SYNTHESIS AND STRUCTURAL ACTIVITY RELATIONSHIP OF 2-HYDROXY-4-METHOXYBENZALDEHYDES AS MOSQUITO LARVICIDES

By

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This thesis is my original work and has not been presented for a degree in any other university.

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CHAPTER 1
INTRODUCTION

During the first part of this century, malaria was the most important tropical disease. The situation has not changed to date. The disease has been one of the most serious obstacles to agricultural development and permanent human settlements.

Globally, mosquitoes transmit disease to more than 700 million people annually and are responsible for 1 in every 17 deaths (Fradin, 1998). Malaria results from infection with a parasitic protozoan transmitted by mosquitoes, causing approximately 3 million deaths annually (WHO, 2000). Mosquitoes transmit the arboviruses responsible for yellow fever (Sanders *et al.*, 1996), dengue, hemorrhagic fever (Briceno-Garcia *et al.*, 1996) epidemic polyarthritis, and several forms of encephalitis (Meslen, 1997). Bancroftian filariasis is also caused by a nematode transmitted by anopheline mosquito (Thompson *et al.*, 1996).

1.1 The disease

Malaria is a parasitic disease, acute, sometimes severe and often chronic, characterized by shaking chill, rapidly rising temperature and a palpable spleen (Vogel, 1974). One of the long-term effects is severe anaemia. It is caused by one of the four parasites: *Plasmodium vivax, P. malariae, P. falciparum* and *P. ovale*. These cause benign tartian, quarten, malignant tertian and ovale malaria, respectively. *P. falciparum* is the most virulent with dangerous complications, such as cerebral malaria and potentially lethal to humans. In man, the parasite develops within the red blood cell or in the liver tissue. Only the female anopheline mosquitoes (Plate 1) that are sufficiently closely associated with man are important as malaria vectors. Malaria is transmitted by an infected, female mosquito (*Anopheles sp*). It can also be rarely acquired from infected blood during transfusion (Guerrero *et al.*, 1983) or even from shared needles among drug addicts (Barata *et al.*, 1993). Human malaria parasites only develop in *Anopheles* mosquitoes (Grassi and Filleti, 1900).

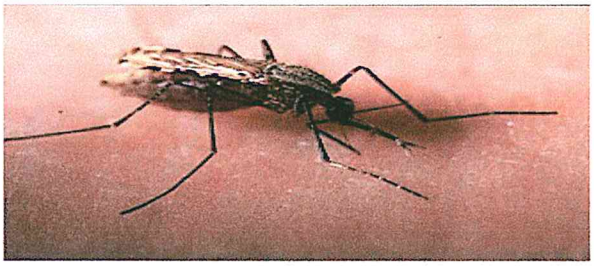


Plate 1: *Anopheles gambiae*

1.2 Occurrence

Malaria occurs predominantly, though not exclusively, in tropical and sub-tropical regions (Africa, Middle East, Asia, China and South America) where it has been directly or indirectly responsible for untold suffering and economic deprivation from the beginnings of recorded history. In non-endemic areas, travelers and immigrants bring it in (Shell, 1997). Epidemics related to political upheavals, economic difficulties, climatic and environmental changes contribute significantly to the death tolls and human suffering. Malaria is endemic to 101 countries and territories: 45 in Africa, 21 in America, 4 in Europe, 14 in eastern Mediterranean, 8 in South East Asia, and 9 in Western Pacific, (Fig. 1).

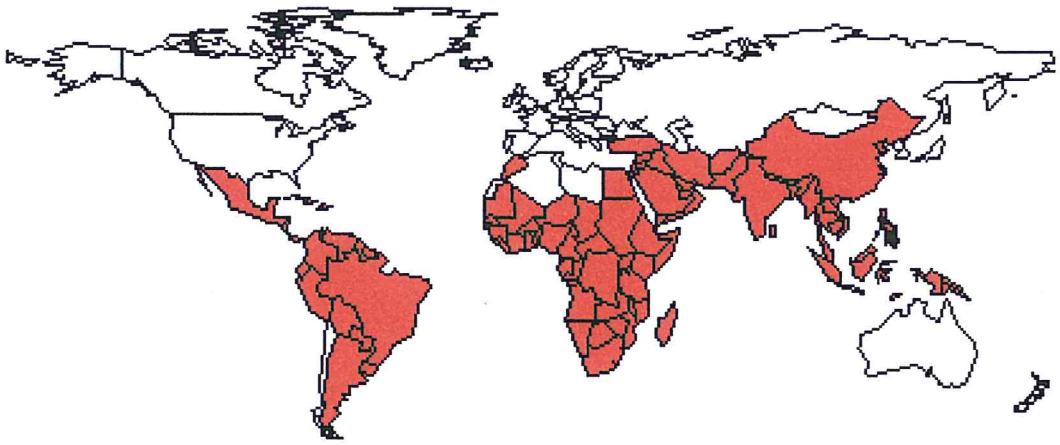


Figure 1: Global malaria distribution map (WHO, 1997)

1.3 Vectors and their ecology

Endemic malaria is found where transmission occurs throughout the year. Malaria is stable in these places. Transmission fluctuates to its maximum depending upon rainfall and output of *An. gambiae* and *An. funestus* in the hot humid high rainfall areas. The high humidity and temperatures favor continuous mosquito breeding. The high temperatures also favor continuous development of the extrinsic cycle of the *P. falciparum* in the mosquito. *An. gambiae* is the main vector but *An. funestus* and *An. arabiensis* are also important, particularly in dry periods, when breeding is restricted to permanent water bodies such as swamps, lakes, ponds among others (Vogel, 1974; Di Deco *et al.*, 1981).

1.4 Socio-economic effects

It is no exaggeration that malaria has been responsible for much of human suffering and misery accompanying the process of social and economic development. It has also "fuelled" the vicious cycle of poverty, ignorance and disease.

Malaria is responsible for 2% of the disease burden worldwide and 90% of this burden is in Africa. The severe complicated form of the disease is most commonly found in Africa, where more than 85% of malaria cases and deaths occur. Over 3000 people, mostly children under five, die as a result of the disease every day (WHO, 1999).

In Kenya approximately 20 million people are exposed to stable malaria transmission (regular parasite exposure every year), including 3.5 million children below the age of five. Of the childhood population, it is estimated that approximately 26,000 die each year from the direct consequences of malaria infection (72 children per day) (Anon, 2001). Malaria is a fact of life for most African people; it interrupts daily life by keeping adults from work and children from school.

Malaria is the bane of motherhood. It causes serious medical complications and deaths during pregnancy; blighting the development of the child before and after birth. Too often it can be the cause of her child's death.

Annual economic growth in countries with high malaria transmission has historically been lower than in countries without. Economists believe that malaria is responsible for a 'growth penalty' of up to 1.3% per year in some African countries. When compounded over the years, this penalty leads to substantial differences in GDP between countries within and without malaria endemic regions and severely restrains the economic growth of the entire region (Anon, 2001).

The human suffering afflicts almost every Kenyan household and an estimated 170 million working days are lost each year as a result of the disease. The health sector in particular is heavily burdened by the cost of drugs and treatment.

1.5 Malaria control

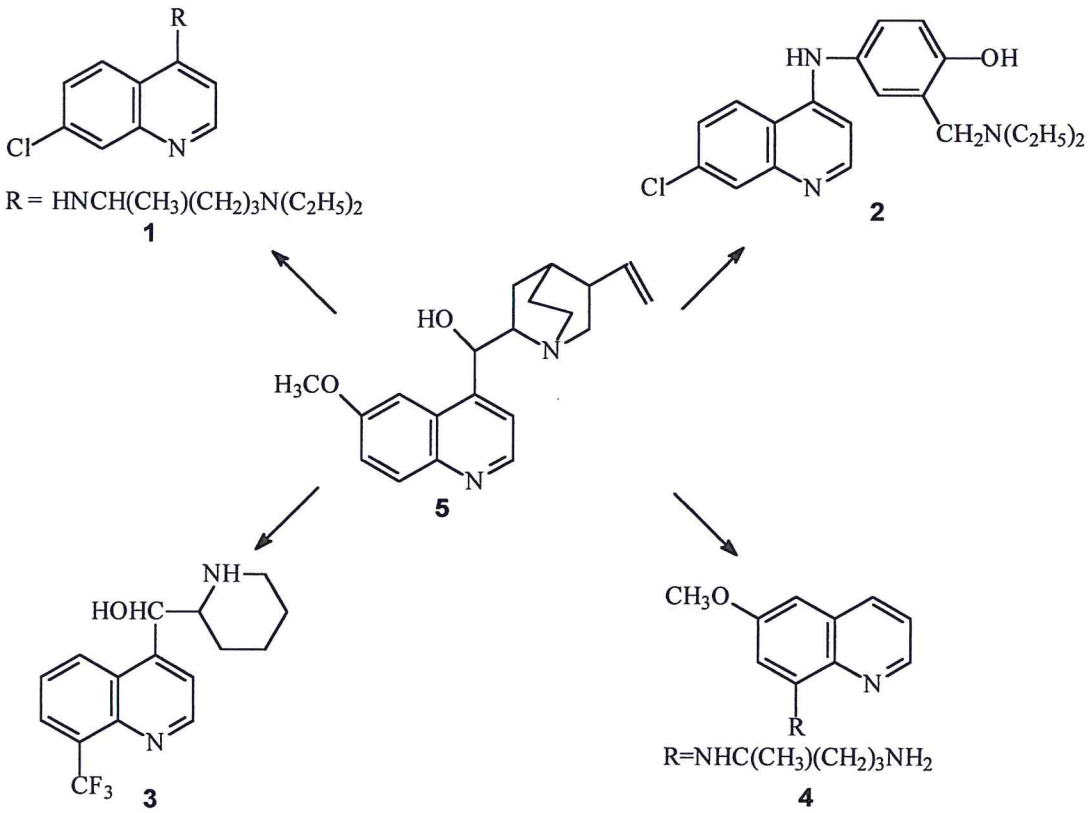
Malaria is a preventable and treatable disease. The deaths it causes can be avoided with the control tools currently available. Prevention of malaria encompasses a variety of measures that may protect against infection or the development of the disease in infected individuals. Measures that protect against infection are directed against the mosquito vector. These can be personal (individual or household) protection measures such as protective clothing, repellents, bed nets, or community/population protection measures like, use of insecticides or environmental management to control transmission. Measures, which protect against disease but not against infection, include chemotherapy, chemoprophylaxis and vaccination (Phillips, 2001).

1.5.1 Chemotherapy/chemoprophylaxis

Chloroquine (CQ) (**1**) and other quinine analogs have been the most widely used drugs in most of the endemic regions. Since its development in the early 1940s, CQ became the drug of choice for malaria management. It is relatively cheap and therefore affordable in the poor countries, which are the worst affected. Other quinoline based synthetic antimalarials include amodiaquine (AQ) (**2**), mefloquine (**3**) and primaquine (**4**) among others.

