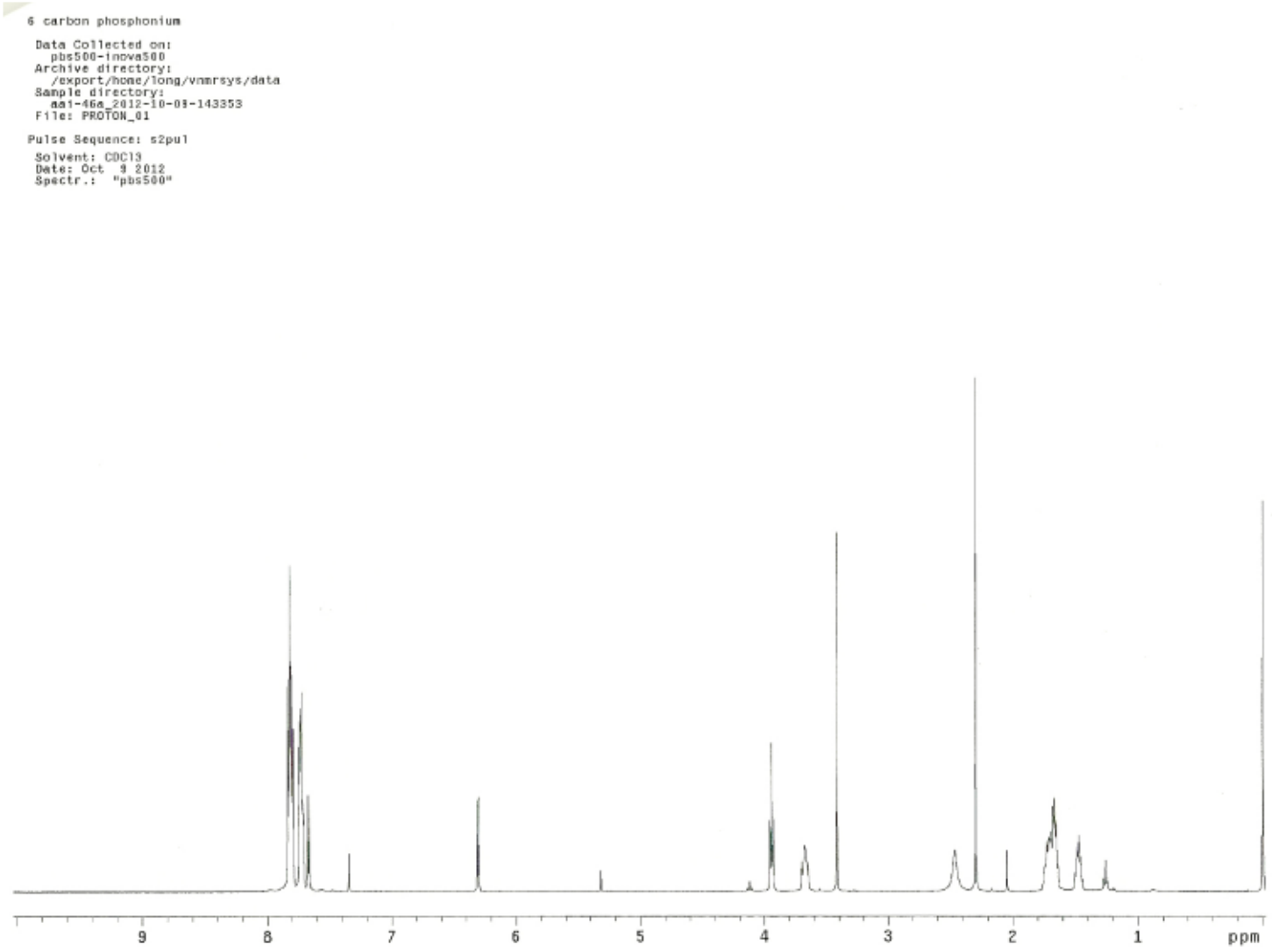


^{1}H of (10-((4-oxo-4*H*-pyran-3-yl)oxy)hexyl)triphenylphosphonium bromide (8c)



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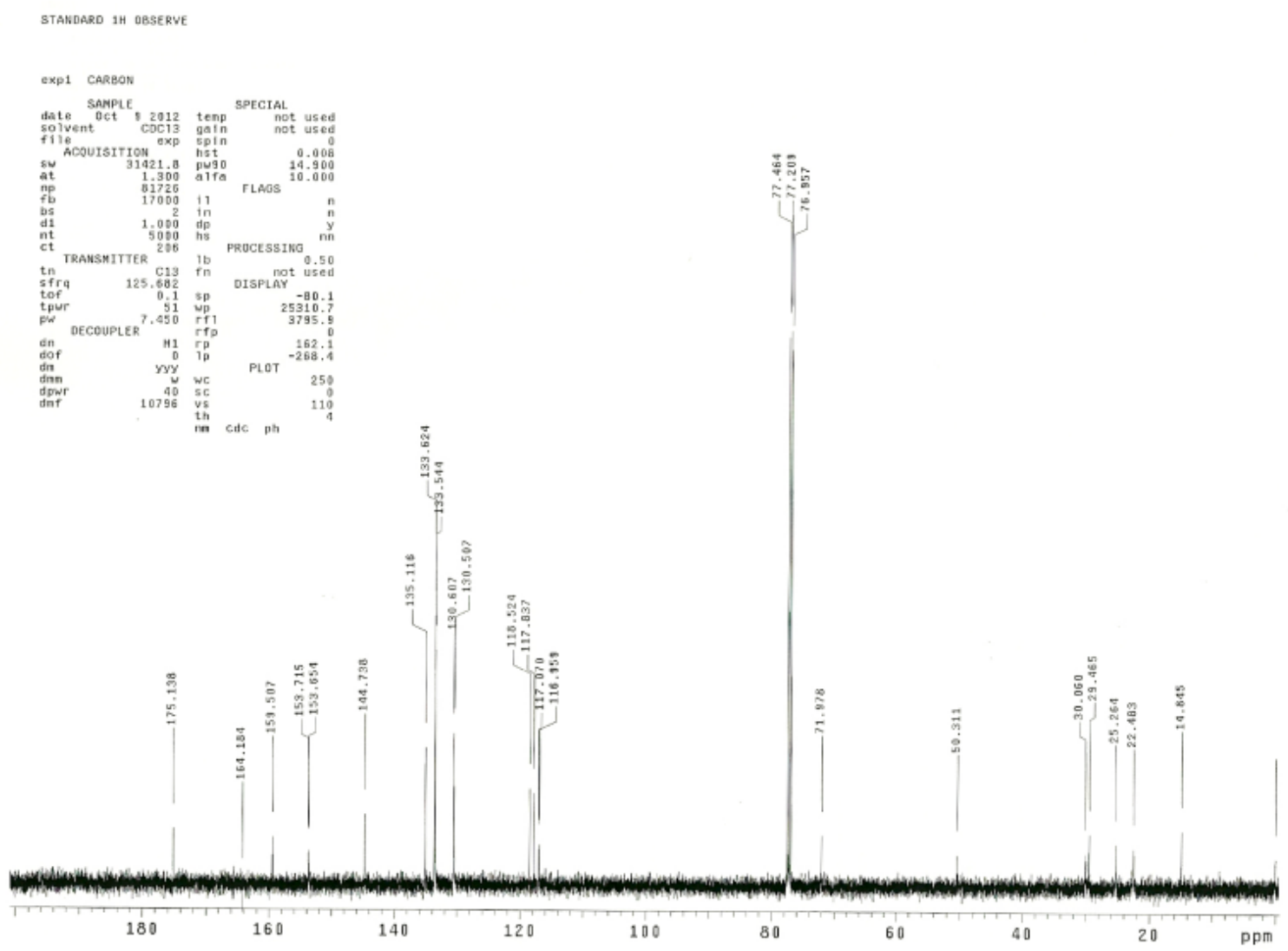
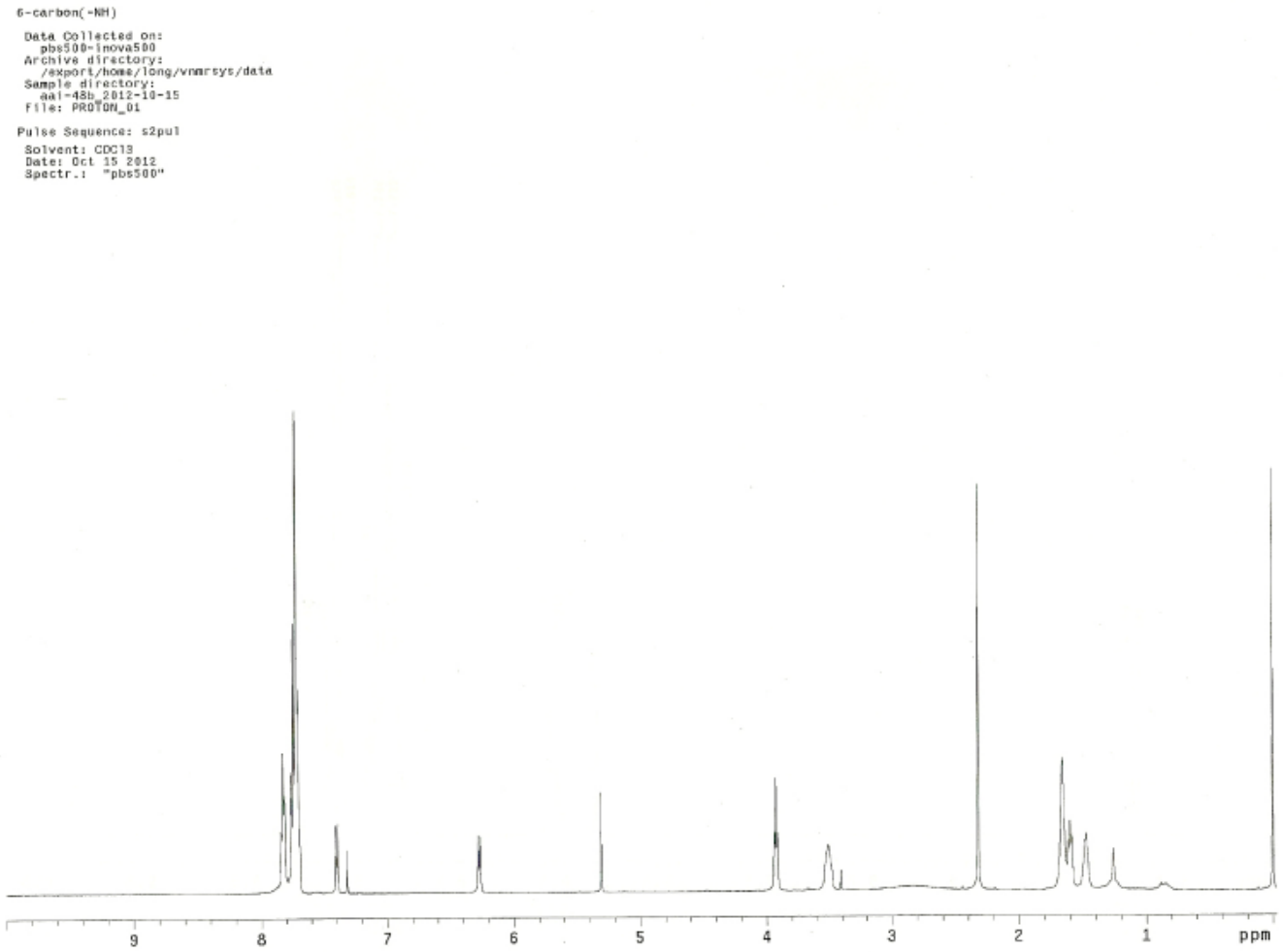
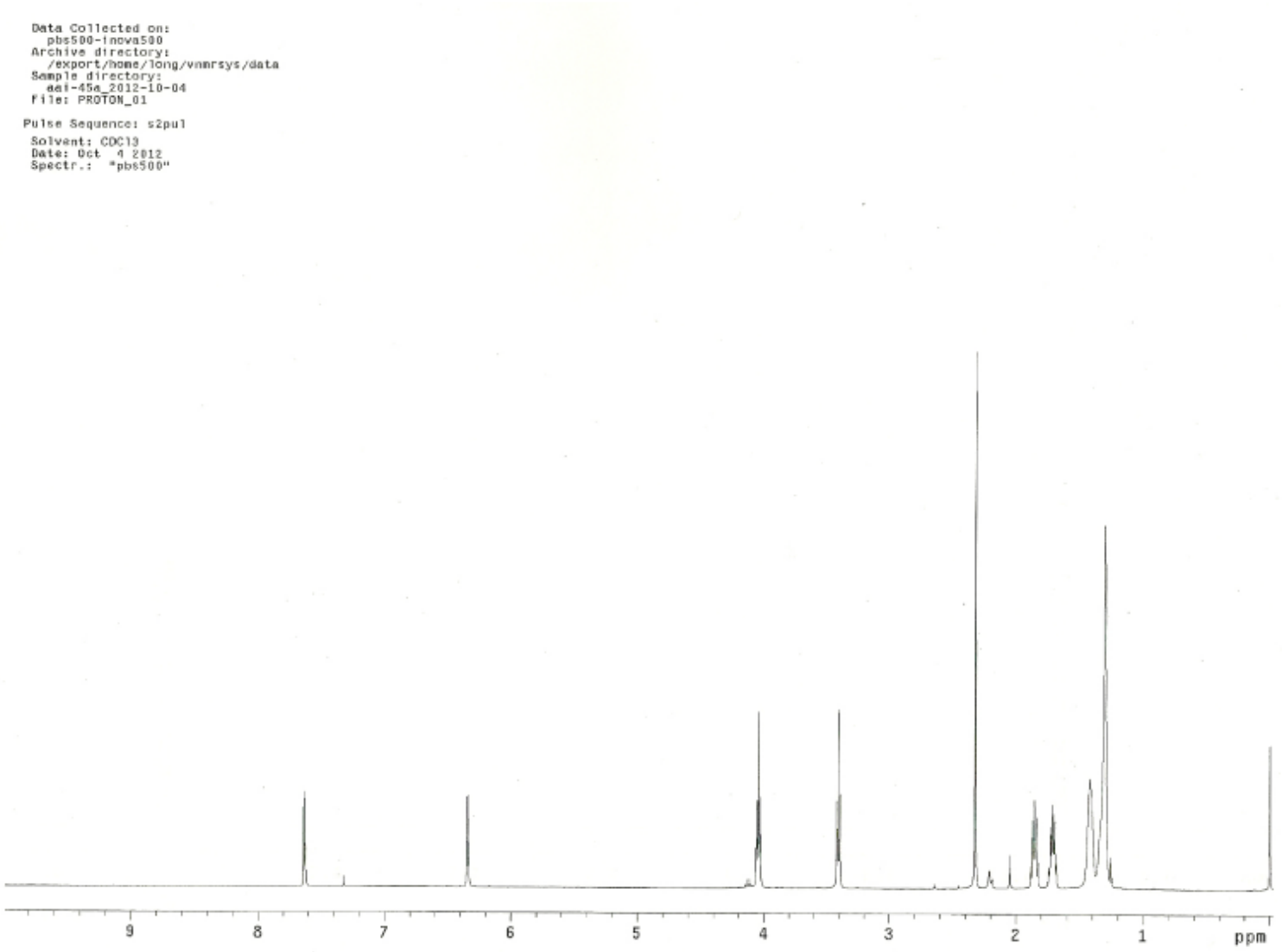


^{1}H of (6-((2-methyl-4-oxo-1,4-dihydropyridin-3-yl)oxy)hexyl)triphenylphosphonium bromide (9c)



^{1}H of 3-((10-bromodecyl)oxy)-2-methyl-4*H*-pyran-4-one (7d)