However, the spread of chloroquine resistance has been reported in most areas in Africa, South America, and South East Asia (Watkins *et al.*, 1988a; Garcia *et al.*, 1989; Lepers *et*

al., 1989; Wellens, 1991; Harinasuta *et al.*, 1983). Although chloroquine resistant *P. falciparum* (CRPF) has spread through almost all endemic areas, CQ remains the most widely used treatment for uncomplicated, *P. falciparum* malaria. Varying degree of CQ resistance has been reported in Kenya (Masaba and Spencer, 1982; Watkins *et al.*, 1984; 1988a,b). Overwhelming evidence indicates that AQ is significantly more effective than CQ in Africa, and should presumably replace it as one of the effective anti-malarial drugs (Muller *et al.*, 1996; Van Dillen *et al.*, 1999). Although at first CRPF strains retained some sensitivity to amodiaquine resistance to this drug soon followed chloroquine (Draper *et al.*, 1988).

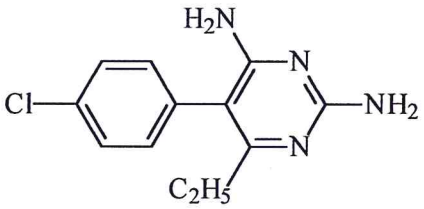


Scheme 1: Quinine derivatives

Mefloquine (**3**) is also widely used for the treatment of acute malaria where multi-drug resistant *falciparum* occurs (Kofi, 1983). However, resistance in non-immune individuals has been reported in Thailand (Mockenhaupt, 1995)

Quinine (**5**), the natural template from which the others were developed (Scheme 1), is used as the last resort (where resistance to the synthetics is rampant) due to the negative side effects. Quinine has remained the main therapeutic drug for CRPF malaria although low sensitivity was reported in Brazil as early as 1910. Success rates with quinine have now fallen to below 50% in some areas (Giboda and Denis, 1988).

The readily available antifoliates include; pyremithamine (**6**), proguanil (**7**) and mepacrine among others.



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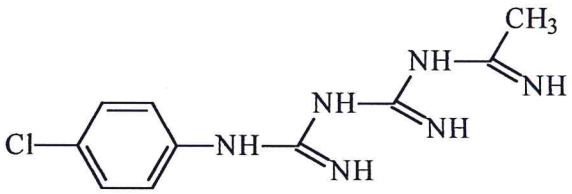

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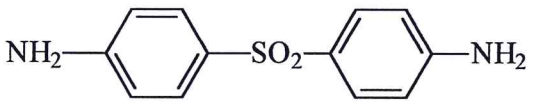
Resistance of *P. falciparum* to pyremithamine develops rapidly. It was first described in 1953 and is now widespread (Onori, 1984). Resistance of the asexual *P. falciparum* to proguanil was also detected early (Edeson, 1950) and lack of prophylactic efficacy was confirmed in Thailand (Pang *et al.*, 1989).

Sulphur based antimalarials like dapsone (**8**), sulphadiazine (**9**) and sulfadoxine (**10**) have also been used.



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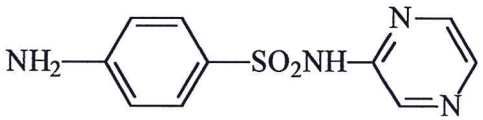

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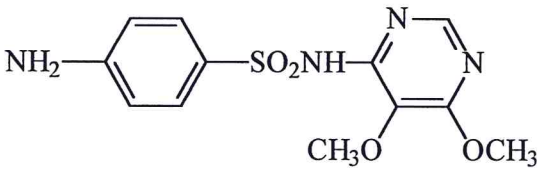

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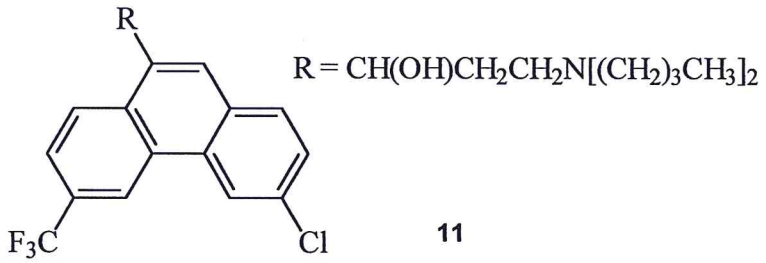
Halofantrine® (**11**), a phenanthrine-methanol compound with activity against the erythrocytic stages of the malaria parasite has been used in areas with multiple-drug resistant *falciparum* (Nosten *et al.*, 1993). However, the drug can produce potentially fatal cardiac conduction abnormalities, limiting its use (Yetman *et al.*, 1998).



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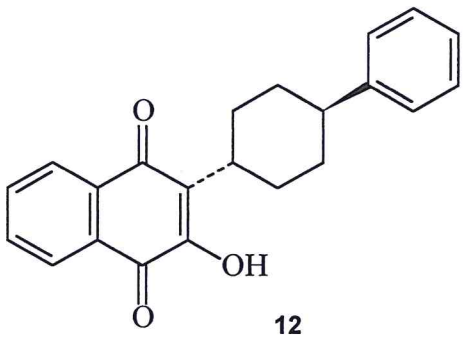
Atovaquone (**12**), a hydroxynaphthoquinone is currently being used mainly for the treatment of opportunistic infections in immuno-suppresed patients. It is effective against CRPF, but resistance develops rapidly when used alone (Looareesuwan *et al.*, 1996). Atovaquone is therefore usually given in combination with proguanil, (Looareesuwan *et al.*, 1996; Radlof *et al.*, 1996).



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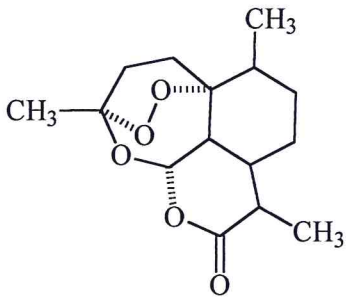
Another class of newer anti-malarials consists of the sesquiterpene lactones like artemisinin (**13**) and derivatives such as artemether, artesunate and dihydroartemisinin (Bloland, 2001).



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Artemisinin derivatives presently show no cross-resistance with known anti-malarials and as such are important for treating severe malaria in areas of multi-drug resistance (Price *et al.*, 1996). However, they require long treatment courses and when used alone, recrudescence may occur (Looareesuwan *et al.*, 1992). Resistance to artemisinin has been demonstrated *in vitro* (WHO, 1987). The drug must therefore be used sparingly to avoid rapid resistance development.

Resistance development to almost all anti-malarial drugs has necessitated research into combination therapy. It involves the combination of at least 2 anti-malarials with different modes of action. The principle is the hope of potentiation of the drugs leading to reversal or delaying of resistance (White, 1999). Although sulfadoxine-pyremithamine (SP) is still reliable in most areas in Africa, resistance is increasing and could potentially develop to render the drug useless in the near future, as in Asia (Trigg *et al.*, 1997). Numerous sentinel cases of resistance to sulfadoxine and SP been reported in non-immune persons visiting Africa during the 1980s (Schapira *et al.*, 1988).

In a recent study done in Mumbai (India), the combination of CQ and SP was found to be safe and had superior efficacy compared to CQ alone (Gogtay *et al.*, 2000). One, generally affordable, drug combination for the treatment of malarial infections resistant to CQ or SP is CQ plus chloropheniramine (Sowunmi and Oduola, 1997).

Malarone™, a combination of atovaquone and proguanil has been investigated and found to be effective in treating drug-resistant *P. falciparum* in the field (Radloff *et al.*, 1996; Lell *et al.*, 1998).

A strategy that has received much attention recently is the combination of antimalarial drugs, such as mefloquine, SP or amodiaquine, with an artemisinin derivative (White, 1999; Sowunmi *et al.*, 2000).

Patients with recrudescent *falciparum* malaria can well be treated with a sequential combination of artesunate and mefloquine (Looareesuwan *et al.*, 1992).

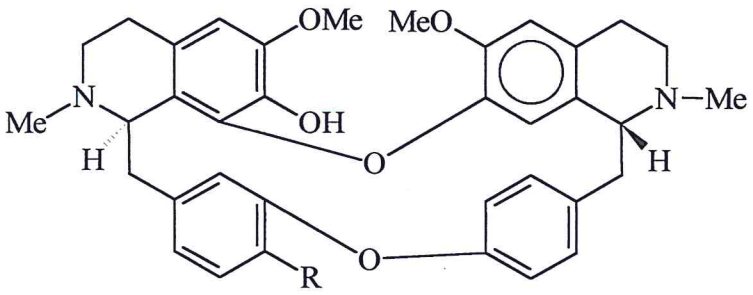
As resistance is developed against anti-malarial drug combinations, a search for alternative agents with novel modes of action different from the commonly used quinoline-based anti-malarials is ongoing. This has resulted in the screening of some anti-biotics like erythromycin, chloromphenical, tetracycline and clindamycin. They have reportedly been studied for their efficacy against *P. falciparum in vitro* and *P. berghei in vivo* with good prospects as combination drugs (Kremsner, 1989; Gingrass and Jensen, 1993).

Beside drug resistance, chemotherapy is limited by lack of effective case management and diagnosis. This is made worse by the existence of unofficial health care systems like traditional herbal healers, unlicenced medicine vendors and spiritual healers which run parallel to the official system (Salako, 1998).

The reduced efficacy of conventional anti-malarial drugs is a matter of great concern. Currently, there are no drugs that can offer protection against malaria in all regions of the world, and the need for novel chemotherapeutic agents is therefore acute. Plants are a potential source of new anti-plasmodial compounds and are therefore the focus of much current research (Jonathan *et al.*, 1999).

Recently, an effective anti-malarial lead was found in *Artemisia annua* (Compositeae) (Klayman, 1985). In a continued search for natural products with anti-malarial activities

several anti-plasmodial naphthoquinones from *Nepenthes therolii* were identified (Kittisac, 1998). The most active of the isolated compounds was plumbagin with an IC_{50} of 0.27 µM. *Abuta grandifolia* a plant used in South America for the treatment of malaria contains three bisbenzylisoquinoline alkaloids, which show potent anti-plasmodial activity (Milliken, 1997). Studies with krukovine (**14**), the most potent of the three isolated compounds, suggest that it may succumb to CQ-type resistance in *P. falciparum* whereas limacine (**15**) has been reported to reverse CQ resistance (Jonathan *et al.*, 1999).



14 R=OH
15 R=OMe

These compounds may therefore provide effective treatment in combination. Nitidine (**16**) from *Toddalia asiatica* (Gakunju *et al.*, 1995) and cryptolepine (**17**) from *Cryptolepis sanguinolenta* (Kanyanga *et al.*, 1997) are other examples of compounds with high anti-plasmodial activity.

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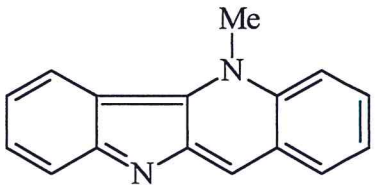

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It is probable that other plants contain as yet undiscovered anti-malarial substances. Much research has focused on trying to isolate and purify the anti-malarial compounds. However, there is little research in the clinical effectiveness of herbal remedies as they are used in real life. National malaria control programmes have largely ignored the

potential of traditional healers, even though they are more accessible and culturally accepted than conventional health care systems (TDR, 1999). The WHO and the Kenya Government recently recognized the role played by the traditional healers and are currently advocating for the inclusion of herbal remedies into the national health system. Due to the many problems encountered in chemotherapy, prevention still offers the best opportunity for malaria control.

1.5.2 Prevention

1.5.2.1 Vaccine Development

It has long been noted that natural exposure to malaria leads to the development of partial immunity in humans, but repeated re-infection is required to maintain this immunity (Bojang *et al.*, 2001). As a result a great deal of research has been directed towards the development of vaccines to protect against infection by *P. falciparum*. The complexity of the life cycle of *P. falciparum* is being exploited in attempts to develop malaria vaccines because it provides several potential targets for immune response (Paul and Allan, 1988). Candidate vaccines are based on various antigens derived from different stages of the malaria parasite life cycle.

Pre-erythrocytic vaccines prevent the malaria parasite sporozoites stage from entering or developing within liver cell. The asexual blood stage vaccines prevent the parasite merozoite stage from entering or developing within red blood cells, and transmission blocking vaccines inhibit the development of the sexual stages of the parasite within the mosquito (Kwiatkowski and Marsh, 1997).

A pre-erythrocytic vaccine, RTS,S" has been tried in the Gambia showing 47% protective efficacy (Bojang *et al.*, 2001). SPf-66, pre-erythrocytic and asexual blood stage proteins of *P. falciparum*, is designed to block the parasite at its later merozoite form, when it emerges from initial incubation in liver. In Tanzania, the efficacy was 31% in children (1-5 yrs) (Alonso *et al.*, 1994), while protective efficacy in Gambia was 8% in infants (6-11 months) (D' Alessandro et al., 1995) and 9% in Thailand for children aged between 2-15 yrs (Nosten *et al.*, 1996).

The development of a suitable vaccine however, has been hindered by lack of suitable source of parasites from which it can be prepared. The main problem being low immunogenecity of malaria parasites (Kwiatkowski and Marsh, 1997). Vaccines would be a useful addition to chemotherapy and mosquito control in combating malaria. However, there is no guarantee that the current approaches to malaria vaccine development will result in a cost-effective vaccine. Unlike less complex organisms, parasites have developed ingenious ways of avoiding the hosts' immune response. For instance, the malaria parasite expresses different antigens at each stage of its life cycle, and is often able to change these antigens when the host mounts an immune response towards them (Phillips, 2001). The difficulty to grow malaria parasites in large enough quantities too has also limited the development of vaccines (Engers and Godal, 1998).

Drugs and vaccines might solve many problems in the industrialized world. In less developed countries, only efficient mosquito control can hold the promise for the future (Karl, 1985).

1.5.2.2. Vector control

Between the 1940s and 1960s malaria eradication was achieved in the USA, USSR, southern Europe and most Caribbean islands mainly by vector control (Bradley, 1996; Johnson, 1966). Much progress was also made in the Indian sub-continent and parts of South America. Now in the 21st century emphasis needs to be placed on vector control once again. Historically, the strategies for reducing the incidence of malaria have been two-pronged; habitat management through environmental, chemical and biological means; and the use of personal protection in the form of insect repellents.

1.5.2.2.1. Adult Control

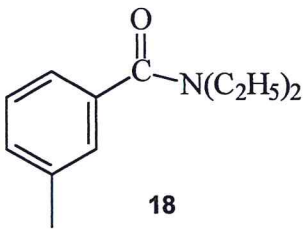
1.5.2.2.1.1 Personal protection

Personal protective measures are often mentioned as the last line of defense. These are measures that protect the vertebrate host from vector bite and therefore preventing pathogen transmission. Since the late 1970s, research in the development of personal protective measures against mosquitoes and other biting arthropods has been prevalent leading to the development of insect repellents (Skinner and Johnson, 1980). The quest

to make humans less attractive to mosquitoes has fueled decades of scientific research on mosquito behavior and control (Fradin, 1998).

Thousands of plants have been tested as potential sources of insect repellents. Plants whose essential oils have been reported to have repellent activity include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and pepper mint. Unlike synthetic insect repellents, plant-derived repellents have been relatively poorly studied. When tested, most of these essential oils tended to give transient protection, usually < 2 hours (Sukumar *et al.*, 1991; Brown and Herbert, 1997; King, 1954; Quarles, 1996).

N,N-diethyl-*m*-toluamide, (DEET), (N,N-diethyl-3-methylbenzamide) (**18**) remains the gold standard of currently available insect repellents (Fradin and Day, 2002).



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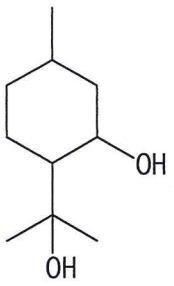

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Thus, the search for an ideal topical insect repellent continues. The ideal agent would repel multiple species of biting arthropods, remain effective for at least 8 hours, cause no irritation to the skin or mucous membranes, cause no systemic or dermal toxicity, be resistant to abrasion and rub-off, and be greaseless and odorless. No available insect repellent meets all of these criteria (Khan *et al.*, 1969; Strauss *et al.*, 1968). Efforts to find such a compound have been hampered by the numerous variables that affect the inherent repellency of any chemical (Wright, 1975; Rutledge *et al.*, 1983). Studies have indicated that components in *Eucalyptus maculata citriodon* oil especially *p*-menthane-3, 8-diols (**19**), (Schreck and Leonhardt, 1991) and eucamol (Satoh, 1995) are as effective as DEET (Watanabe and Shono, 1993; Trigg, 1996). Medical review indicates that eucalyptus oils may cause seizures, one of the toxicological problems caused by DEET

(Burkhard *et al.*, 1999). Eucalyptus oils have also caused skin irritation and sensitization (Schaller and Korting, 1995).

Bed nets and window screens have been used for decades to protect against mosquitoes. Although they are useful barriers, bed nets and screens are often poorly fitted, easily torn, and if human contact is made with the net the mosquitoes can still bite (Roberts and Andres, 1994). This prompted the use of insecticide treated nets to control or prevent human vector contact (WHO, 1989). This became feasible with development of synthetic, photostable pyrethroids (Roberts and Andres, 1994).

Field studies with permethrin-impregnated clothing have been shown to be effective against mosquitoes, ticks and other biting flies (Lindsay and McAndless, 1978, Schreck *et al.*, 1978). Permethrin-impregnated mosquito nets have been shown to provide better protection than untreated nets against mosquitoes, (Darriet *et al.*, 1984). Unfortunately, studies have demonstrated problems with the use of impregnated bed nets. They are ineffective against mosquitoes biting outdoors, restrict ventilation, not easily accepted by prospective users and rendered ineffective by washing. Lack of compliance and improper use of nets may reduce their impact in malaria control. Widespread use of insecticide treated nets increases the selection for resistance (Vulule *et al.*, 1996).

1.5.2.2.1.2. Genetically modified mosquitoes

There is considerable interest among biologists, including biochemists, in the idea of rendering mosquito populations genetically harmless by introduction of genes, which make them non-susceptible to *Plasmodium* infection (Collins and Paskewitz, 1994) or change them from being strongly attracted to humans. The real problem with these concepts is not so much in producing harmless strains, but propagating the genes extensively. Thus, if the desirable genes are to be spread in anopheline populations, genetic systems that will reliably cause genes to spread from a small "seeding" of a wild population to the rest will have to be developed (Jinitsu *et al.*, 2002). Although this approach is appealing, implementation may present insurmountable difficulties. Besides, their impact on the ecosystem is currently unknown. It therefore remains a theoretical tool and a long shot.

1.5.2.2.2. Larval control

Larval control also contributes to malaria vector management programs, especially where breeding sites are limited in extent and are clearly defined (wells, permanent pools and water tanks). Various methods have been applied towards the control of mosquito larvae and they include environmental management, biological control, and the use of synthetic and plant derived chemicals.

1.5.2.2.2.1. Biological control

Bacillus thuringiensis var. *israelensis* (B.t.i.) (serotype H-14) is a safe, effective biological control agent (Davidson and Sweeney, 1983; Legner and Sjogren, 1984). Pest control using highly specific toxins produced by *B.t.* or *B. sphaericus* is currently favored because of low environmental impact and the high success rates. One of the major drawbacks is that the efficiency is affected by environmental conditions (Garcia and Des Rochers, 1979). This coupled with rapid biodegradation could result in *B.t.i.* producing less than 100% mosquito mortality in many habitats (Garcia *et al.*, 1980). However, *B.t.i.* resistance by *Pletulla xylostella* L., *Culex pipiens* L., and *Cx. quinquefaciatus* has been documented (Rao *et al.*, 1995). Fungi, such as *Leptolegnia chapmani* (Zattau and McInnis, 1987) and *Toplocladium cylindrosporum* (Ravallec *et al.*, 1989), have shown some potential for mosquito control.

Invertebrate predators of mosquito larvae also exist (Hinman, 1934; Jenkins, 1964). Under natural conditions in rice fields, invertebrate predators (Coleptera, Dystiscidae, Hydrophylidae, Hemiptera, Belostomidae and Notonectidae among others) can be responsible for drastic reductions in larval populations. The mermethid or mosquito-attacking nematode, *Ronanomesis culcivorax* has also been used against mosquito larvae (Nickle, 1972; 1979). Due to the use of vertebrate predators and agrochemicals coupled with natural population fluctuations, invertebrate predators can be eliminated or severely reduced (Washino, 1970).

Vertebrate predators like larvivorous fish have been used as a biological tool for mosquito control for nearly 100 years (Meisch, 1985). *Gambusia affinis* has been successfully used in controlling populations of *Cx. tarsalis* and *An. freeborni* in California rice fields

(Craven and Steelman, 1968; Hoy and Reed, 1970; Cech and Moyle, 1983), and is by far the most commonly used fish for mosquito control. However, it is expensive to rear and transport (Hoy, 1985) and is a menace to many native fishes when introduced into other ecosystems (Menon, 1977). The guppy, *Poecilia reticulata* Peters, and the Argentine pearl fish, *Cynolebias bellottii* Steindachner are also useful for mosquito control (Bay, 1966; 1967). Fishes may not survive in temporary breeding habitats thus limiting their use in mosquito control programs.

For a number of reasons biological control proves to be more difficult to achieve than control with insecticides or environmental management. They are also slow and may not be used in emergency situations like epidemics. This requires supplementary methods in mosquito control.

1.5.2.2.2.2. Environmental management

With the problems inherent in the over use or misuse of insecticides, many control programs are going back to basics, using environmental management (EM) measures, to reduce vector population by eliminating potential vector habitats or targeting the immature (early) stages. The tactical options fall into 3 broad categories: Environmental modification; environmental manipulation; and modification/manipulation of human habitation or behavior.

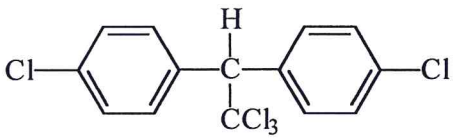
Environmental modification includes permanent physical transformations such as drainage, land filling and grading. Environmental manipulation includes recurrent measures that produce temporary conditions that are unfavorable to larval production, including regulation of water level, removal of vegetation, stream flushing, among others. The third category involves activities that are designed to reduce contact with vectors and therefore pathogen transmission. These include location of human settlements away from vector sources, mosquito proofing of houses, use of bed nets, zooprophylaxis and other personal protection measures (Lawrence and Cynthia, 1990). Some of these procedures were effective in the past but were de-emphasized or discarded due to their high cost and labour-intensive nature.