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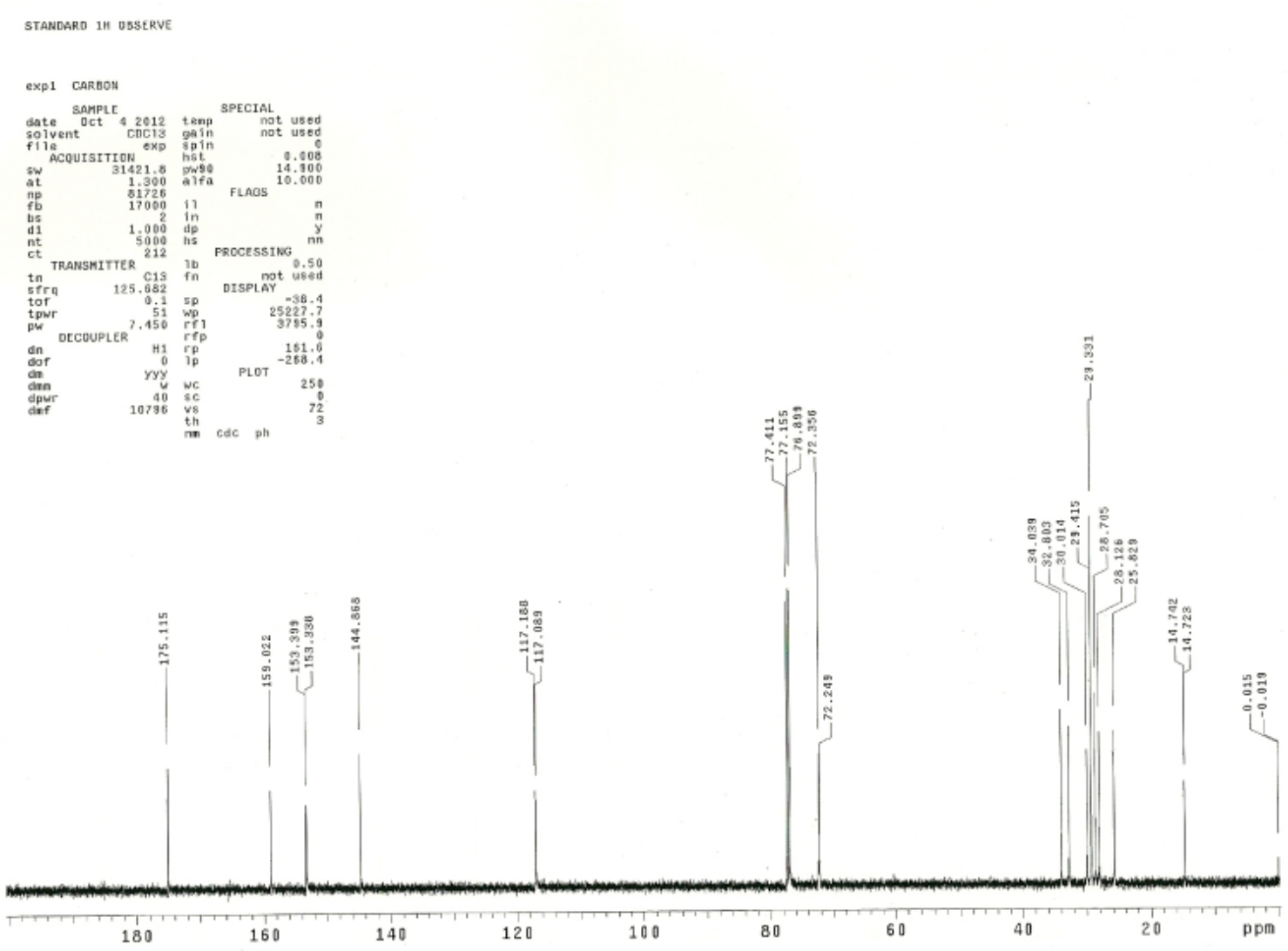

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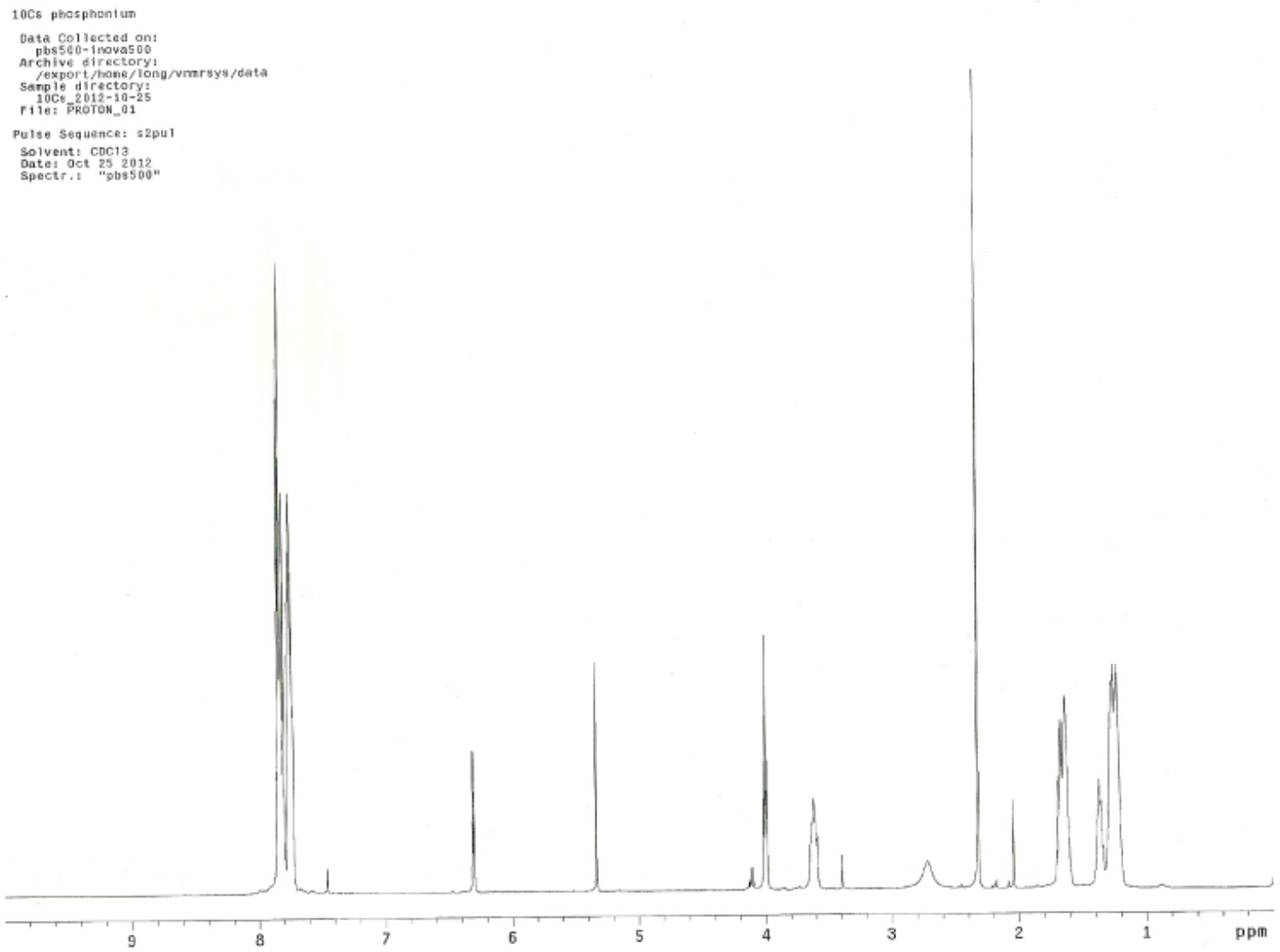

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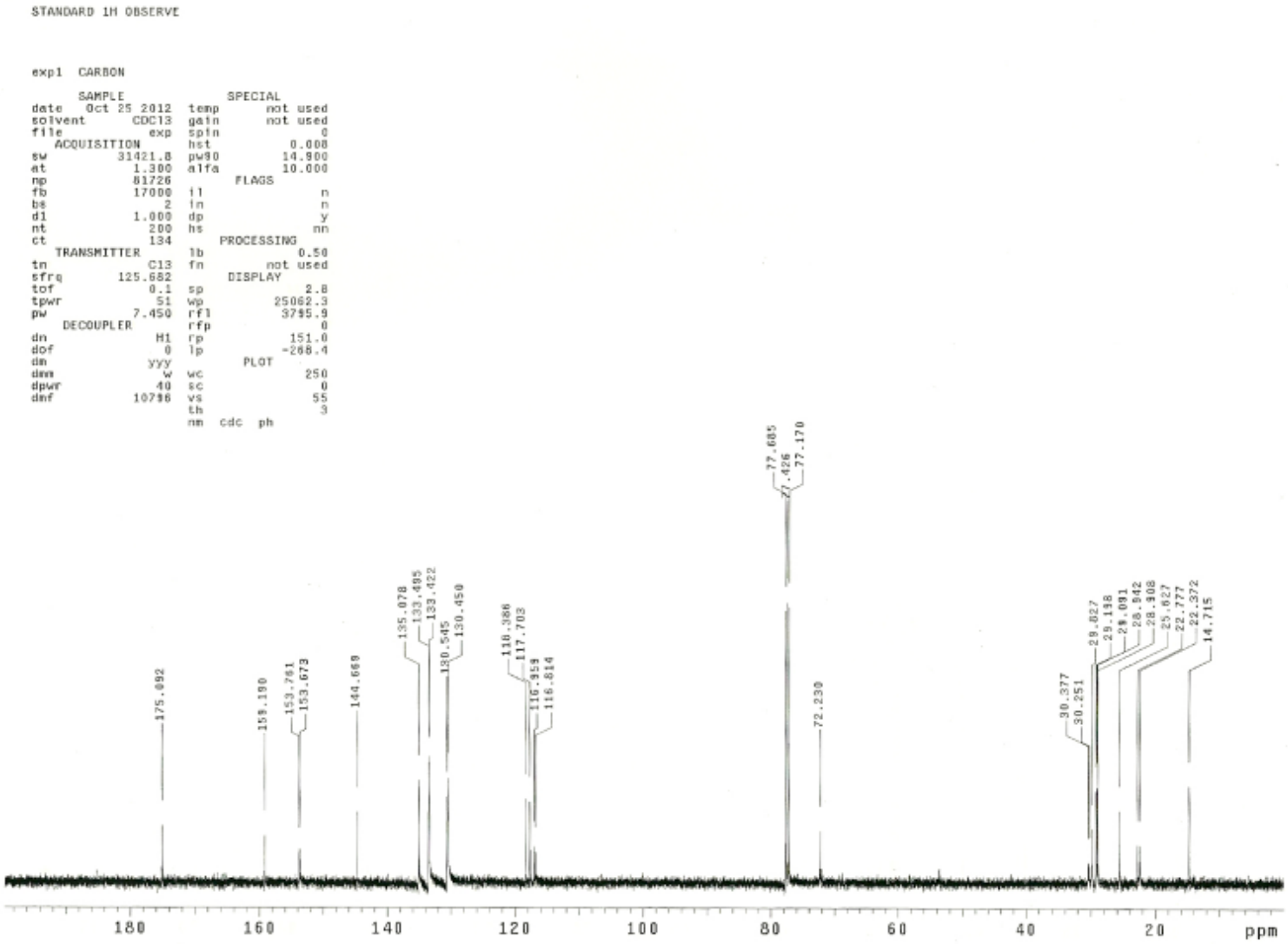