1.5.2.2.2.3. Chemical larvicides

Insecticides are at the moment the main weapon against insect pests. Before the 2nd world war (WW II), the chemicals used for destroying pests were largely inorganic compounds of lead and arsenic, which are well known poisons to other non-target organisms including man (Metcalf *et al.*, 1962).

The era of synthetic and cheap chemicals began during WW II with the discovery of DDT (**20**) (Forest *et al.*, 1946). The spectacular success achieved in suppressing arthropod-borne disease among allied troops during WW II convinced many biologists that pest control had moved into a new era. DDT has continued to be recommended for indoor spraying long after it was banned for agricultural use in the USA and many other countries (Curtis, 1994). It has been recommended because of the low cost and durability. Other compounds that have been used include organophosphates and carbamates (Hutson and Roberts, 1985).

Most adulticides have also been used as effective larvicides. The choice of such larvicides for mosquito control has been based on the species and habitat of the mosquito, hazards to humans, animals, aquatic organisms and other wildlife, presence of insecticide resistant mosquito strains and cost factors. The earliest used synthetic organic larvicides were organochlorines (DDT (**20**), methoxychlor (**21**), and benzene hexachloride (**22**). For instance, DDT was used in the San Joaquin valley in 1945 with toxaphene being exclusively used in the USA in 1949. In special situations benzene hexachloride and lindane were used (Gjullin and Richards, 1952). However, organochlorines are toxic to fish, other aquatic animals and birds. They also accumulate in the food chain, are persistent in the environment and harmful to the ozone layer.



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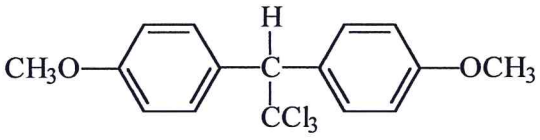

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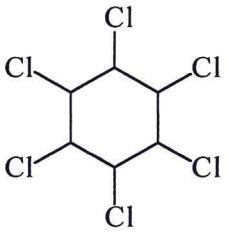

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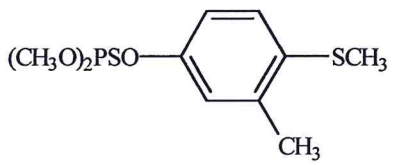
Due to development of resistance in mosquitoes and gnats the organochlorine insecticides were gradually dropped from use in vector control programs (Mulla, 1963) and replaced by organophosphate (OP) materials (malathion, parathion (**23**), fenthion (**24**), and temephos (**25**) among others). On the other hand, OPs are also toxic to mammals.



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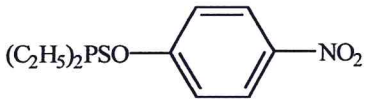

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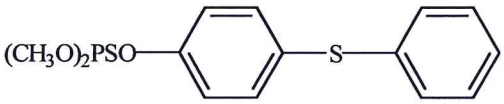

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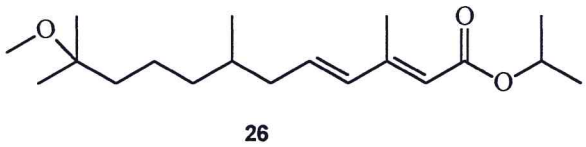
Carbamates have also been widely used against mosquito larvae (Kirk and Othmer, 1981; Metcalf *et al.*, 1962). However, carbamates are also highly toxic to birds and other economically important insects such as bees and can poison mammals as well.

Synthetic pyrethroids have also been used for larval control. Decamethrin and its chloro-analogue FMC-45497 have been found to be 50-100 times more effective than OPs against the resistant and susceptible strains of *Aedes nigromaculis* (Ludlow) larvae (Mulla *et al.*, 1980).

IGRs have become an important tool for the control of mosquitoes. The juvenoid methoprene (**26**) has been employed in mosquito control since 1975, and the chitin synthesis inhibitor, diflobenzuron (**27**) was registered for mosquito control in non-crop ecosystems in 1985 (Jose and Mulla, 1986). Resistance to juvenoids has been found in *Cx. quinquefasciatus* in USA and Tanzania. Resistance to diflubenzuron has also been detected in *Cx. quinquefasciatus* in Tanzania (Brown, 1986).



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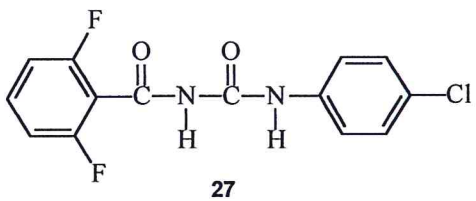

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The development of resistance by mosquitoes to the synthetic compounds used as larvicides and adulticides was first observed in 1947, when the salt-marsh mosquitoes *Ae. taeniorhynchus* and *Ae. sollicitans* began to show resistance to DDT (Kennedy, 1947). Since then, resistance to organochlorines (DDT and/ or dieldrin) has been reported in 109 mosquito species globally; 58 species have developed resistance to organophosphorus insecticides, of which 4 had not been recorded as organochlorine resistant (Brown, 1986). In addition, 17 species have now shown adult resistance to carbamates (propuxur or bendiocarb), and have also shown either resistance or cross-resistance to certain synthetic pyrethroids (Chandre *et al.*, 1999; Hodjati and Curtis, 1999; Hargreaves *et al.*, 2000). Multiple resistance to all 4 of the insecticide classes in the same population of a mosquito species has been observed in *Ae. aegypti, Cx. pipiens, Cx. quinquefacsiatus, An. albimamus, An. culicifacies, An. pseudopunctipennis, An. sacharovi* and *An. stephensi* (Brown, 1986).

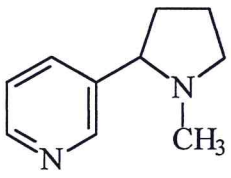
The OPs constitute almost all of the present-day larvicides, and their use is increasing as residual adulticides also. In the worldwide campaign against malaria, OP-resistance is now known in 31 species of *Anopheles* (Brown, 1986). Resistance to malathion was first detected in *An. albimanus* in Elsalvador and western Nicaragua (Georghiou, 1972; Georghiou *et al.*, 1987), 7 years after it was introduced in 1965 because of the ineffectiveness of organochlorine residual sprays. Among the 31 species records of organophosphate resistance in anophelines, 26 have involved malathion, 20 fentropthion, 10 fenthion, 6 chlorpyrifos and 5 temephos (Brown, 1986; Mekuria *et al.*, 1994). The carbamates have not proved effective as larvicides, but have been employed as residual adulticides and in adulticidal mists. Carbamate resistance has been found in *Cx. Pipiens* (Georghiou *et al.*, 1966). The most severe resistance-related problems have developed in agricultural areas where large volumes of OP insecticides such as parathon, fenthion, and certain carbamates such as carbarul and propoxur, are applied to crops (Georghiou, 1977).

Synthetic pyrethroid resistance is emerging despite early optimism that its rapid toxicological action would not produce resistance (Malcolm, 1988). In Guatemala, pyrethroid resistance was first reported in an *An. albimanus* population resistant to fenitrothion. When deltamethrin was used, the esterase conferring fenitrothion resistance was enhanced by selective pressure to produce deltamethrin cross-resistance (Brogdon and Barber, 1990). A far more threatening development in pyrethroid resistance is the appearance of target-site resistance (knockdown resistance) to pyrethroids in several important vectors in multiple locations. The knockdown resistance mechanism has been detected in the dengue and yellow fever vector *Ae. aegypti* from Puerto Rico and Indonesia and in the encephalitis vector *Cx. quinquefasciatus* from Louisiana . French researchers have also detected the resistance mechanism in *An. gambiae*, the primary African malaria vector in several countries of West Africa (Elissa *et al.*, 1993). The resistance mechanism may be a legacy of similarities in the site of action of pyrethroids and DDT.

The botanical insecticide pyrethrin is derived from the flowers *Chrysanthemum*. The insecticidal properties of the plant was first recognized around 1800 and originally used

as powders or dusts from the dried flowers (Casida, 1980). The cost of extraction, volatility and the quick bio-degradability has limited their widespread use. Interestingly, no resistance has been reported for the natural pyrethrins. Knowledge of the insecticidal principles pyrethrins I and II, jasmolins I and II and cinerins I and II led to the development of synthetic pyrethroids (Kirk and Orthmer, 1981). Unlike natural pyrethrins, resistance to synthetic pyrethroids is now widespread.

The insecticidal properties of nicotine were recognized as early as the mid-1700s. It is an alkaloid derived from certain members of the plant family Solanaceae. Commercially, nicotine (**28**) and the closely related nornicotine are obtained from the leaves of *Nicotiana tobaccum* and *N. rustica*. Anabasine (**29**) occurs in *Anabasis aphylla*, (Chenopodiaceae) and is used commercially as an insecticide in Russia (Campbell and Sullivan, 1933).



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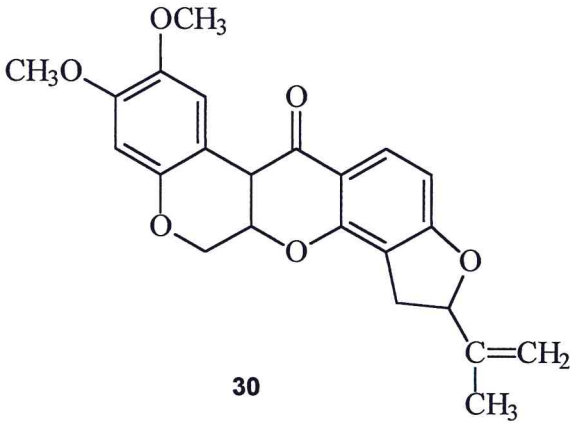
The toxicity of nicotine and related alkaloids to non-target organisms including man limited its widespread use as an insecticide.

Another group of plant-derived insecticides comprise of rotenoids, derived from roots of certain genera in the family Leguminoseae. There are 6 active principles in the genus *Derris* with rotenone (**30**) being the most active. They are also found in *Tephrosia* and



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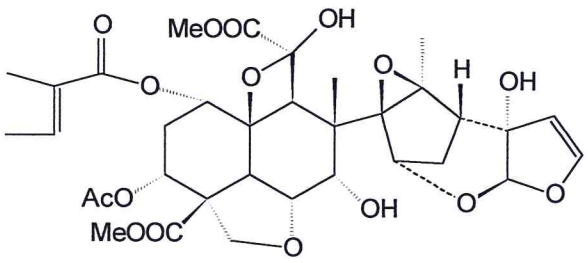
Lonchycarpus (Manuel and James, 1985). Rotenoids are also effective fish toxins and have been used by native fisherman and to rid undesirable species from lakes and streams (Kirk and Orthmer, 1981; Metcalf *et al.*, 1962).

The toxicity of rotenoids to non-target aquatic organisms and their skin irritation in man has limited their use as insecticides. It is also destroyed by light and hence of limited persistence in the field (Kenneth, 1982)

Azadirachtin (**31**) is believed to be the insecticidal agent in the neem tree (*Azadirachta indica*) that grows widely in Asia and Africa (Zebitz, 1984).



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Long recognized for its insecticidal properties in India, it has recently received a great deal of attention around the world as a potentially useful and safe "bio-pesticide" (Stone, 1992). Neem products are currently used for vegetable and flower pest control in USA (Joris and Gerda, 1997).

Plants have provided useful lead compounds in insecticide development. A good example can be found in the development of synthetic pyrethroids through SAR studies of the natural pyrethrins. These helped in the stabilization of pyrethroids, enhanced potency and reduced cost thus ensuring wide affordability and accessibility.

CHAPTER 2

LITERATURE REVIEW

2.1 Structureactivity relationship

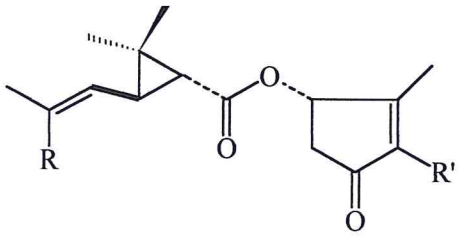
Recently, successful applications of quantitative structure activity relationship (QSAR) analysis to the molecular design have been accumulated in the field of pesticide synthesis (Jolles and Wooldridge, 1984). In most of these applications, the designed compounds are of the same type of structure as those included in the primary set of the correlation analysis.

The conventional strategy for pesticide and drug synthesis has been based largely on identification of an active lead followed by analogue synthesis programs based on structure activity relations (SAR). Most of the insecticides represented by organoposphorus esters, N-methyl carbamates, the chlorinated hydrocarbons, and benzophenylureas were derived largely from analogue synthesis and SAR optimization. Natural products of plant, animal or microbial origin are a vast source of bioactive substances, which have been exploited only to a limited extent as models in the development of commercial insecticides. Undoubtedly, the most important and significant application of a natural model from botanical origin centers on the insecticidal properties of pyrethrins and synthetic pyrethroids.

2.1.1 Pyrethroids

The increased usage of chlorinated hydrocarbons and OPs during the 1950s and 1960s resulted in decreased demand for the more expensive and less stable natural pyrethrins. However, problems associated with these synthetic insecticides, such as resistance, environmental persistence and high mammalian toxicity gave renewed attention to the natural pyrethrins.

At least six closely related compounds occur in the dried inflorescence of *Chrysanthemum cinerariaefolium*. The six principal active ingredients in the pyrethrum flowers include; pyrethrins I (**32**) and II (**33**), cinerins I (**34**) and II (**35**) and jasmolins I (**36**) and II (**37**) (Kirk and Orthmer, 1981).



32 Pyrethrin I, $R = CH_3$, $R' = C_5H_7$

33 Pyrethrin II, $R = COOCH_3$, $R' = C_5H_7$

34 Cinerin I, $R = CH_3$, $R' = C_4H_7$

35 Cinerin II, $R = COOCH_3$, $R' = C_4H_7$

36 Jasmolin I, $R = CH_3$, $R' = C_5H_9$

37 Jasmolin II, $R = COOCH_3$, $R' = C_5H_9$

Early this century, structural identification and synthesis of the insecticidal components of natural pyrethrins was accomplished. Although the natural pyrethrins are effective insecticides, their extreme photolability has limited their use. This work laid the foundation for development of techniques to synthesize analogs of the natural compounds, (Casida, 1980).

Table 1: Mosquitocidal activity of synthetic Pyrethroids (Zerba, 1988)

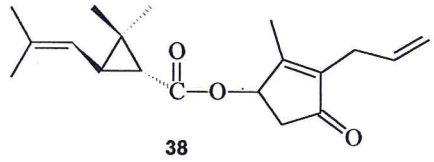
Compound	**Topical LD50 (ng/insect)**	
	An. stephensi	*Ae. aegypti*
Natural pyrethrins	7.9	11.4
Allethrin	8.5	20
Tetramethrin	3.5	22.9
Resmethrin	1.6	3.0
Bioresmethrin	1.1	1.6
Cismethrin	0.7	1.0
Phenothrin	4.1	-
Permethrin	1.1	-
Cypermethrin	0.2	-
Deltamethrin	0.04	-
Fenvalerate	1.5	-

The early synthetic Pyrethroids like allethrine were simply replacements for the natural compounds and did not serve to enhance toxic properties or stability. A major advance

in pyrethroid chemistry was the synthesis of analogs with increased photostability, like allethrin (**38**), that allowed their use in a variety of settings (Pap *et al.*, 1996).

The complexity of pyrethroid molecules provides a great potential for synthetic modification. Today, hundreds of pyrethroid modifications are tested each year. Many compounds show enhanced photostability, faster knockdown, enhanced insecticidal potency, lower mammalian toxicity, or a different insecticidal spectrum when compared with the natural pyrethrins. The first major development in pyrethroid chemistry was discovered by replacing the isobutenyl side-chain of the acid by a dichlorovinyl group and the alcohol with 3-phenoxybenzyl alcohol resulting in the first photostable pyrethroid, permethrin (**39**). This development greatly extended the range of potential applications of pyrethroids. Further enhanced insecticidal activity was achieved through introduction of a cyano substituent at the benzyl carbon of the 3-phenoxybenzyl moiety of phenothrin and related dihalovinyl esters to give compounds such as cyphenothrin and, cypermethrin (**40**). The capacity for synthetic variation was further increased by the important observation that the presence of a cyclopropane ring next to the ester linkage was not essential for high insecticidal activity leading to fenvalerate (**41**) (Nisha and Kalyanasundaram, 1992).

Deltamethrin (**42**), is one of the most active insecticides with a potency of 600 times that of DDT against *An. stephensi* and 34 times that of bioresmethrin (**43**) against *Musca domestica* (Elliot, 1989).



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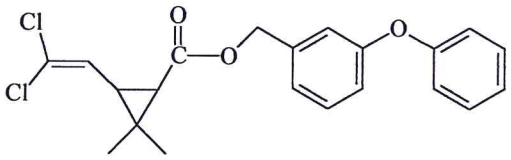

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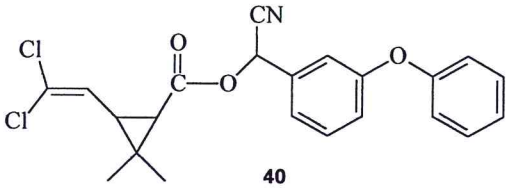

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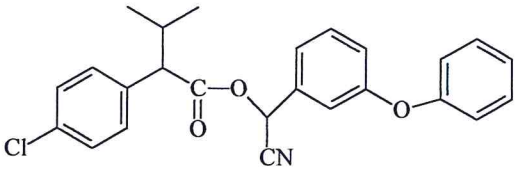

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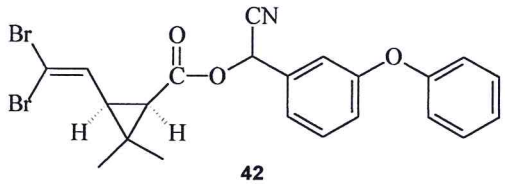

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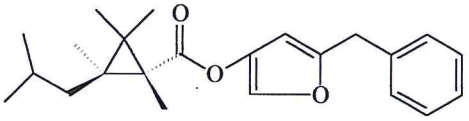

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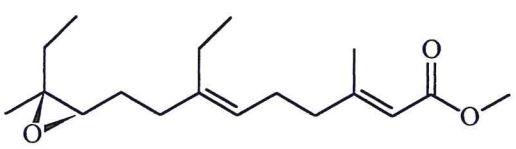
It should be noted that small changes in substituents and stereochemistry are sufficient to produce compounds differing in their insecticidal potency, spectrum of activity and mammalian toxicology. The successful development of more potent synthetic pyrethroids strongly suggests the untapped modeling potential that exists in nature and awaits further scientific exploitation.

2.1.2 Insect growth regulators

Identification of juvenile hormone (JH) in insects and its physiological effects led to the proposal that JH type compounds could be developed as insect-specific control agents (Williams, 1967). The juvenile hormone mimics are compounds bearing a structural resemblance to the juvenile hormones of insects. Juvenile hormones (JH I (**44**), JH II (**45**), JH III (**46**) are lipophilic sesquiterpenoids containing an epoxide and methyl ester groups (Shemshedini and Wilson, I990).



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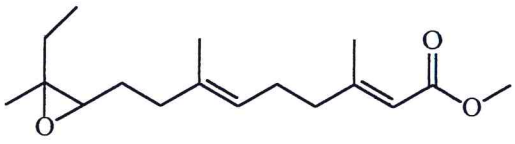

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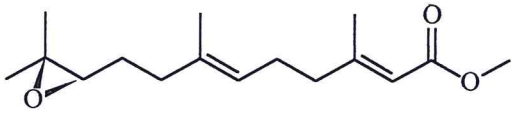

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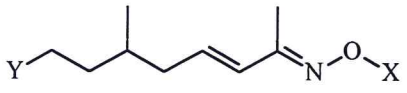
Based on " mode of action" maps and other QSAR parameters Iwamura and Toshio (1986) designed the terpenoid-like, undecen-2-one oxime O-ethers (**47**) and undecen-2-yl carbamate (**48**).



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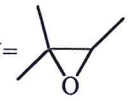

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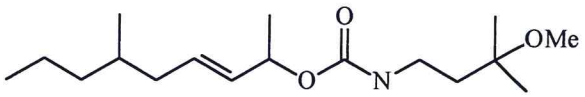

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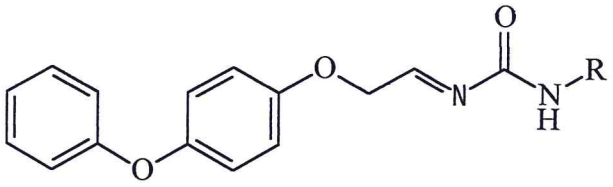
The activities were examined against *Cx. pipiens*, *Chilo suppressalis* and *Musca domestica*. The activity of the oxime O-ethers having an epoxy or a methoxy function at the y-end were considerably higher than those corresponding 9-ene- and 9,10 unsubstituted compounds. The activity of the most active member was comparable to or higher than that of the JH 1 (**44**) but much lower than that of methoprene (**26**) (Iwamura and Toshio, 1986). To obtain higher activity, they transformed the terpenoid-like structure to non-terpenoid one. Since the non-terpenoid, 2-(4-phenoxyphenoxy)ethyl carbonate, had been reported to show higher JH activity (Karrer and Farooq, 1981) they chose 2-(phenoxyphenoxy)ethane structure to obtain (4-phenoxyphenoxy)acetaldoxime O-ether (**49**) type of compounds (Iwamura and Toshio, 1986) .



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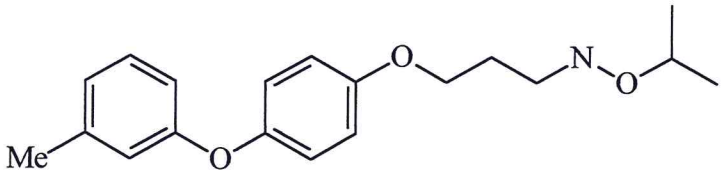
The activity of these compounds on *Cx. pipiens* was ~10^2 higher than the terpenoid-like oximes. Acetaldoxime series of compounds that had systematically varied substituents at both ends were prepared revealing that a methyl group at *meta* position of the terminal benzene raised the activity several times (Iwamura and Toshio, 1986).