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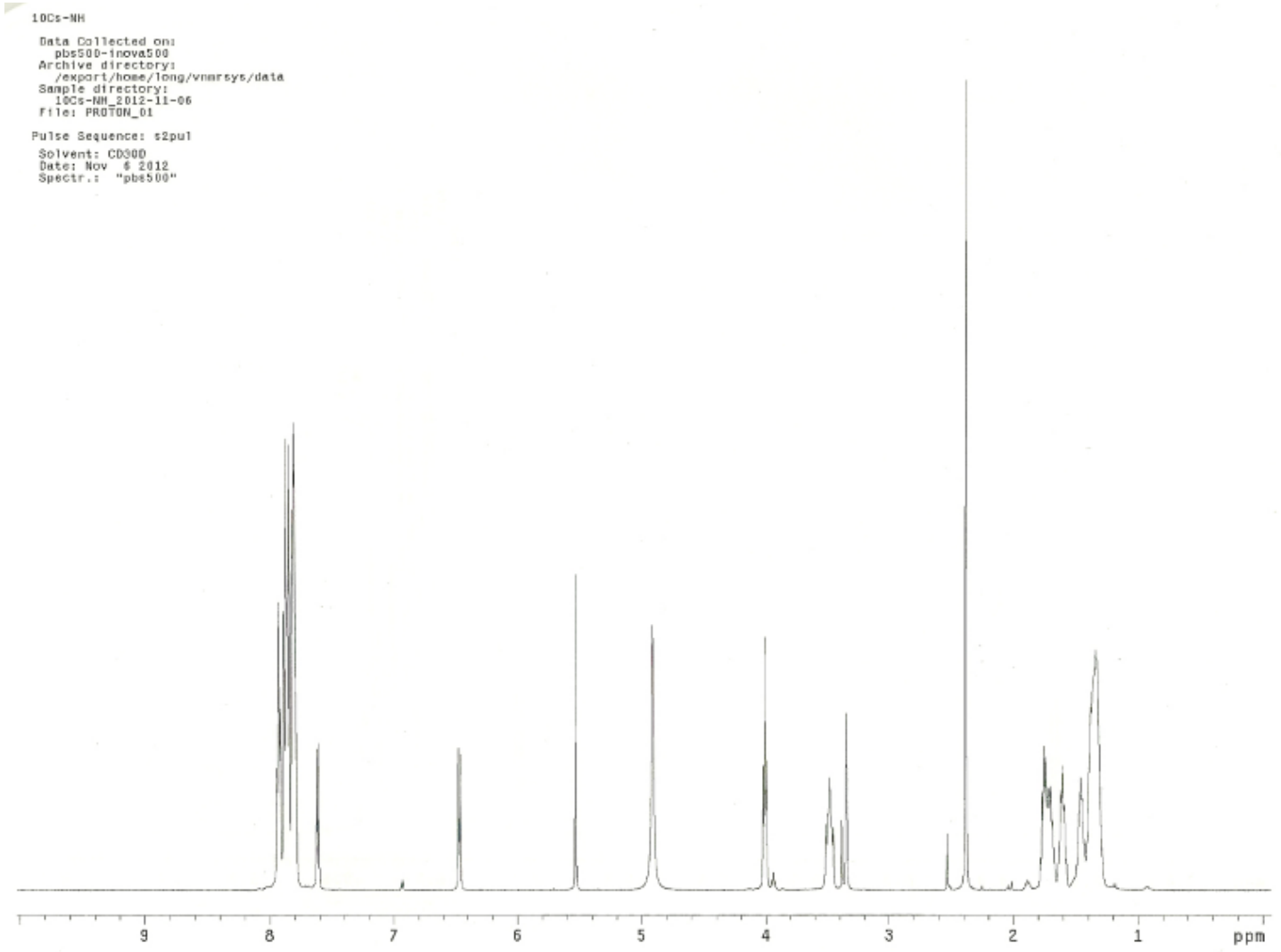
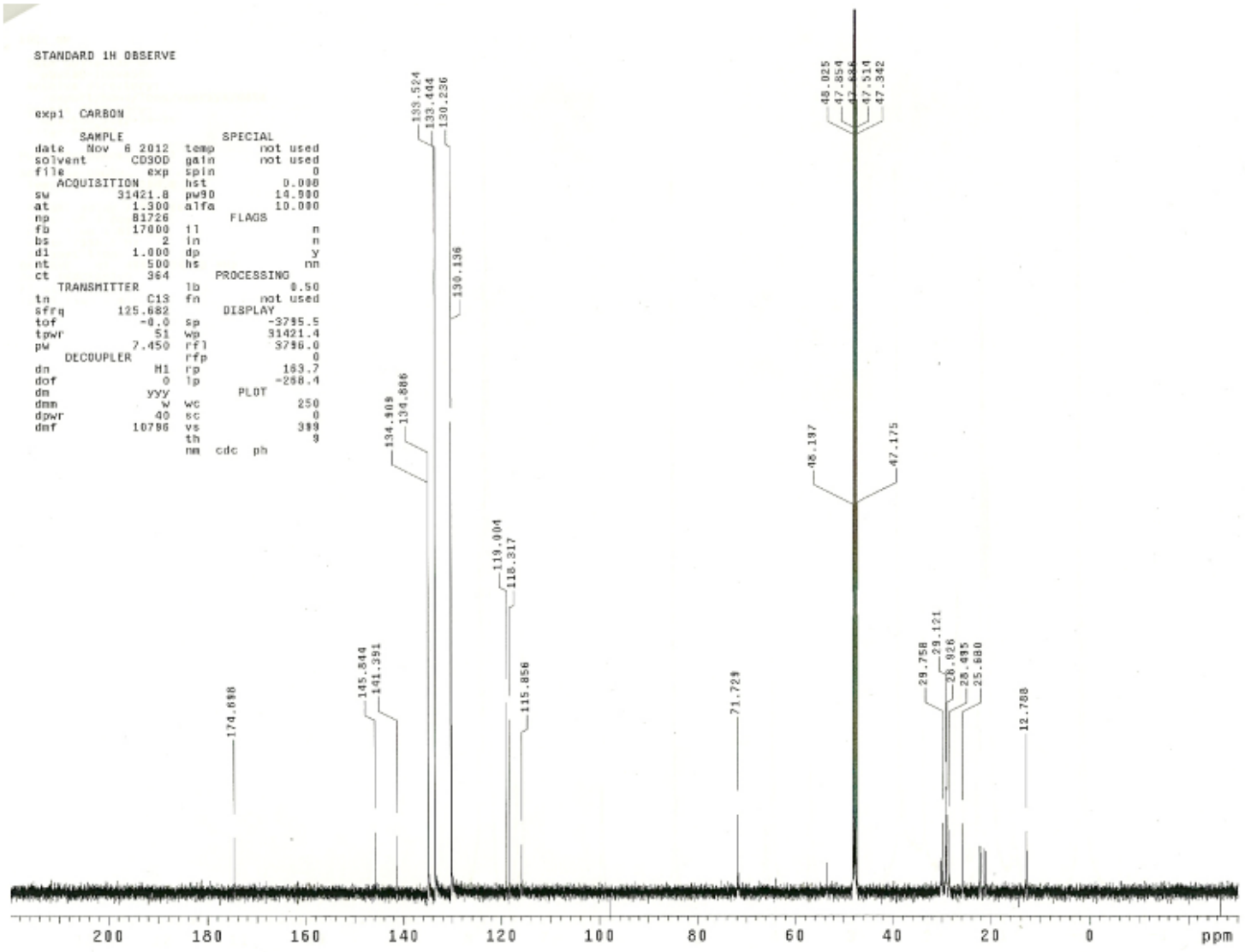
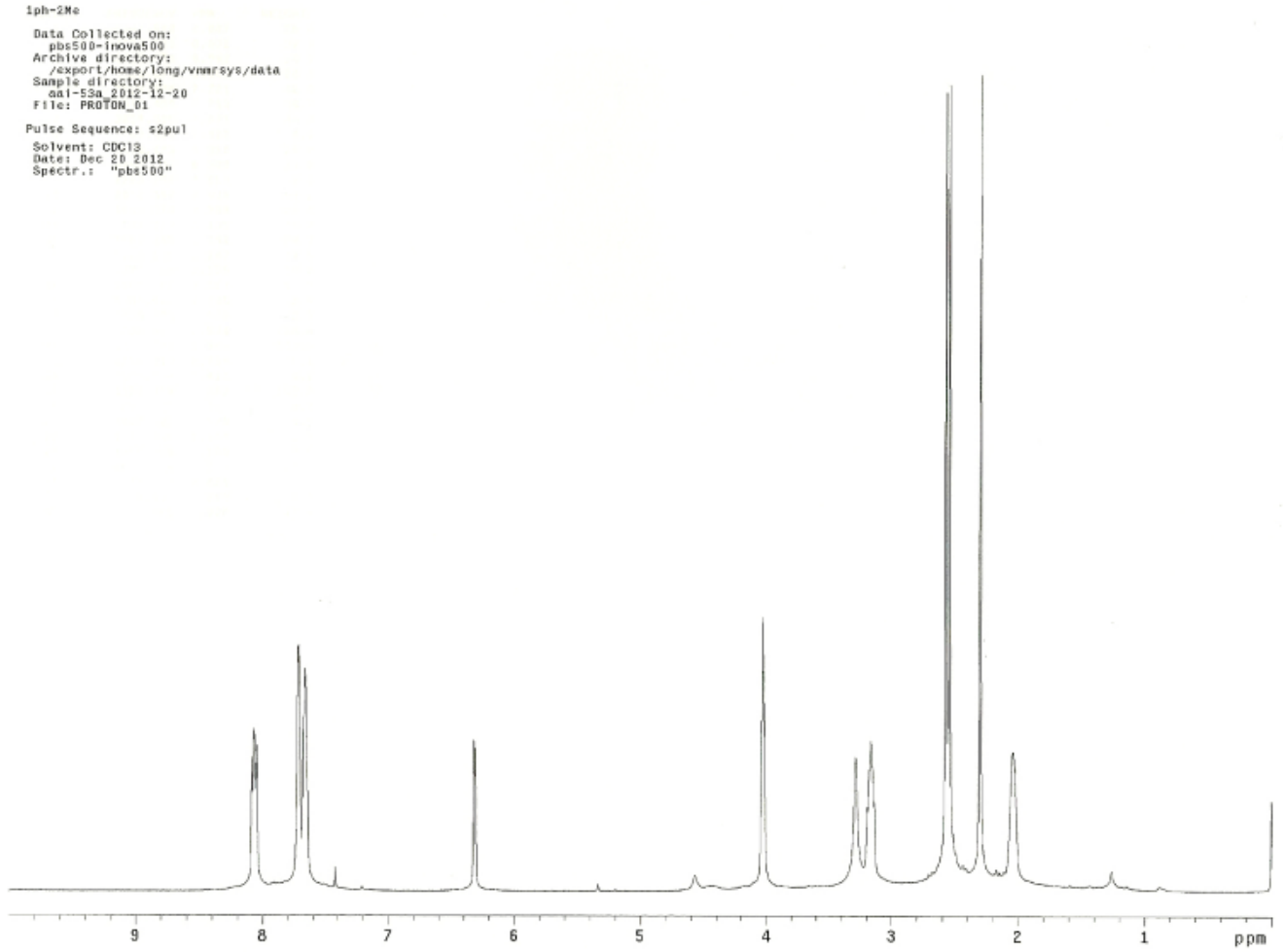


^{13}C of (10-((4-oxo-1,4-dihydropyridin-3-yl)oxy)decyl)triphenylphosphonium bromide (9d)



^{1}H of dimethyl(3-((2-methyl-4-oxo-4H-pyran-3-yl)oxy)propyl)(phenyl)phosphonium bromide (8f)



^{13}C of dimethyl(3-((2-methyl-4-oxo-4H-pyran-3-yl)oxy)propyl)(phenyl)phosphonium bromide (8f)

STANDARD 1H OBSERVE

exp1 CARBON

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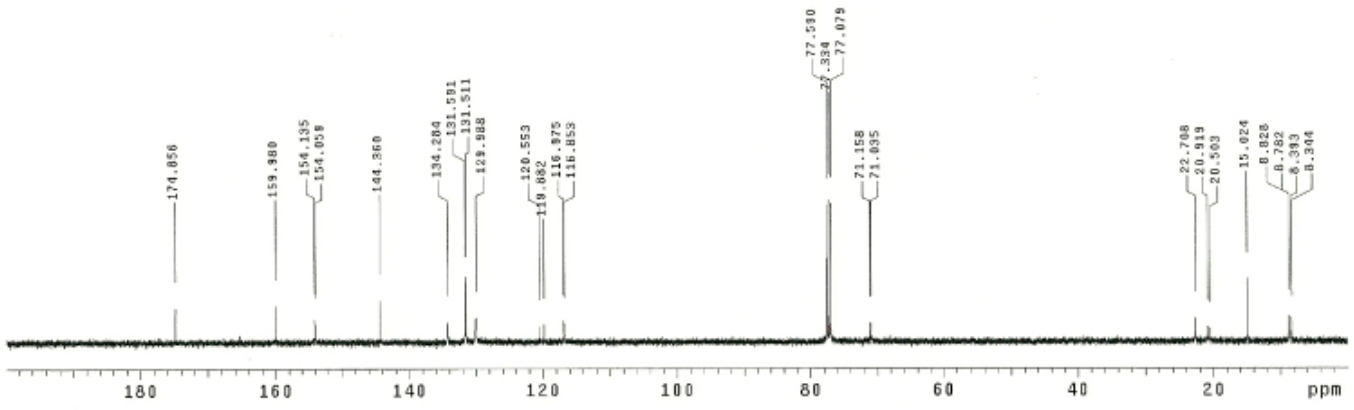
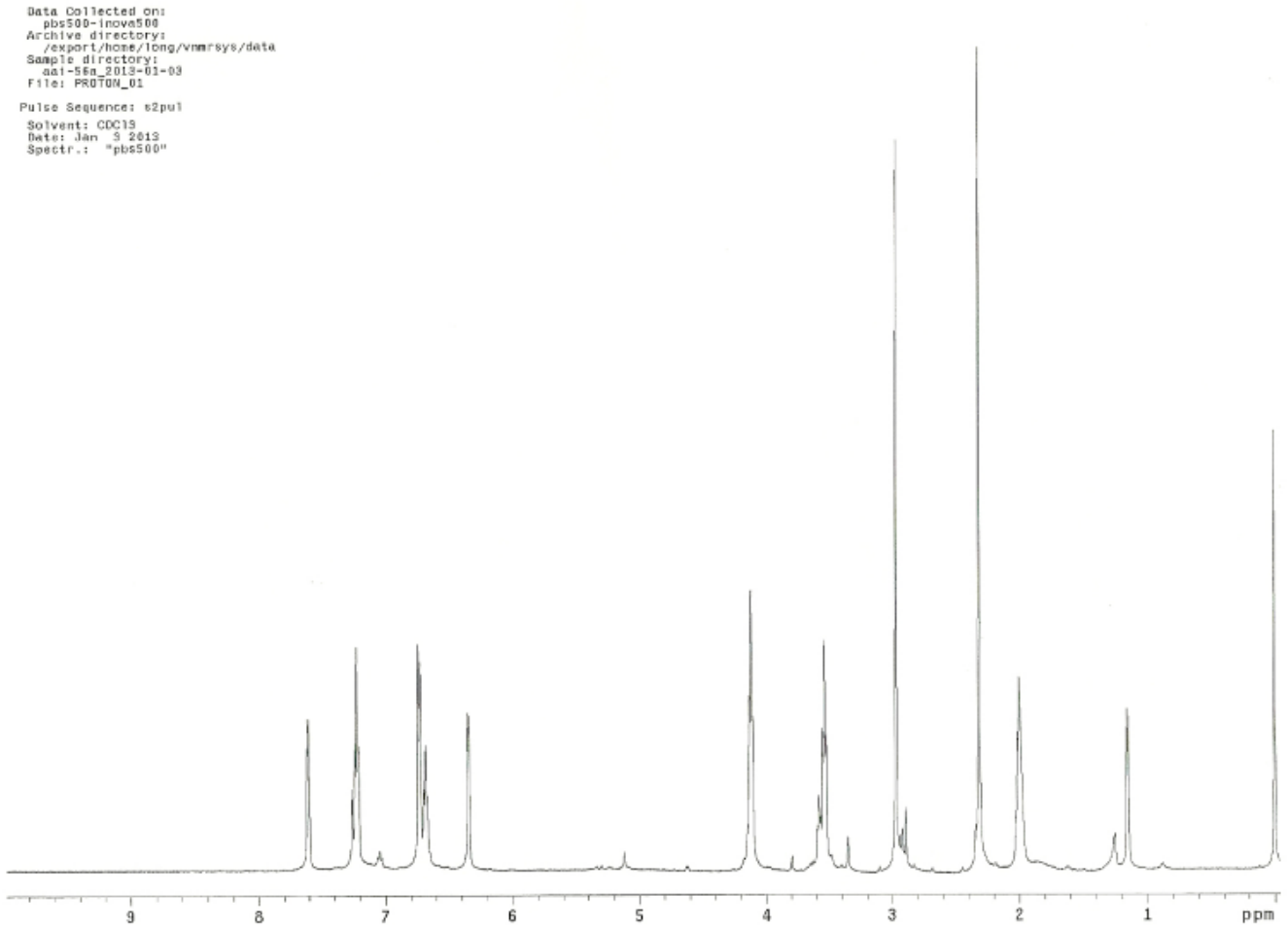
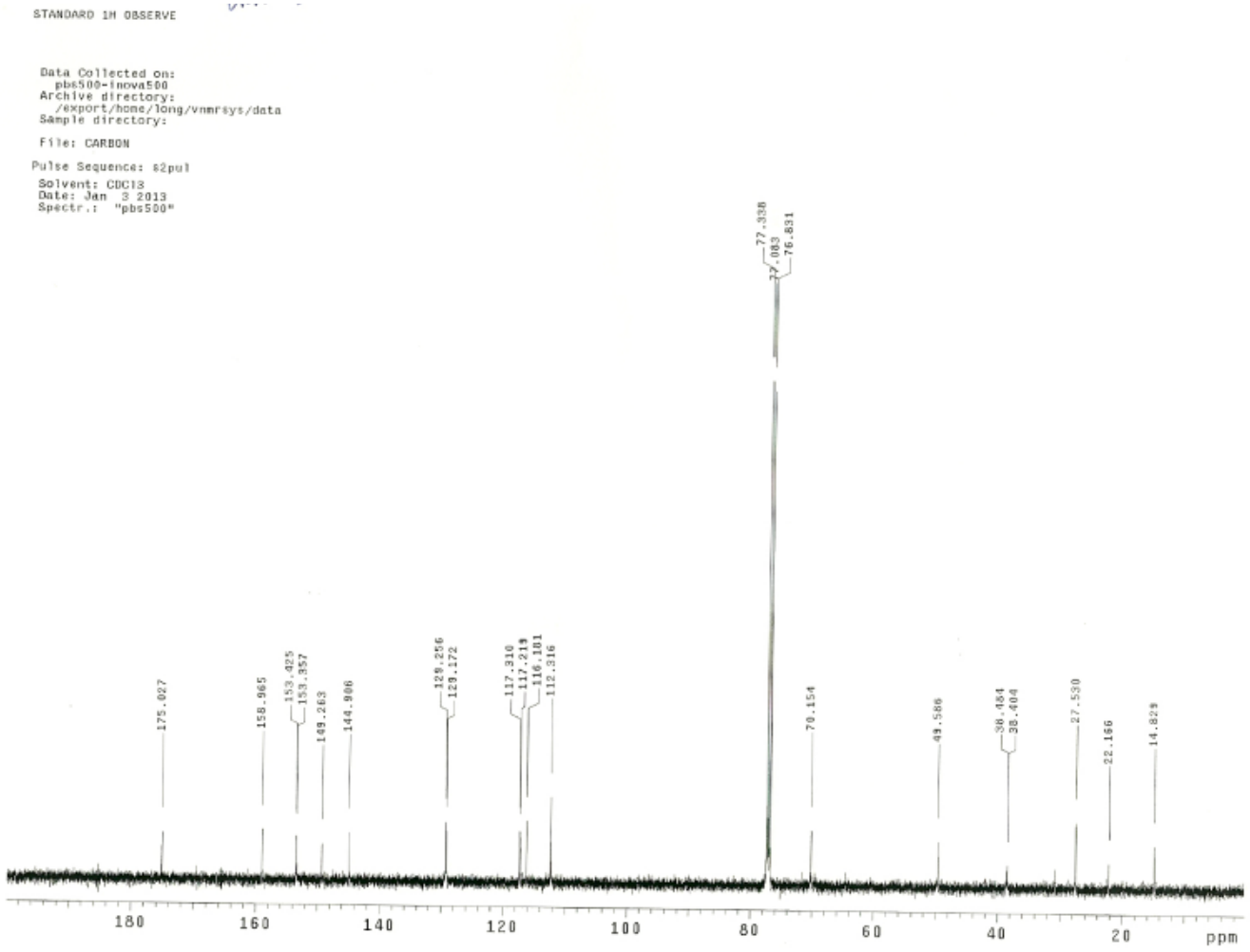
date	Dec 20 2012	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	0
ACQUISITION		hst	0.088
sw	31421.8	pw90	14.980
at	1.300	alfa	10.000
np	81726	FLAGS	
fb	17000	il	n
bs	2	in	n
d1	1.000	dp	y
nt	500	hs	nn
ct	236	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.682	DISPLAY	
tof	0.1	sp	2.8
tpwr	51	wp	25104.0
pw	7.450	rf1	3795.9
DECOUPLER		rfp	0
dn	H1	rp	145.4
dof	0	lp	-244.6
dm	yyy	PLOT	
dmm	w	wc	250
dpwr	40	sc	0
dmf	10796	vs	26
		th	2
		nm	cdc ph