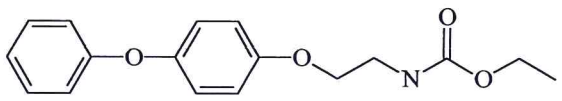
Propinaldoxime O-ether series of compounds were also explored with activities dozen times higher than the corresponding aceteldoxime O-ethers. By optimization of the terminal structures 3-[4-(3-methylphenoxy) phenoxy] propionadoxime-O-isopropylether (**50**) the most potent member of the class was obtained.



50

The activity (1.8 ppm) of this compound on *Cx. pipiens* was 10^4 higher than that of methoprene (**26**).

Such studies successfully afforded phenoxyphenoxy carbamate (**51**), hydroprene (**52**), tripene (**53**) and the phenylgeranyl ether (**54**) that have found applications as insect control agents.



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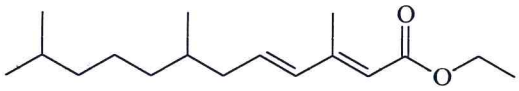

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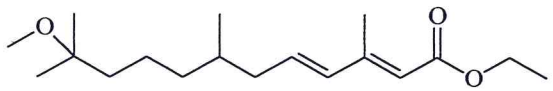

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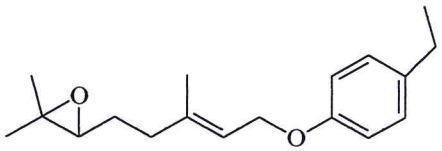
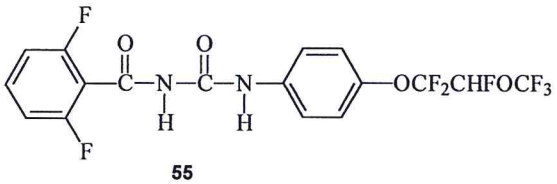


54

The discovery of chitin synthesis inhibitors was a result of efforts to develop new herbicides. While these newly developed compounds were ineffective as herbicides, they proved to be potent insecticides. These compounds classified, as benzoylphenylureas possess a number of halogen substituents. Diflubenzuron (DFB) (**27**) is the prototypical compound in this series. More recent modifications have resulted in, novaluron (**55**), hexaflumuron (**56**), teflubenzuron (**57**), chlorfuazuron (**58**), with considerably higher toxicity level than DFB (Sparks and Hammock, 1983; Hoffmann and Lorenz, 1998; Londershausen *et al.*, 1996).



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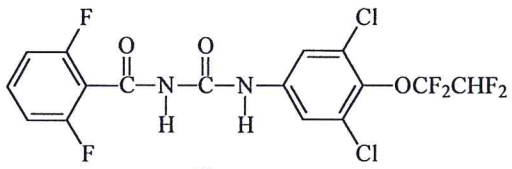

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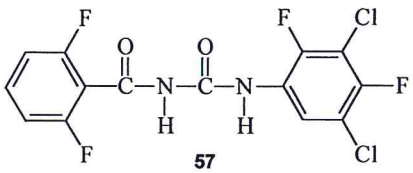

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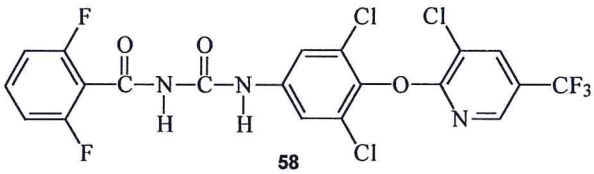


58

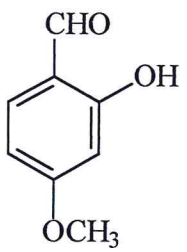
The successful development of synthetic pyrethroids and juvenile hormonoids from natural products-based leads strongly suggests the untapped modeling potential that exists in nature and awaits further scientific exploitation.

It is evident that only a very minute proportion of naturally occurring plant or animal derived toxicants have been studied as possible models for the development of insecticides.

2.2. 2-Hydroxy-4-methoxybenzaldehyde (59)

Some medicinal plants have been observed to have deleterious effect on larvae or adults of *An. gambiae* mosquitoes. One such plant is *Mondia whytie* (Hook) Skeels (Asclepiaceae). The plant is found in western and coastal parts of Kenya (Kubo and Ikuyo, 1999). In eastern, southern and central Africa the powdered roots of *Mondia whytei* are added to porridge, beer, soup or tea and taken orally as an aphrodisiac and also to treat anorexia, schitosomiasis, constipation and gonorrhea (Gelfand *et al.*, 1985).

2-Hydroxy-4-methoxybenzaldehyde (**59**) was isolated from the plant and assayed on *An. gambiae* larvae and adult. The larvicidal effect may be explained by its role as a tyrosinase inhibitor (Kubo and Ikuyo, 1999), preventing the larvae from moulting to the next instar/stage of development. It is also a repellant of adult female mosquitoes. Recently, the compound was shown to be responsible for the taste modifying properties and the characteristic aromatic smell of the plant (Mukonyi and Ndiege, 2001). 2-Hydroxy-4-methoxybenzaldehyde is a corresponding aglycone of 2-hydroxy-4-methoxybenzaldehyde-2-O-β-*D*-glucopyranos-1, 6-O-β-*D*-xylopyranoside (**60**) isolated from the plant (Msonthi, 1991).



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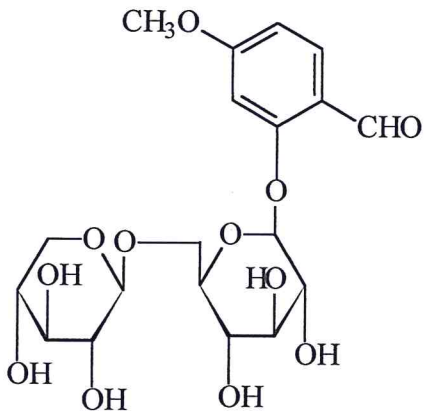

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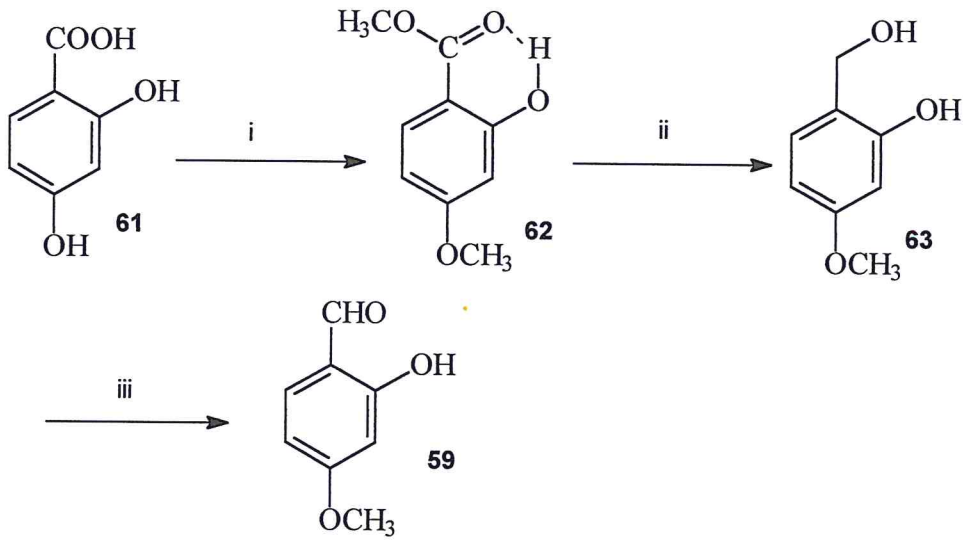
The aglycone may be obtained synthetically according to scheme 2 (Msonthi, 1991).



Reagents/conditions: i. CH_3I, K_2CO_3 ii. $LiAlH_4$, dry Et_2O iii. PCC, CH_2Cl_2

Scheme 2: Synthesis of 2-hydroxy-4-methoxybenzaldehyde

On finding the tyrosinase inhibitory activity of the compound, Kubo and Ikuyo (1999) examined several closely related compounds and established that despite their close structural similarity, the benzaldehyde derivatives did not show significant inhibitory activity. The reasons for the exceptionally potent tyrosinase inhibitory activity of the compound are still unclear.

This study examined several structural derivatives of benzaldehyde and other closely related benzene derivatives, some of which were synthetically obtained, for their larvicidal activities against *An. gambiae* larvae.

2.3. Synthetic strategies

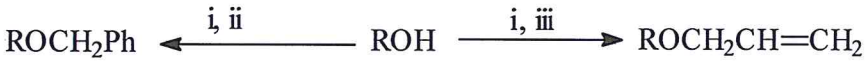
2-Hydroxy-4-methoxybenzaldehyde (**59**) can be presumed to undergo a number of reactions based on the presence of the carbonyl and the hydroxyl functionalities. These include; benzylation and benzoylation reactions, which target the hydroxyl group; Wittig, Grignard, and Claisen-Schmidt's reactions, which target the carbonyl group.

2.3.1 Benzylation

It is a common requirement in synthesis that a hydroxyl group be masked as a derivative lacking an active hydrogen. An example of this is in reactions involving Grignard and/or other organometallic reagents. The acidic hydrogen of a hydroxyl group destroys one equivalent of strongly basic organometallic reagents and possibly adversely affects the reaction in other ways (Francis and Richard, 1990).

Allyl and benzyl groups are commonly employed for the protection of alcohol moieties due to the ease of their removal. Solvent free benzyl ether preparation using a combination of potassium carbonate and KOH bases is possible in presence of tetrabutylammonium bromide under microwave irradiation. However, the reaction requires drastic conditions such as high temperatures and specialized apparatus (Rao and Senthilkumar, 2001).

Therefore, in most reactions solvent-based methods of benzylation are used as shown in scheme 3 (Rao and Senthilkumar, 2001).

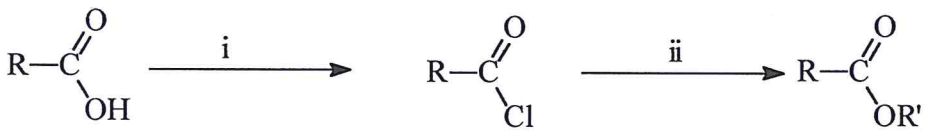


Reagents/conditions: i. K_2CO_3 ii. $BrCH_2Ph(EtOH)$ iii. $BrCH_2CH=CH_2$ (EtOH)

Scheme 3: Benzylation

2.3.2 Benzoylation

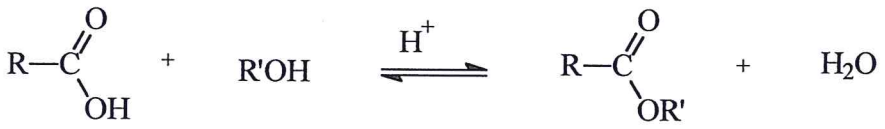
Esters are usually prepared by the reaction of alcohols/phenols with acids or acid derivatives. Alternatively, acids are usually converted into their corresponding esters via the acid chlorides as shown in scheme 4 (Khadilkar and Rebeiro, 2000).