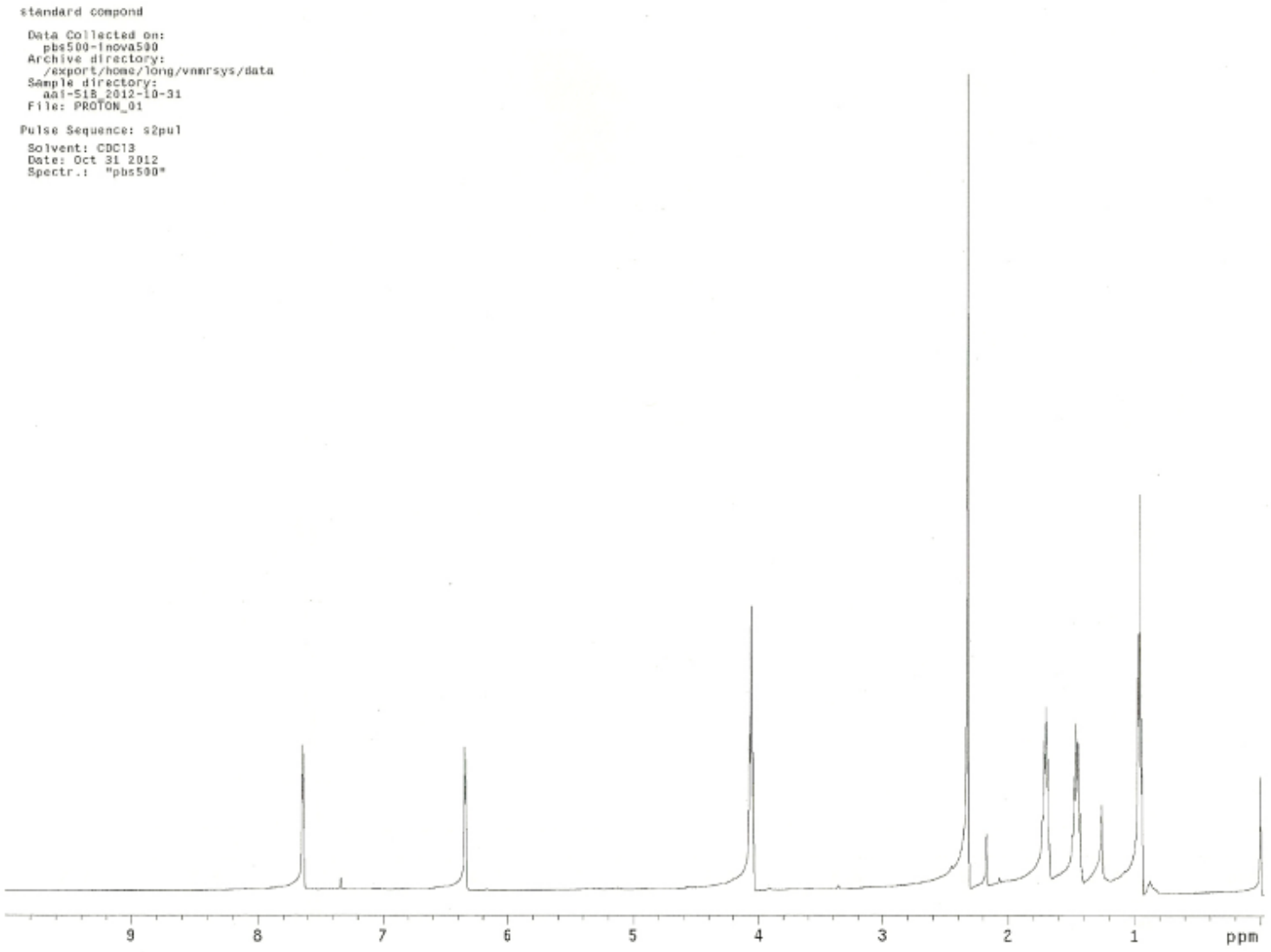
^{1}H of N,N-dimethyl-N-(3-((2-methyl-4-oxo-4H-pyran-3-yl)oxy)propyl)benzenaminium bromide (10)



^{13}C of N,N-dimethyl-N-(3-((2-methyl-4-oxo-4H-pyran-3-yl)oxy)propyl)benzenaminium bromide (10)



^{1}H of 3-butoxy-2-methyl-4H-pyran-4-one (12)

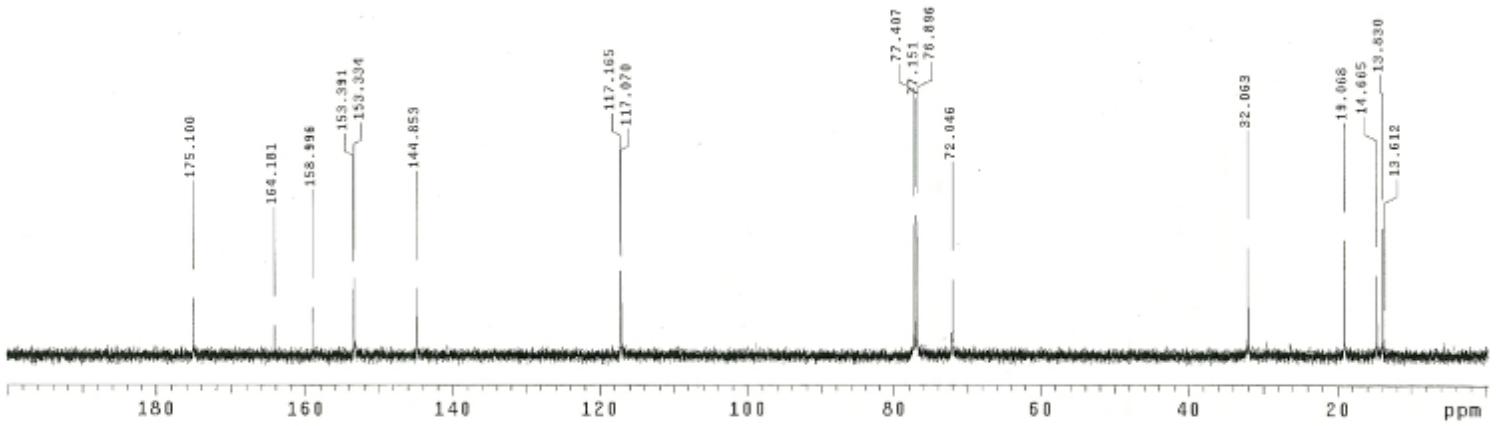


^{13}C of 3-butoxy-2-methyl-4H-pyran-4-one (12)

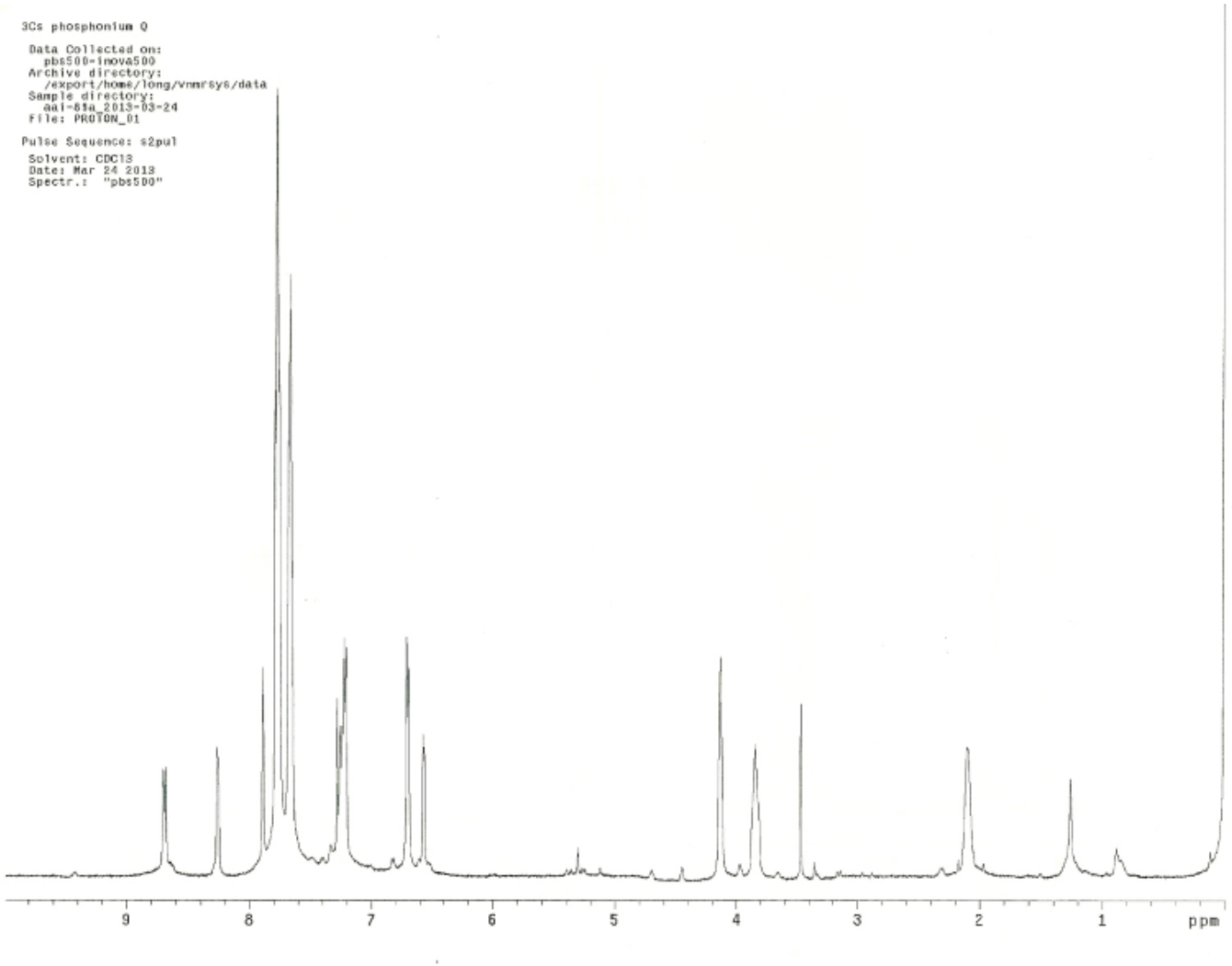
STANDARD 1H OBSERVE

exp1 CARBON

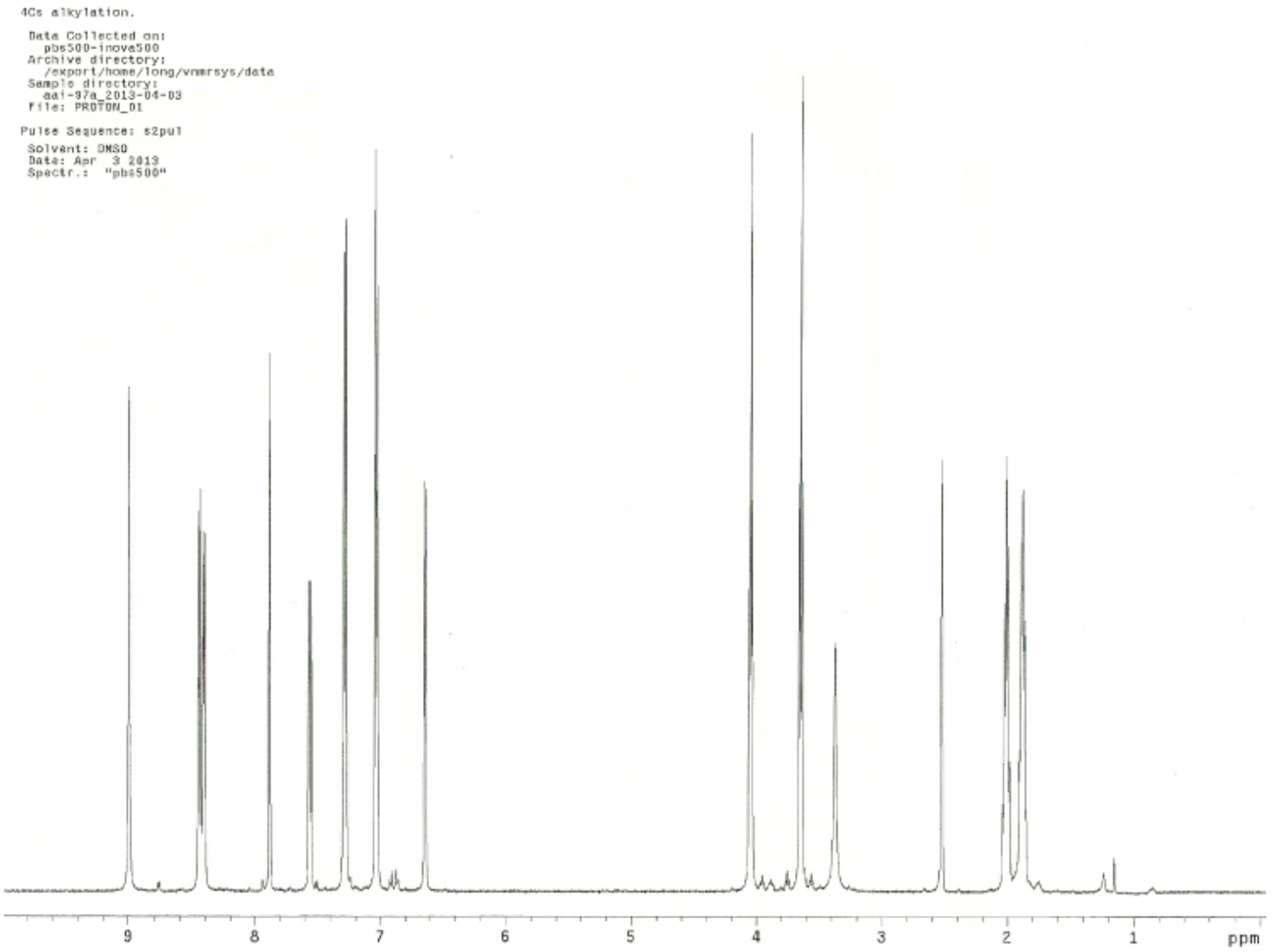
SAMPLE		SPECIAL	
date	Oct 31 2012	temp	not used
solvent	CDCl3	gain	not used
file	exp	spin	0
ACQUISITION		hst	8.808
sw	31421.8	pw90	14.900
at	1.300	alfa	10.000
np	81726	FLAGS	
fb	17000	il	n
bs	2	in	n
d1	1.000	dp	y
nt	500	hs	nn
ct	106	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	125.682	DISPLAY	
tof	0.1	sp	-38.4
tpwr	51	wp	25186.5
pw	7.450	rfl	3795.1
DECOUPLER		rfp	0
dn	H1	rp	151.8
dof	0	lp	-268.4
dm	yyy	PLOT	
dmm	w	wc	250
dpwr	40	sc	0
dmf	10796	vs	24
		th	3
		nm	cdc ph



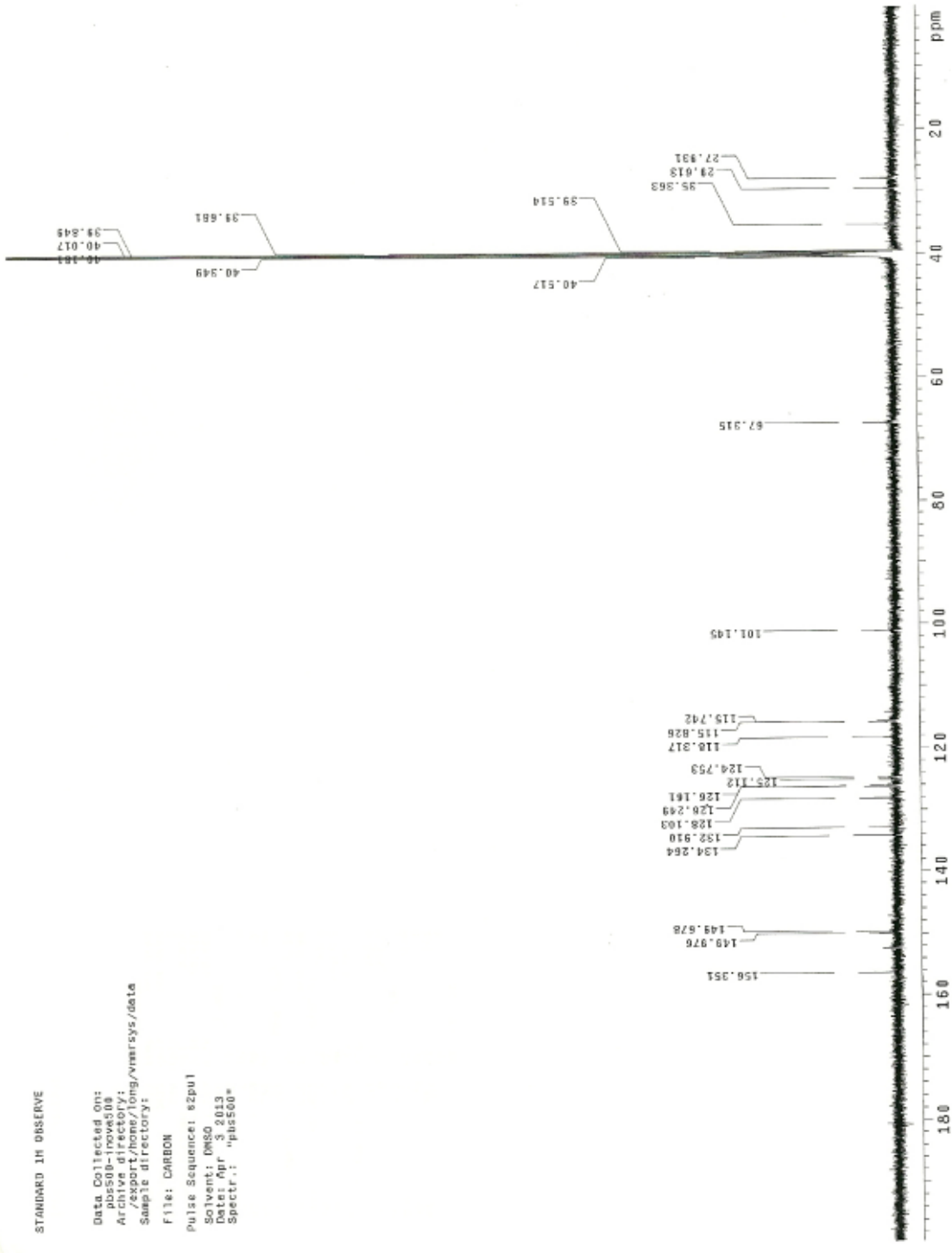
^{1}H of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)propyl)triphenylphosphonium bromide (16a)



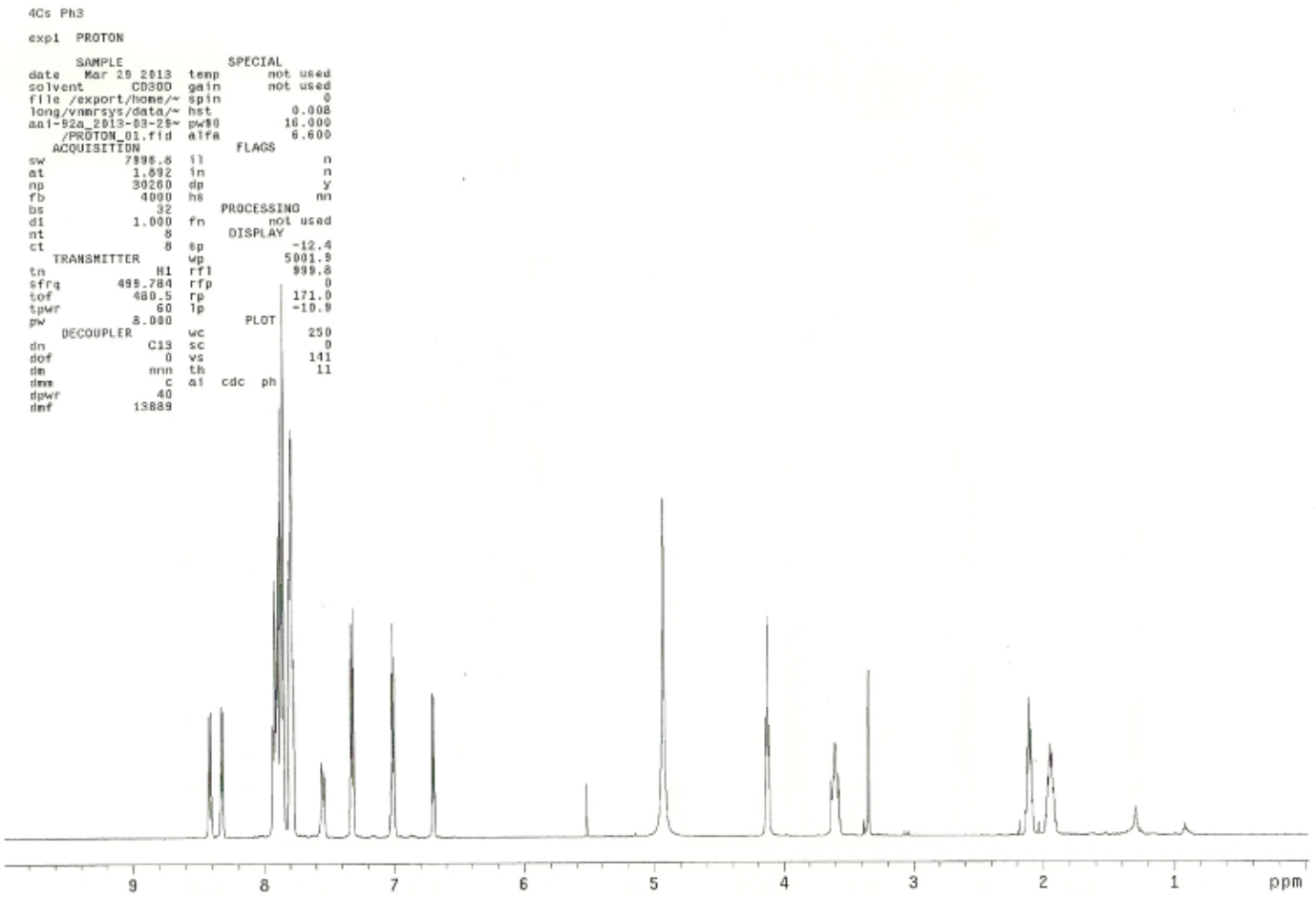
^{1}H of N-(4-(4-bromobutoxy)phenyl)-7-chloroquinolin-4-amine (14b)



^{13}C of N-(4-(4-bromobutoxy)phenyl)-7-chloroquinolin-4-amine (14b)



^{1}H of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl)triphenylphosphonium bromide (16b)

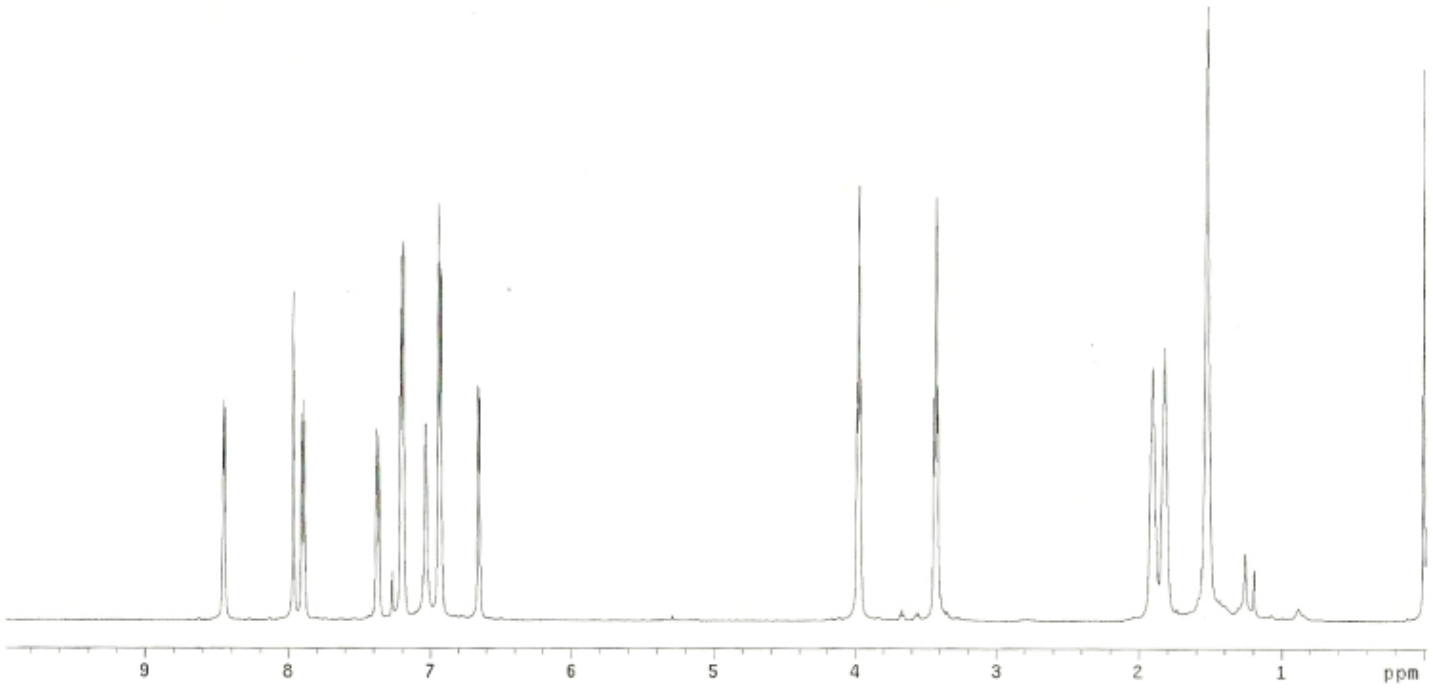


^{1}H of N-(4-(4-bromohexoxy)phenyl)-7-chloroquinolin-4-amine (14c)

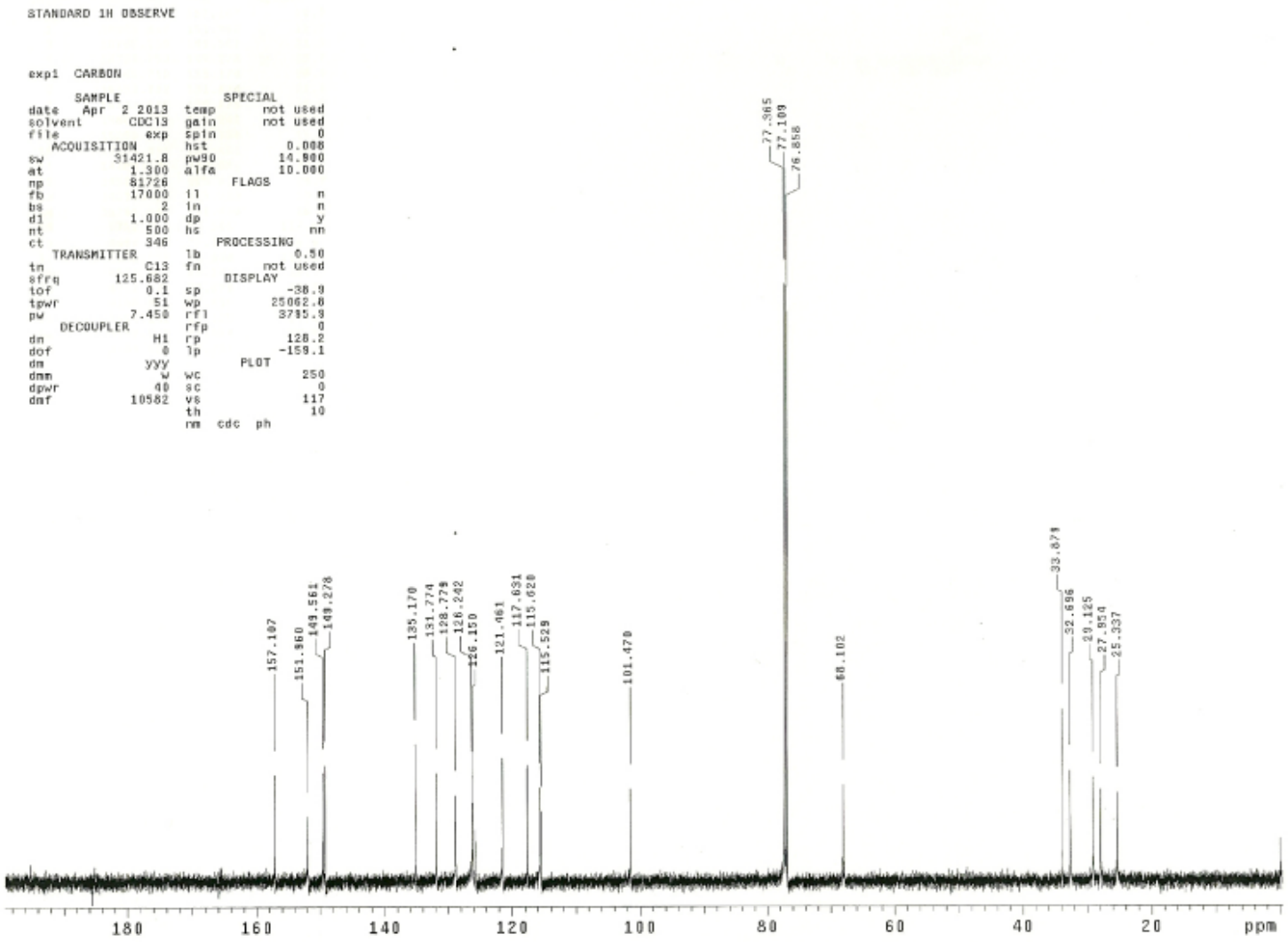
6Cs alkylation.