Reagents/conditions: i. SOCl ii. R'OH, reflux

Scheme 4: Esterification

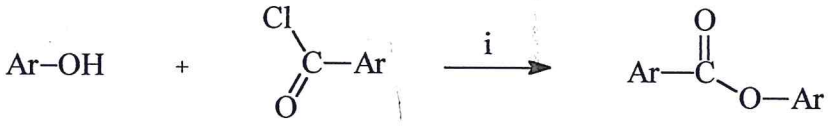
However, a carboxylic acid can be converted directly into an ester when heated with an alcohol in presence of mineral acid, usually concentrated sulphuric or hydrochloric acid (Scheme 5). This reaction is reversible and requires the removal of one of the products for it to proceed to completion.



Scheme 5: Esterifiction in mineral acid

The acid chloride route is more preferred due to the fact that in both steps preparation of acid chloride from acid and ester from the acid chloride are essentially irreversible and go to completion (Morrison and Boyd, 1991).

Esterification using aromatic acid chlorides leading to the formation of benzoates is often carried out in the presence of a base, usually pyridine (Schotten-Baumann technique) (Scheme 6). The base serves not only to neutralize the hydrogen chloride liberated, but also to catalyze the reaction. Pyridine, in particular, seems to convert the acid chloride into acyl pyridinium salt which is a more powerful acylating agent (Pandita and Goyla, 1997).



Ar = Aromatic nucleus

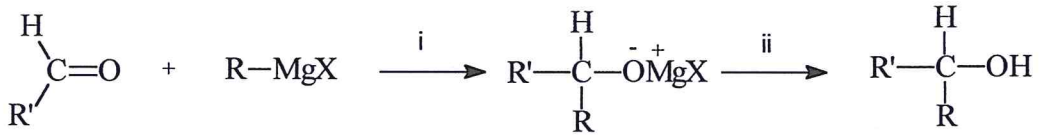
Reagents/conditions: i. Pyridine, 50 ^{0}C

Scheme 6: Schotten-Baumann benzoylation

2.3.3 Alcohol synthesis by Grignard reaction

By far, the most important method of preparing alcohols is the Grignard synthesis that also leads to the formation of C-C bonds. One of the most important uses of the

Grignard reagent lies in its reaction with aldehydes and ketones. The carbon-magnesium bond of the Grignard reagent is a highly polar bond, carbon being negative relative to electropositive magnesium (Ebsworth *et al.*, 1971). In the addition to carbonyl compounds, the organic group becomes attached to carbon, and magnesium to oxygen. The product is the magnesium salt of the weakly acidic alcohol and is easily converted into the alcohol itself by the addition of the stronger acid or water (Scheme 7). Since the Mg(OH)X formed is gelatinous, dilute mineral acid is commonly used instead of water to facilitate the formation of a water soluble magnesium salt. The class of alcohol that is obtained generally depends upon the type of carbonyl compound used (Morrison and Boyd, 1991).

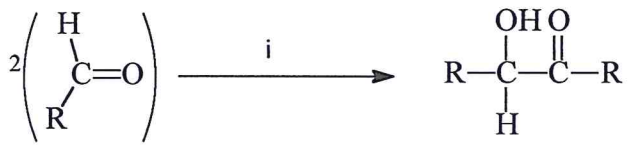


Reagents/conditions: i. Et_2O ii. H_2O, ~5°C

Scheme 7: Grignard reaction

2.3.4 Benzoin condensation

Under the influence of catalytic amounts of cyanide in aqueous alcohol, benzaldehyde undergoes condensation to the α-hydroxyketone (Furniss *et al.*, 1989) as represented in scheme 8.



Reagents/conditions: i. KCN/NaCN, reflux

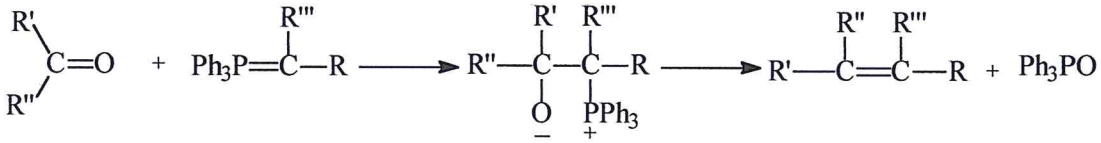
Scheme 8: Benzoin condensation

Other aromatic aldehydes form benzoins (acyloins) under catalysis by cyanides. Treatment of two different aromatic aldehydes with cyanide usually results in formation of one of the mixed acyloins (Miyashita *et al.*, 1996).

2.3.5 Chalcones

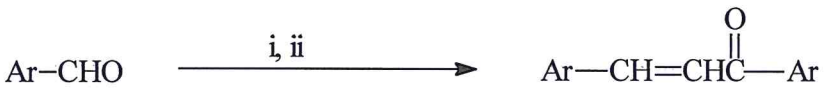
The Wittig reaction is a versatile synthesis and can be used for the preparation of mono-, di-, tri- and tetra-substituted ethylenes and cyclic compounds as well. This reaction can occur under mild conditions without being affected by the presence of groups such as hydroxyl, ether, ester, halogens and terminal acetylene, (Ebsworth *et al.*, 1971). The principle is based on the nucleophilic attack on the carbonyl oxygen by a *ylide* to form a betaine, which often spontaneously undergoes elimination to yield the product.

The reaction is stereoselective. Carbonyl compounds may contain a wide variety of substituents, and so may the *ylide* (Scheme 9). The preparation of the ylide is a two-step process, from the nucleophilic attack on an alkyl halide, to the abstraction of a proton by a base (Cadogan, 1974).



Scheme 9: Wittig reaction

Chalcones may also be obtained without stereoselective control of the α, β-protons using the Claisen-Schmidts' reaction, (Furniss *et al.*, 1989). This is a mixed aldol reaction between two different carbonyl compounds that involves the condensation of an aromatic aldehyde with an aliphatic/aromatic aldehyde or ketone. This is carried out in presence of a relatively strong base (hydroxide or alkoxide ion) to form α,ß-unsaturated aldehyde or ketone as illustrated in scheme 10.



Ar = Aromatic nucleus

Reagents/conditions: i. $PhCOCH_3$ ii. NaOH, 25 ^0C

Scheme 10: Claisen-Schmidt's reaction

1.6 HYPOTHESIS

The structure activity studies of 2-hydxoxy-4-methoxybenzaldehyde may provide insight into the functional groups responsible for its bio-activity and afford other compound(s) with higher larvicidal activity.

1.7 OBJECTIVES

To carry out structure activity relationship studies of 2-hydroxy-4-methoxybenzaldehyde and simpler analogs as larvicidal compounds against *An. gambiae* larvae.

1.7.1 Specific objectives

1. To determine the larvicidal activity of 2-hydxoxy-4-methoxybenzaldehyde and its closely related congeners against *An. gambiae* larvae
2. To determine the larvicidal activity of structurally simpler benzene derivatives against *An. gambiae* larvae.
3. To synthesize analogs of 2-hydxoxy-4-methoxybenzaldehyde and determine their larvicidal activities against *An. gambiae* larvae.
4. To compare the bioactivities of 2-hydxoxy-4-methoxybenzaldehyde, derivatives and analogues and identify the functional groups responsible for the potency.

1.8 JUSTIFICATION

Recent epidemics are telling reminders of how quickly malaria can strike and how great its devastation can be, causing untold suffering and tragedy. The epidemics represent only the tip of the malaria iceberg. Even more serious, in terms of the everyday countless illnesses and deaths in regions where malaria is endemic.

In addition to suffering and death, the disease penalizes poor communities, as it perpetuates poverty through work loss, school drop-out, decreased financial investment and increased social instability creating sizeable social and economic costs. There is no 'magic bullet'; we must fight it on many fronts. We need concerted action to use existing tools more effectively, both widely and wisely.

Malaria is getting out of our control. Resistance to both drugs and insecticides is growing, there are not many new drugs on the shelves, and some of the old strategies are past their sell-by date. The much-vaunted vaccine is apparently not imminent. Any efforts towards malaria control must be encouraged supported and appreciated.

The negative environmental impact caused by some of the synthetic chemical insecticides are dangerous and persistent. New chemicals, which are more effective, environmentally safe, with low toxicity to non-target organisms need to be developed.

Considerations are being made in the application of insecticidal plants as an alternative cost-effective approach for the control of mosquitoes and the plant-derived insecticidal principles as templates in the development of environmentally safe insecticides. The successful use of pyrethrins and the synthetic pyrethroids in insect vector and pest control makes this approach appealing.

Several plants have been screened and found to contain insecticidal and larvicidal activities against *An. gambiae*. One such plant is *Mondia whytei* from which 2-hydroxy-4-methoxybenzaldehyde was isolated. The compound serves as the lead compound for this study in a bid to develop a new class of potent larvicides. Success of larvicides would be the termination of the mosquitoes at an early stage before they can bite and infect humans with the malaria causing parasites among other mosquito-transmitted diseases.

CHAPTER 3

PRELIMINARY STRUCTURAL ACTIVITY STUDIES

3.1 Activity of 2-hydroxy-4-methoxybenzaldehyde and closely related congeners

In a separate study, 2-hydroxy-4-methoxybenzaldehyde (**59**) was found to have tyrosinase inhibitory activity thus providing clues towards the control of insect pests (Kubo and Ikuyo, 1999).

The larvicidal activity of **59** against *An. gambiae* was obtained by a plot of probit transformation against log of the concentration (Busvine, 1971) and the regression equations obtained as Y=8.7936x-15.596, Y=11.186x-21.051 and Y = 9.9042x-17.954 for 24, 48 and 72 hours, respectively.

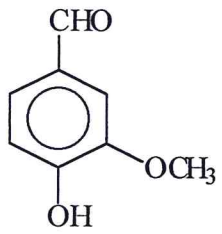
The compound showed a dose dependent larvicidal activity against the *An. gambiae* larvae recording an LD_{50} of 0.022 mg/ml after 24 hours of exposure. The LD_{50} after 48 and 72 hours exposure was 0.021 mg/ml (Table 2). From the LD_{50} values, there is no significant difference between the results obtained after 24, 48 and 72 hours of exposure, probably implying a dose and not time dependent bioactivity relationship.

Table 2: Probit analysis of 2-hydroxy-4-methoxybenzaldehyde (**59**) larvicidal data

Conc. mg/ml	**Log(+3) conc**	**Corrected % mortality**			**Probit transformation**		
Time (hrs)		24	48	72	24	48	72
0.040	2.6021	97±1.73	100±0.0	100±0.0	6.88	-	-
0.030	2.4771	90±2.54	91±2.65	91±2.65	6.28	6.34	6.34
0.025	2.3979	85±2.65	87±0.58	87±0.58	6.04	6.13	6.13
0.020	2.3010	43±1.00	43±0.58	43±0.58	4.82	4.82	4.82
0.015	2.1761	3±2.31	3±1.00	7±1.00	3.12	3.12	3.12

The compound has three functional groups on an aromatic ring namely carbonyl, hydroxyl and the methoxyl groups. The results prompted a structural activity relationship

study to identify which of the three functional groups is/are responsible for the potent larvicidal activity. Bio-assays of the readily available closely related congeners such as 4-hydroxy-3-methoxybenzaldehyde (vanillin) (**64**), and 4-methoxysalicylic acid (**65**) were done. Vanillin showed no activity upto 0.1 mg/ml while 4-methoxysalicylic acid had a relatively low activity (LD_{50} 0.058, 0.52, 0.50 mg/ml after 24, 48 and 72 hours, respectively).