Data Collected on:
pbs500-inova500
Archive directory:
/export/home/long/vnmrsys/data
Sample directory:
aai-96a_2013-04-02
File: PROTON_01

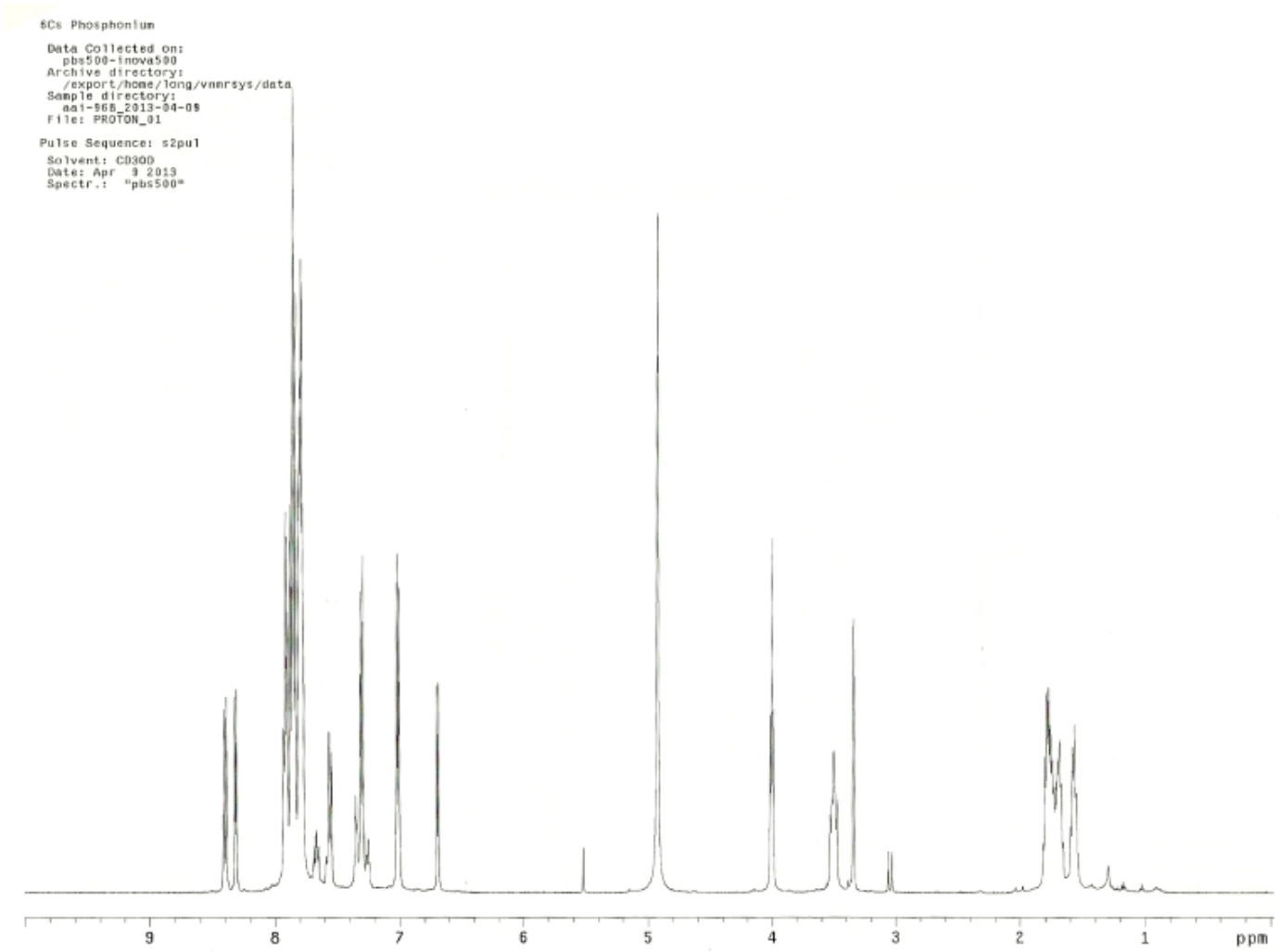
Pulse Sequence: s2pul
Solvent: CDCl3
Date: Apr 2 2013
Spectr.: "pbs500"



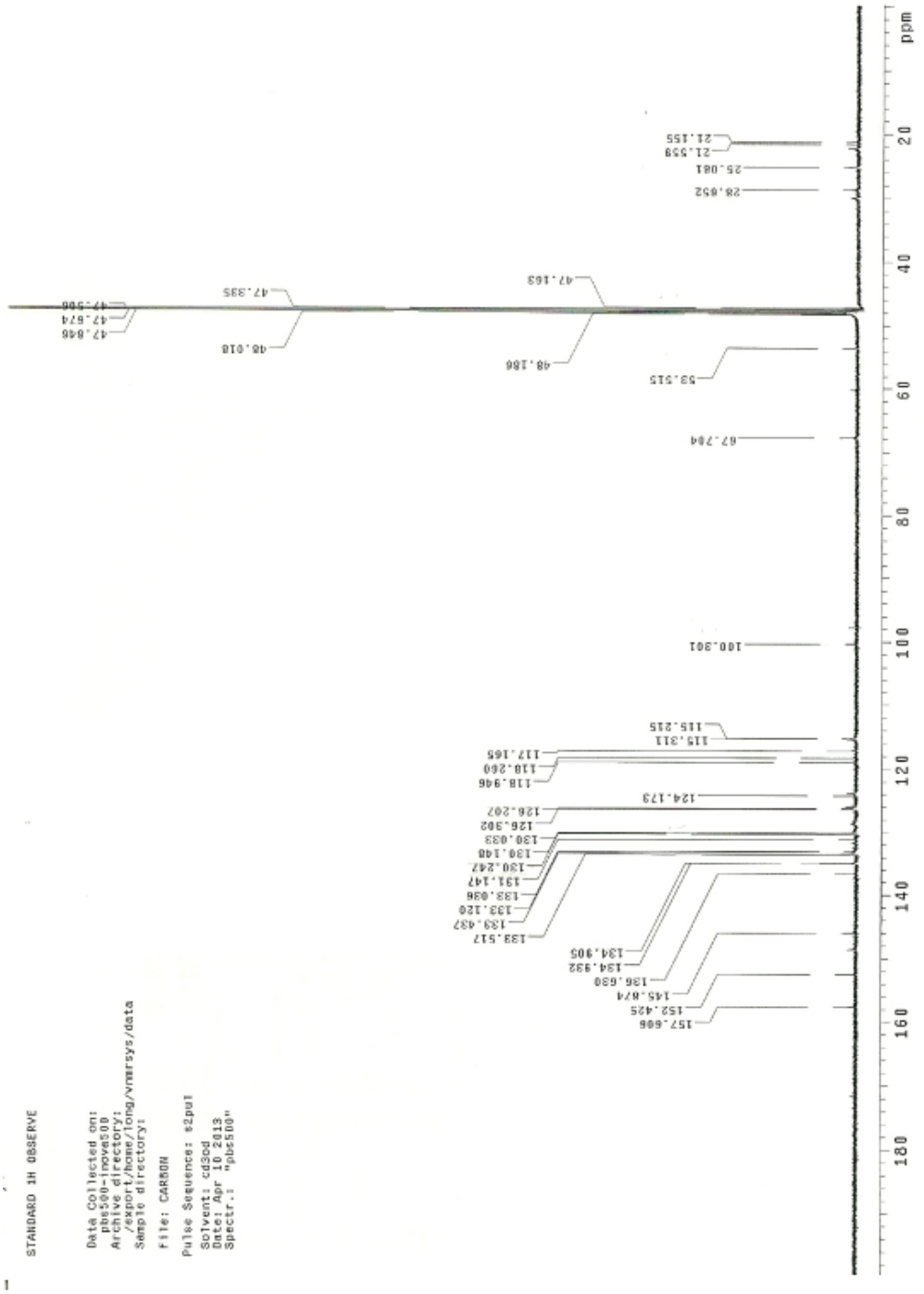
^{13}C of N-(4-(4-bromohexoxy)phenyl)-7-chloroquinolin-4-amine (14c)



^{1}H of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl)triphenylphosphonium bromide (16c)



^{13}C of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl)triphenylphosphonium bromide (16c)



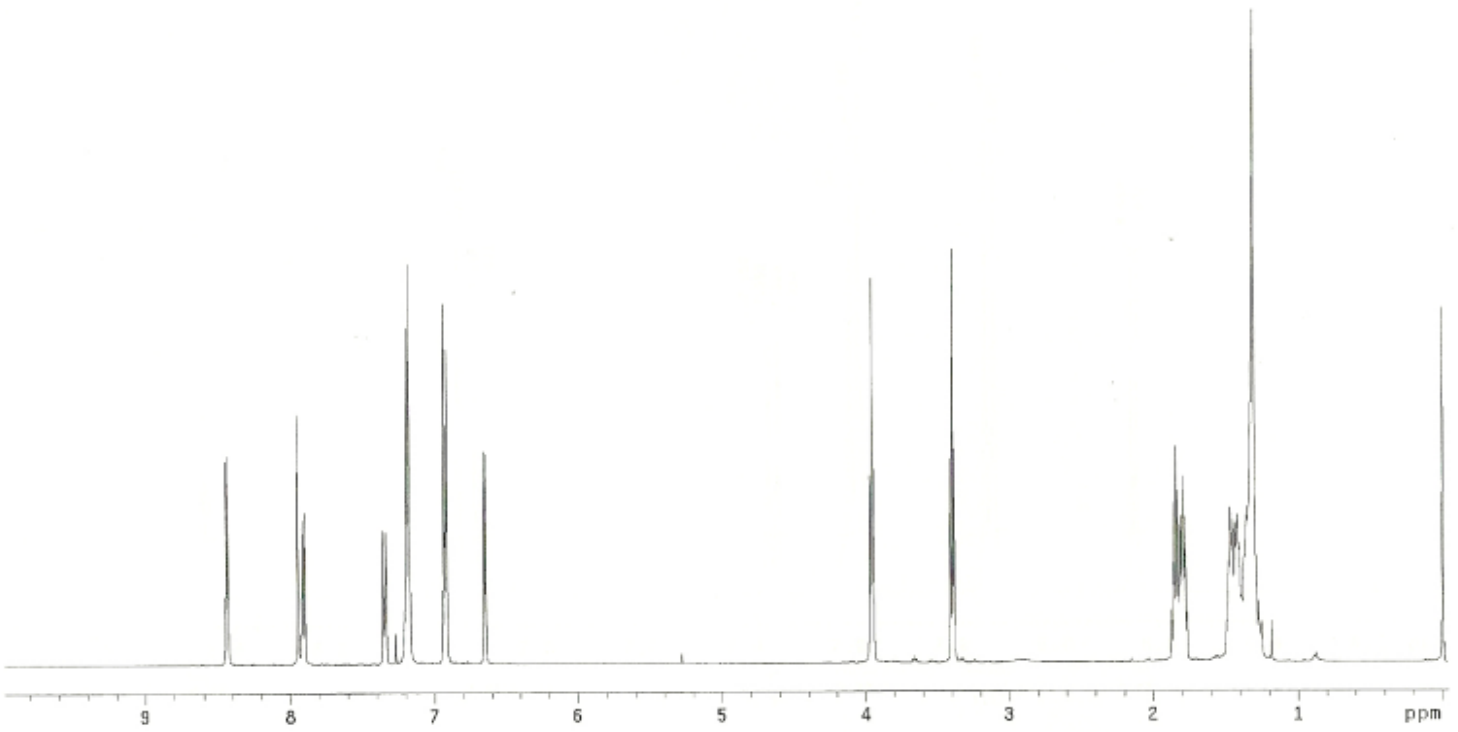
^{1}H of N-(4-(4-bromodecyloxy)phenyl)-7-chloroquinolin-4-amine (14d)

10Cs 4,7-Q intermediate

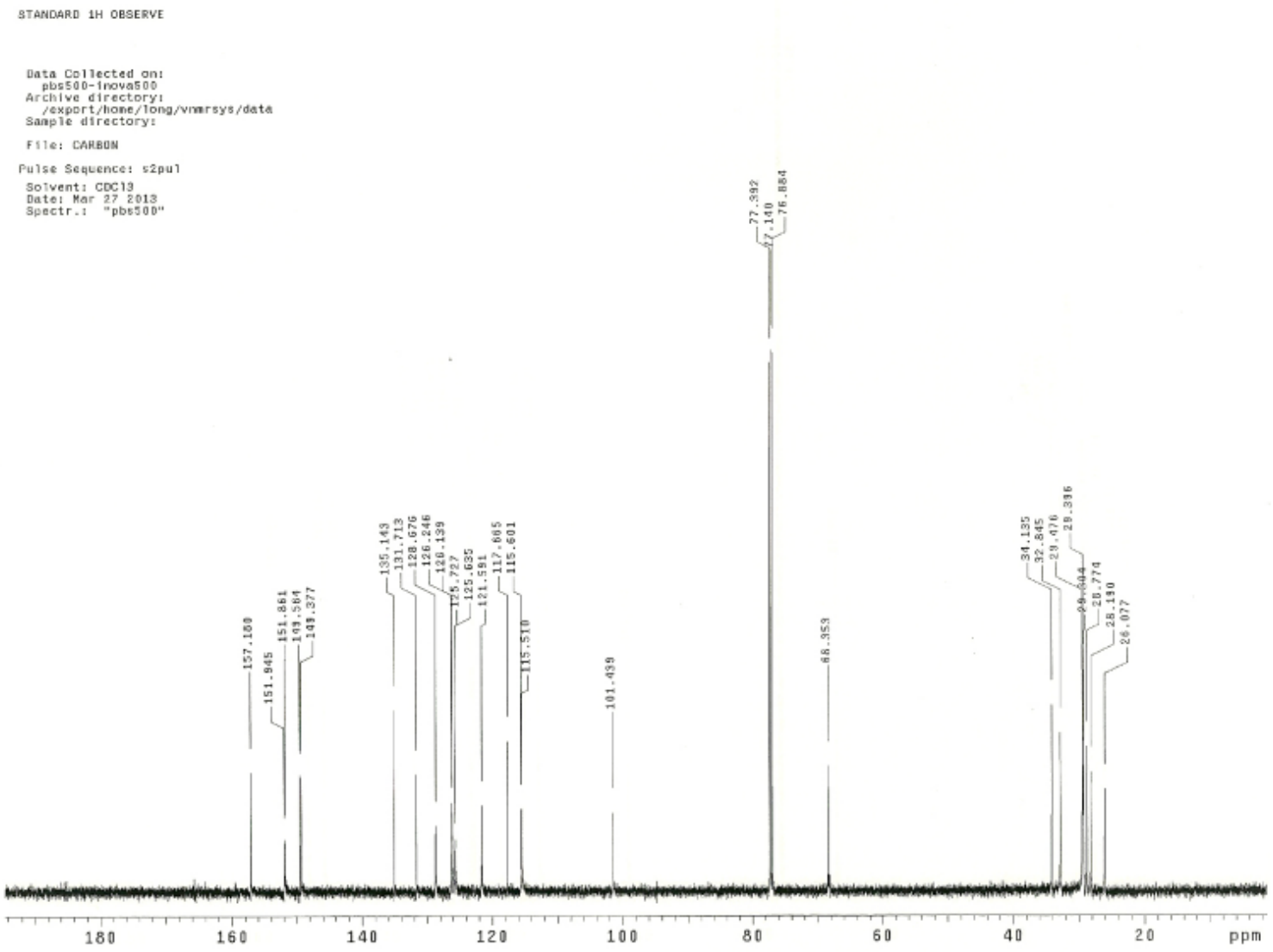
Data Collected on:
pbs500-inova500
Archive directory:
/export/home/long/vnmrsys/data
Sample directory:
aai-93a_2013-03-27
File: PROTON_01

Pulse Sequence: s2pul

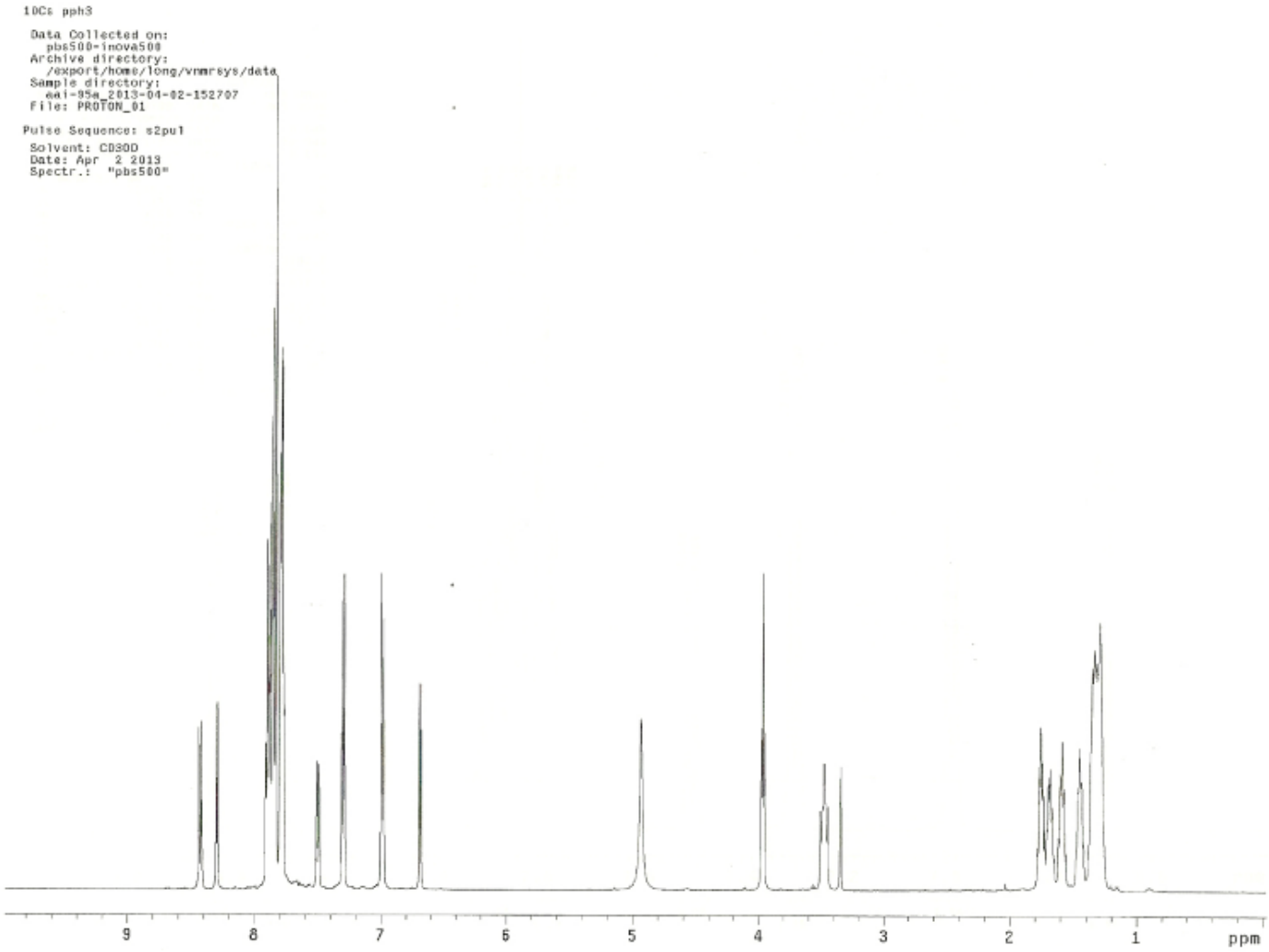
Solvent: CDCl3
Date: Mar 27 2013
Spectr.: "pbs500"



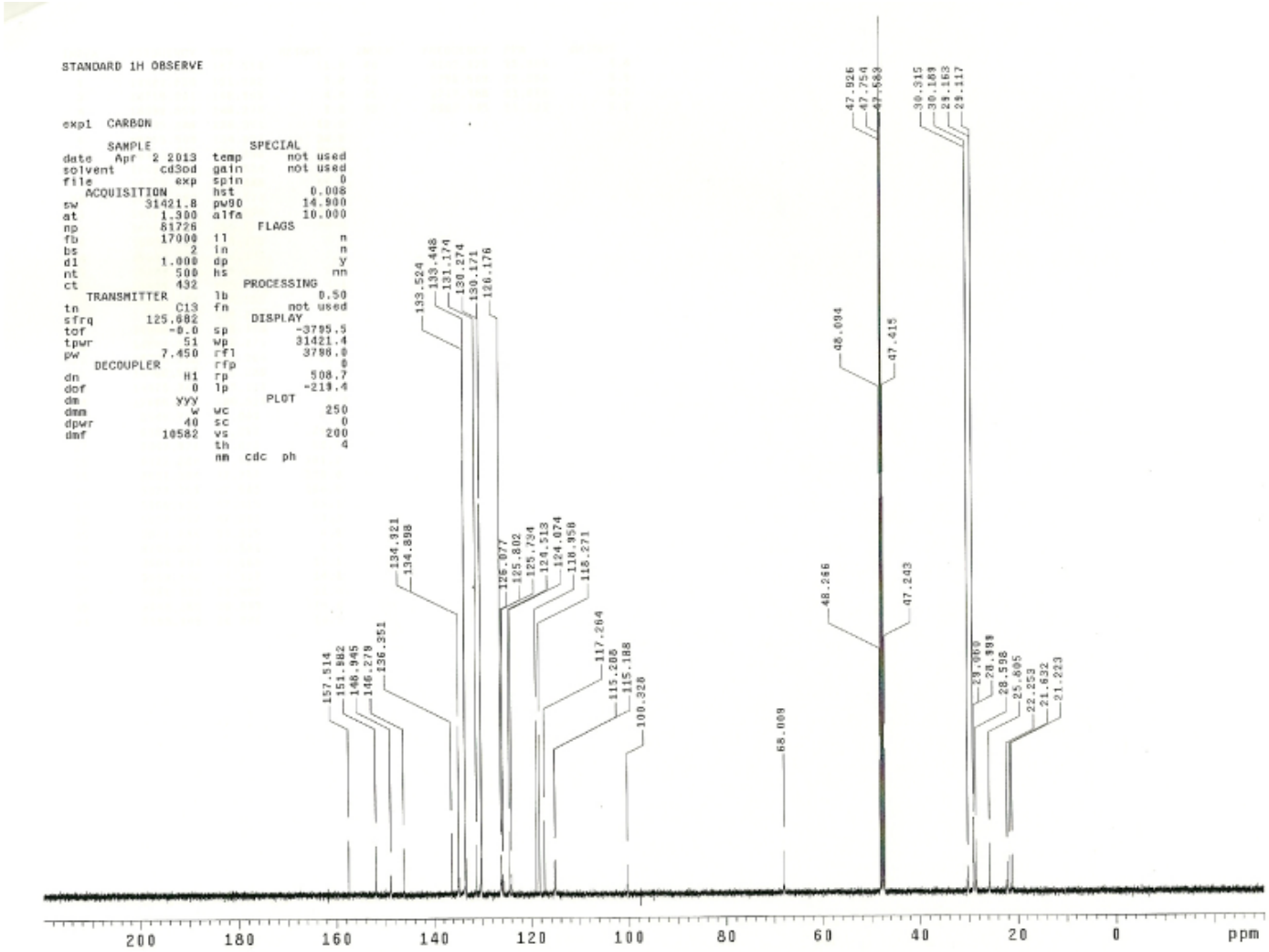
^{13}C of N-(4-(4-bromodecyloxy)phenyl)-7-chloroquinolin-4-amine (14d)



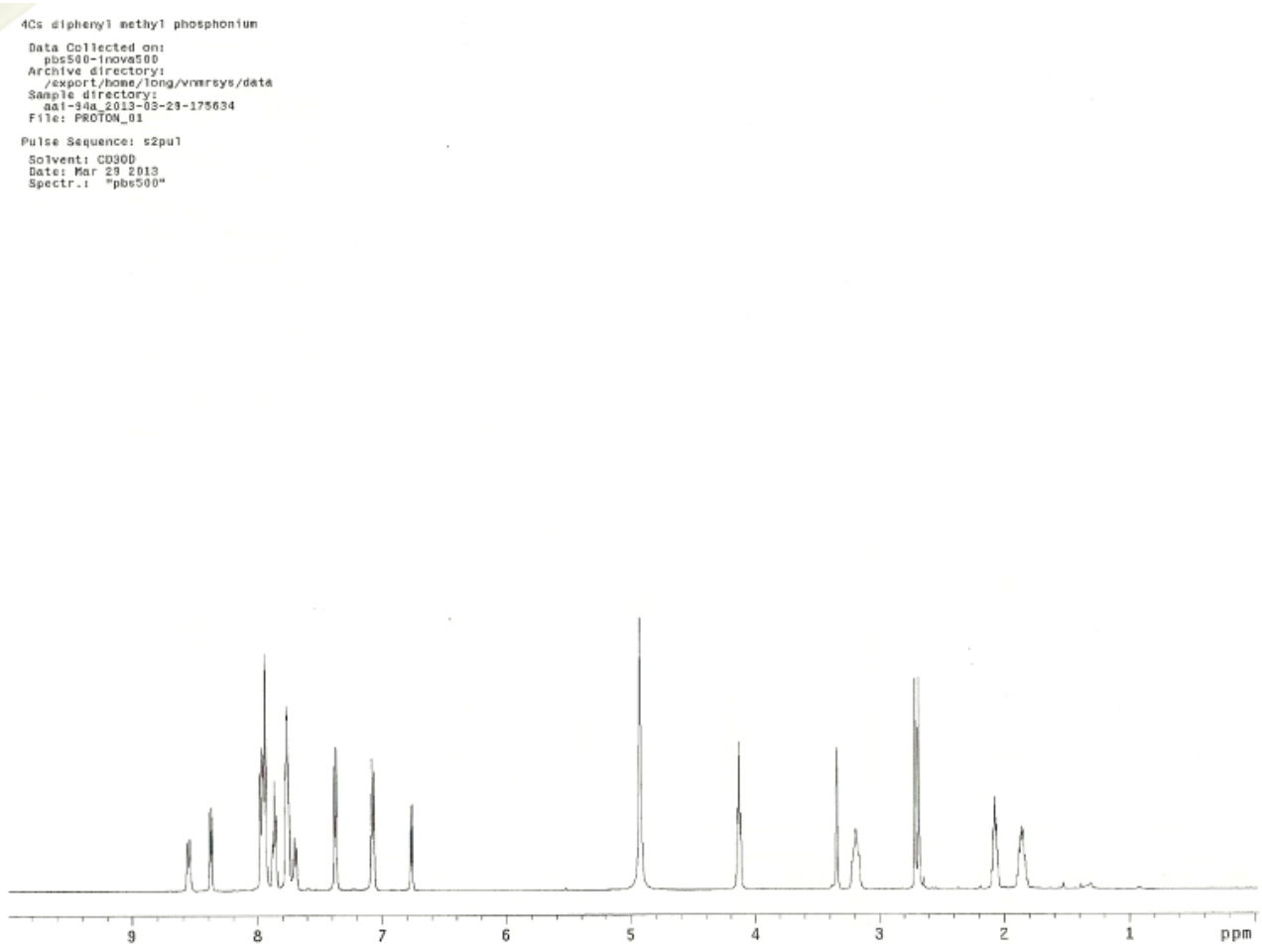
^{1}H of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)decyl)triphenylphosphonium bromide (16d)



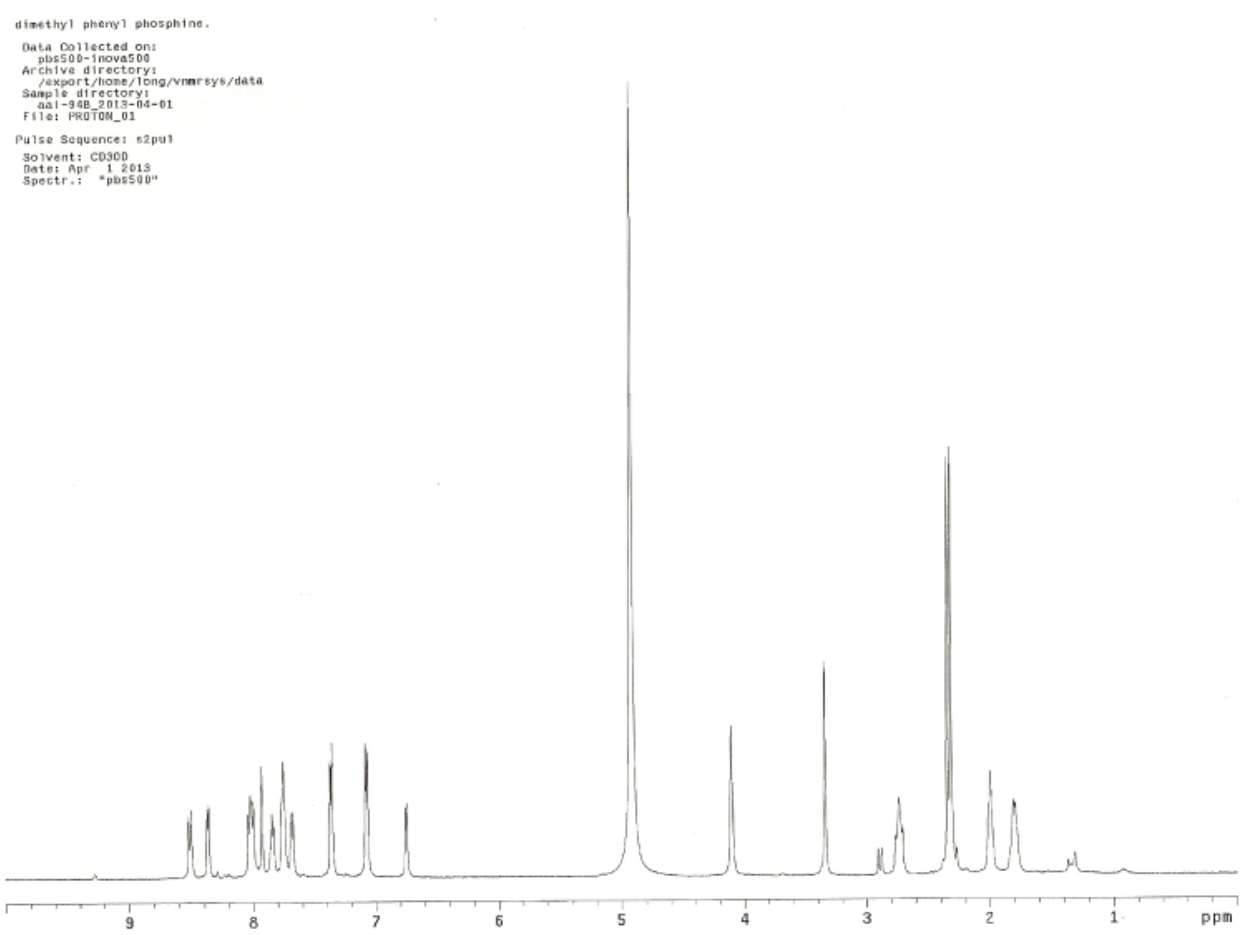
^{13}C of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)decyl)triphenylphosphonium bromide (16d)



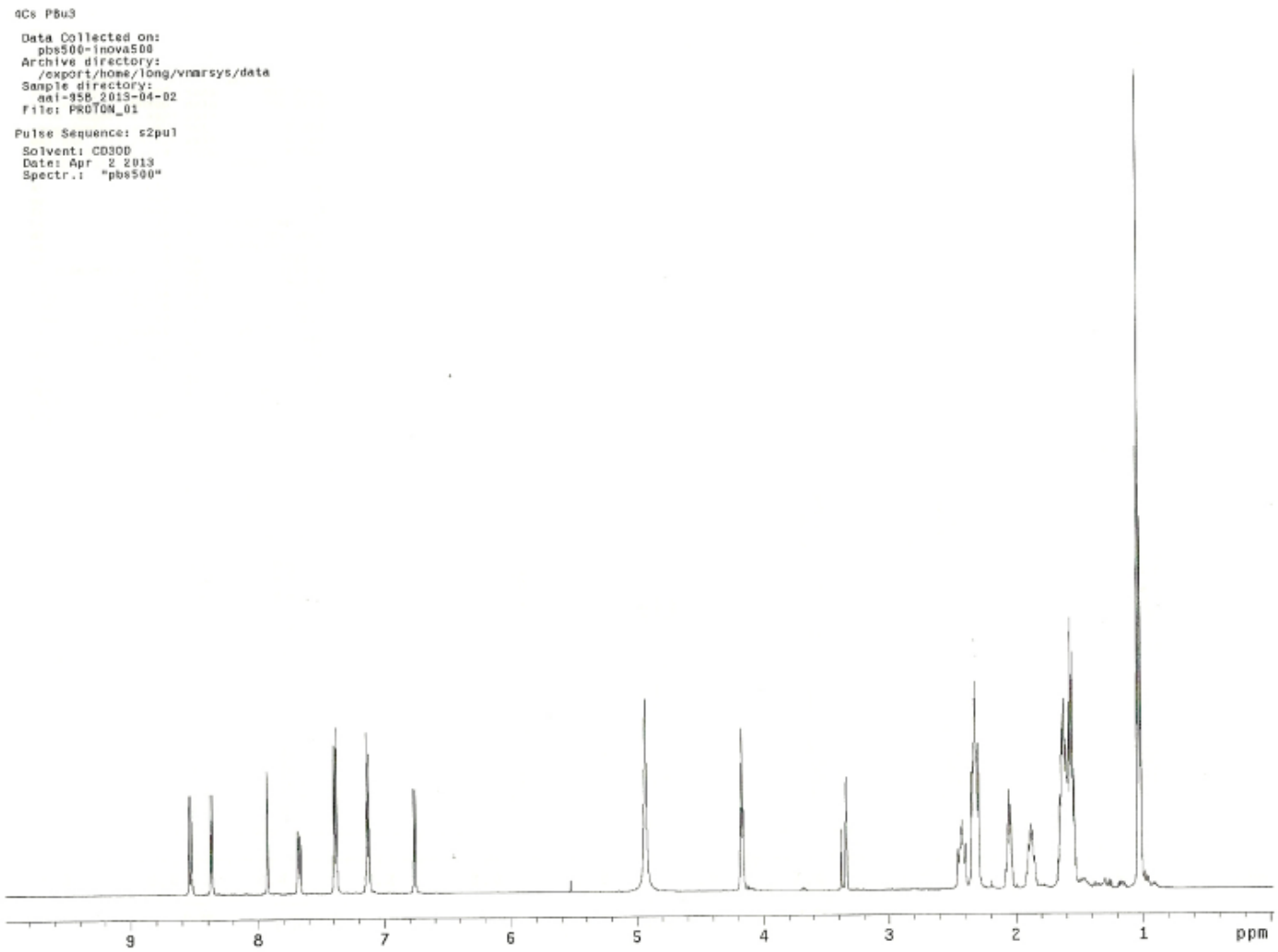
^{1}H of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl)(methyl)diphenylphosphonium bromide (16e)



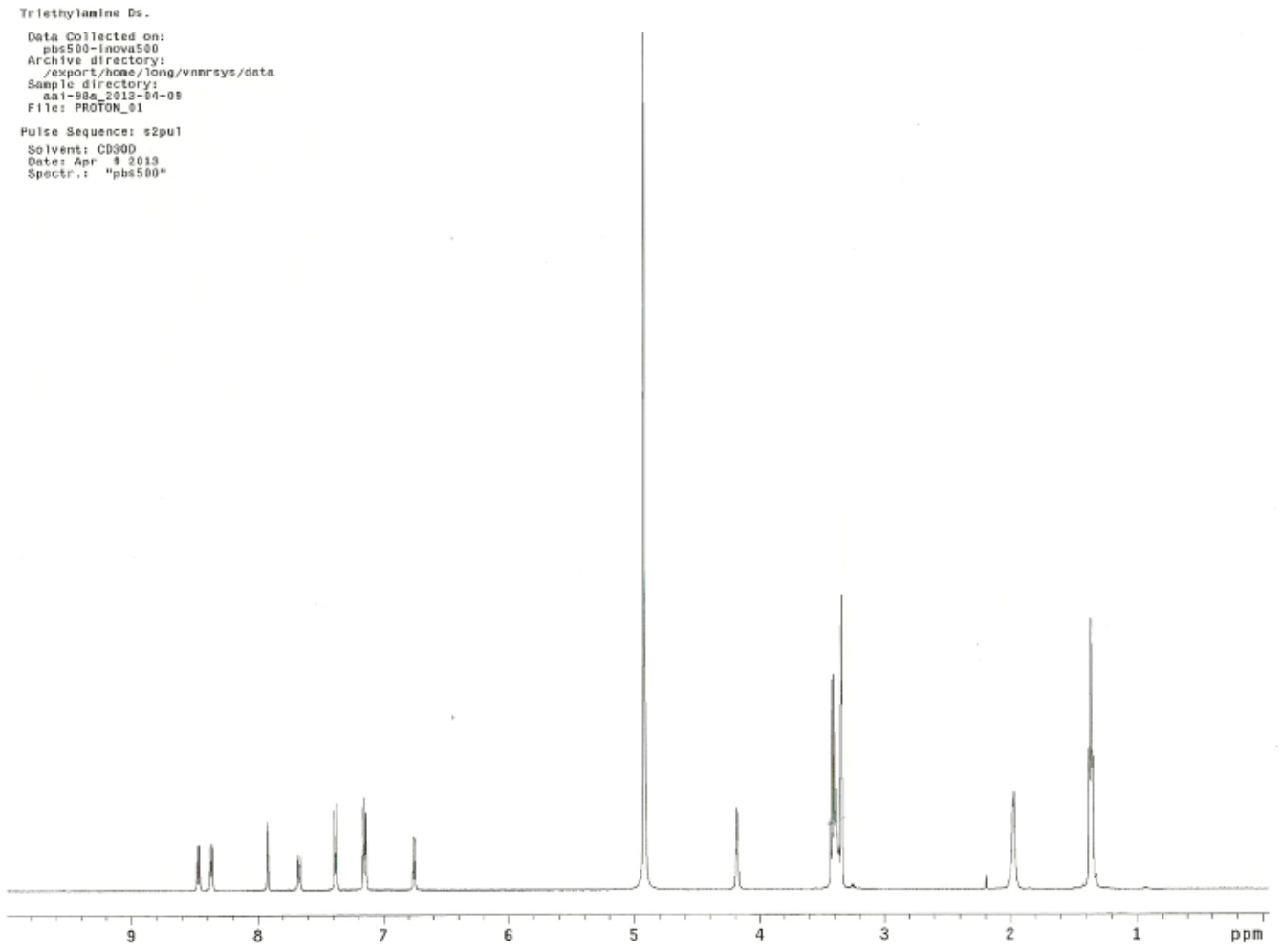
^{1}H of (4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl)dimethyl(phenyl)phosphonium bromide (16f)



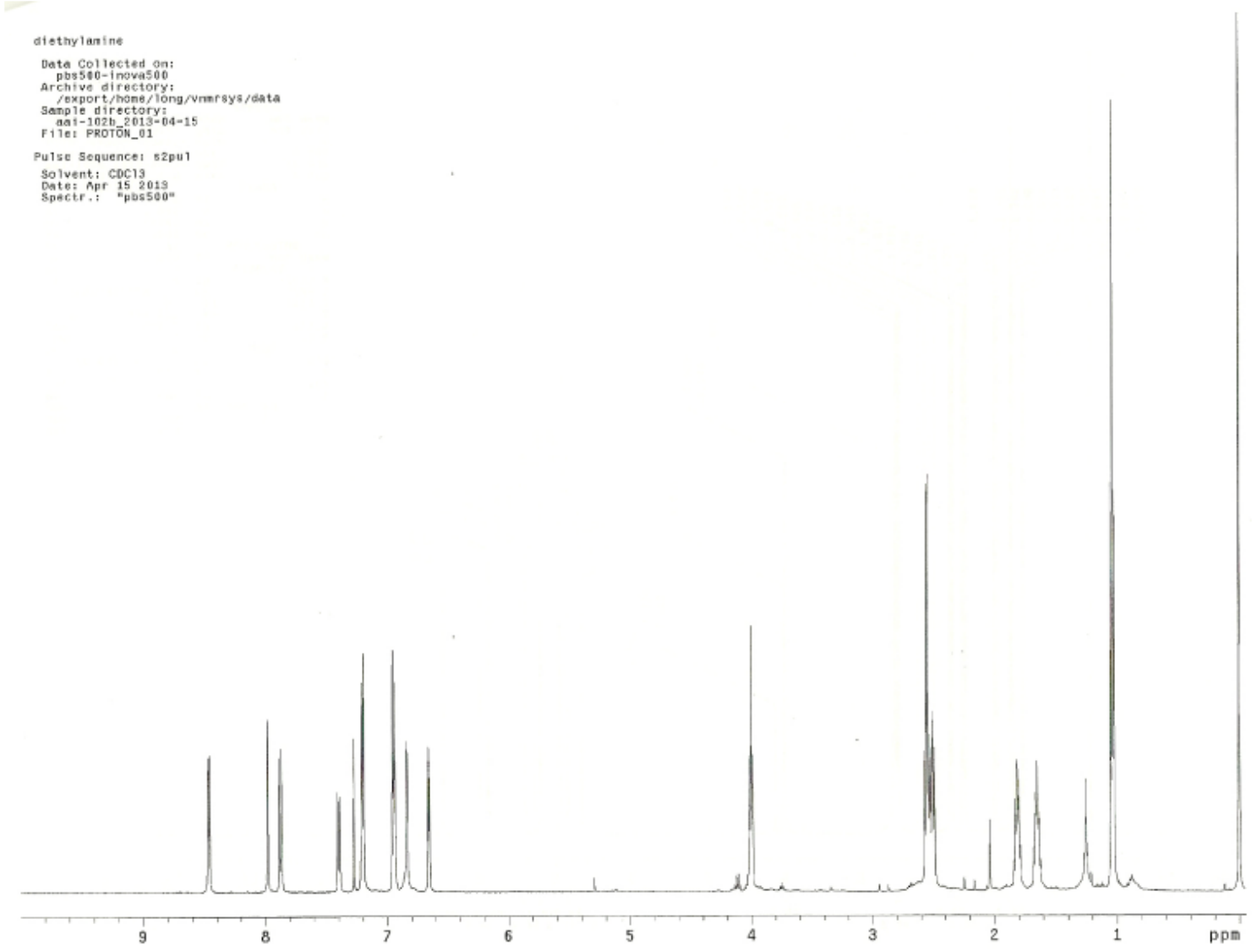
^{1}H of tributyl(4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)butyl)phosphonium bromide (16g)



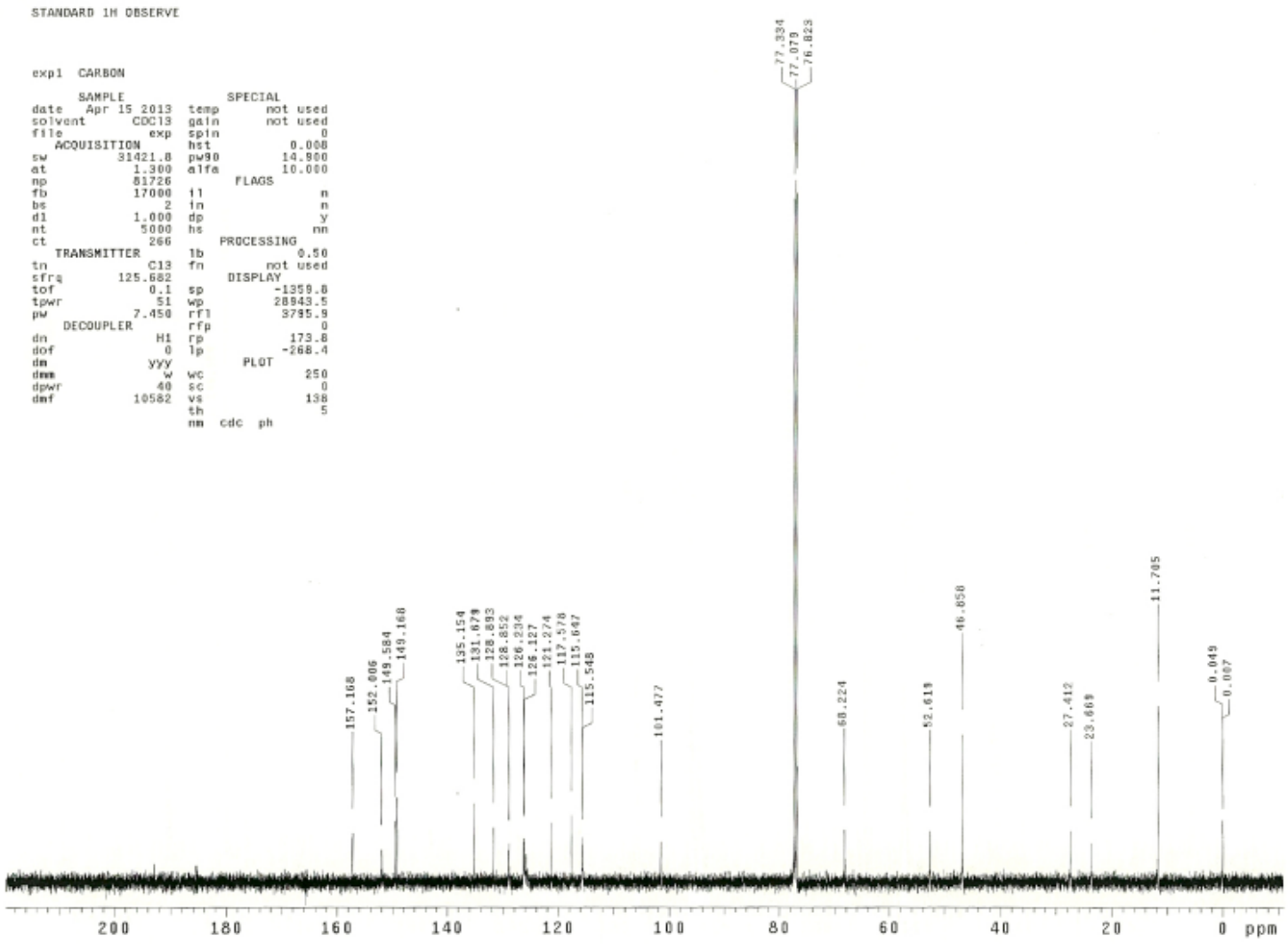
^{1}H of 4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)-*N,N,N*-triethylbutan-1-aminium bromide (16h)



^{1}H of 7-chloro-*N*-(4-(4-(diethylamino)butoxy)phenyl)quinolin-4-amine (18)



^{13}C of 7-chloro-*N*-(4-(4-(diethylamino)butoxy)phenyl)quinolin-4-amine (18)



^{1}H of *N*-(4-butoxyphenyl)-7-chloroquinolin-4-amine (19)