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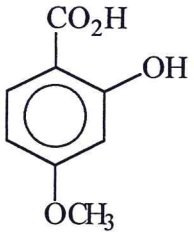

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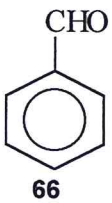
Kubo and Ikuyo (1999) reported very low tyrosinase inhibitory activity of some of the closely related compounds evaluated with vanillin (**64**) showing 10^3 times less potency than **59**.

3.2 Activity of mono- and disubstituted benzenes

Simpler analogues were also examined for further comparison and to understand the roles of each of the functional groups on larvicidal activity. The compounds tested included the mono-substituted benzene derivatives; benzaldehyde (**66**), phenol (**67**), and methoxybenzene (**68**).



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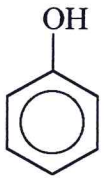

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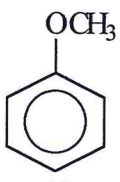

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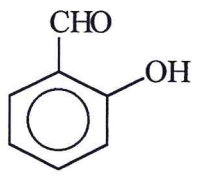
From previous tyrosinase inhibition studies (Kubo and Ikuyo, 1999), it was expected that **66** would show the highest insecticidal activity among the mono-substituted compounds. However, there was no significant difference between the activity of **66** and **67** (LD_{50} 0.055 and 0.054 mg/ml, respectively, after 24 hours). Of the three compounds in this

group, **68** had no activity upto 0.1 mg/ml. The calculated LD_{50} values after 24, 48 and 72 hours are summarized in table 3.

Table 3: Summary of LD_{50} values for the mono-substituted benzenes

Compound	**LD_{50} (mg/ml)**		
Time (hrs)	24	48	72
66	0.055	0.054	0.052
67	0.054	0.054	0.051
68	> 0.1	> 0.1	> 0.1

The disubstituted benzenes studied included 2-hydroxybenzaldehyde (salicyldehyde) (**69**), 2-methoxyphenol (guaiacol) (**70**), 3-methoxyphenol (**71**), *p*-methoxyphenol (**72**), 4-hydroxybenzaldehyde (**73**) and *p*-methoxybenzaldehyde (**74**). The compounds also showed varying degrees of larvicidal activity (Table 4) with **69** showing the highest activity (LD_{50} 0.009 mg/ml after 24 hours). Despite having both the aldehyde and hydroxyl groups, **73** had a much lower activity (LD_{50} of 0.578 mg/ml). This value is about 64 times less than that of **69**. Interestingly, **70** with an *ortho*-methoxy group showed no activity upto 0.1 mg/ml. 3-Methoxyphenol (**71**) and 4-methoxyphenol (**72**) on the other hand had activity levels comparable to the lead compound (**59**) (LD_{50} 0.077 and 0.062 mg/ml, respectively).



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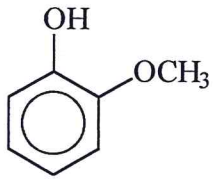

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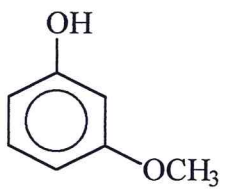

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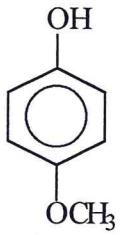


72

73

74

Table 4: Summary of LD_{50} values for the di-substituted benzenes

Compound	**LD_{50} (mg/ml)**		
Time (hrs)	24	48	72 hours
69	0.009	0.009	0.008
70	>0.1	> 0.1	>0.1
71	0.077	0.075	0.072
72	0.062	0.054	0.052
73	0.578	0.223	0.137
74	0.032	0.027	0.027

The $LD_{25,\ 50,\ 75}$ and $_{90}$ were calculated for 2-hydroxy-4-methoxybenzaldehyde (**59**) and congeners, **70-74** (Table 4).

Table 5: Summary of LD values for mortality after 24 hours

Compound	**LD (mg/ml)**			
	LD_{25}	LD_{50}	LD_{75}	LD_{90}
59	0.019	0.022	0.026	0.031
64	> 0.1	> 0.1	>0.1	> 0.1
65	0.048	0.058	0.071	0.085
69	0.007	0.009	0.014	0.019
73	0.248	0.578	1.347	2.918
70	> 0.1	> 0.1	>0.1	> 0.1
74	0.023	0.032	0.045	0.060
72	0.054	0.062	0.075	0.089
71	0.066	0.077	0.089	0.102
67	0.048	0.054	0.065	0.076
66	0.045	0.055	0.063	0.083
68	> 0.1	> 0.1	> 0.1	> 0.1

Only 2 compounds **69** and **74** gave improved larvicidal activity. It can therefore be inferred that particular functional group(s) attached to the aromatic ring in a particular order is/are responsible for larvicidal activities.

Building the compounds from the simple mono-substituted test compounds serves to explain this postulation. The addition of a hydroxyl group at *ortho* position in **66** changing it to **69** increased the activity 6-fold (LD_{50} 0.009 mg/ml). However, shifting the hydroxyl group to the *para*-position as in **73** lowered the activity 64-fold (LD_{50} 0.578 mg/ml). The addition of an electron donating methoxy group at 4-position changes **66** to 4-methoxybenzaldehyde (**74**) increasing its activity 2-folds (LD_{50} 0.032 mg/ml). It can therefore be concluded that a free hydroxyl group is antagonistic at *para* but protagonist at *ortho* position. The presence of methoxy group *ortho*, *meta* or *para* to a hydroxyl group is therefore antagonistic.

Phenol (**67**), which showed a relatively high activity (LD_{50} 0.054 mg/ml) among the mono-substituted derivatives, had its activity lowered by the addition of a methoxy group at *ortho* as in **70** (LD_{50} > 0.1 mg/ml), *meta* as in **71** (LD_{50} 0.077 mg/ml) and para position as in **72** (LD_{50} 0.062 mg/ml) positions. This further confirms the earlier observation.

The addition of a methoxy group at the *para* to the carbonyl in **69** gives the lead compound **59**, which had a comparatively lower activity (LD_{50} 0.022 mg/ml). Interestingly, the replacement of the aldehyde group with the carboxylic group giving 4-methoxysalicylic acid (**65**) lowers the activity 6 times (LD_{50} 0.059 mg/ml) compared to salicaldehyde (**69**). Similarly, 4-hydroxybenzaldehyde (**73**) with a very low activity completely losses activity when converted to **64**. Again the antagonistic effect of methoxy group *para* to the aldehyde and *meta* to the hydroxyl group is demonstrated likewise, the antagonistic effect of the carboxylic acid is evident.

The methoxy group seems to exert little diminishing effect when *para* to the aldehyde and *meta* to the hydroxyl group as shown by compound **59**. Similarly, methoxy group has little effect on the activity when *ortho* or *meta* to the hydroxyl group as shown in **70** and

71, respectively. However, the effect is not pronounced when the methoxy group is *para* to the hydroxyl as illustrated by the relatively high activity of **72**.

The hydroxyl group on the other hand is an activity-potentiating group. Introduction of the hydroxyl *ortho* to the aldehyde group in benzaldehyde (**66**) gives salicyldehyde (**69**) resulting in substantial increment in activity. Although this seems to be the case, it is apparent that the position of the hydroxyl relative to the aldehyde group is a factor in eliciting larvicidal activity. In comparison to benzaldehyde (**66**), an antagonistic effect is observed when the hydroxyl group is *para* to the aldehyde in 4-hydroxybenzaldehyde (**73**).

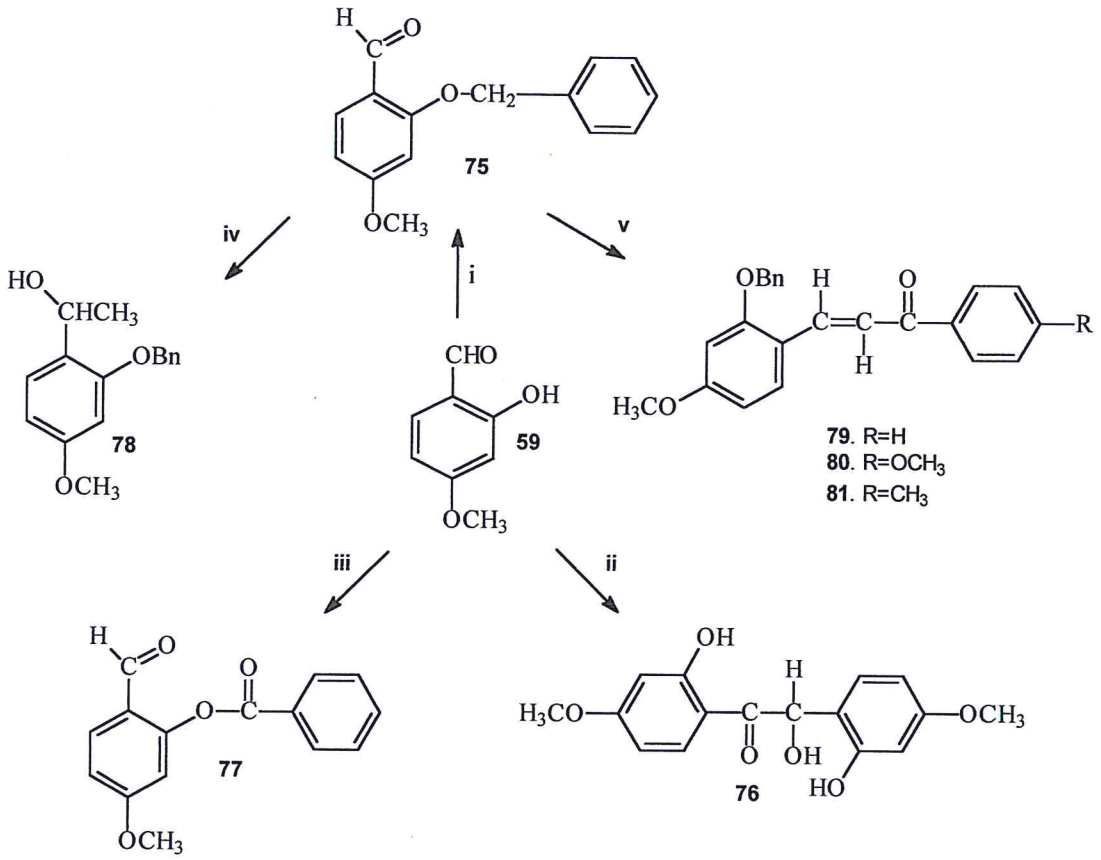
These results suggest that each functional group and its position on the ring has different influence on the larvicidal activity of the compound. Where two or more functional groups are present, the relative positions to each other also have an influence on the activity.

The relevance of the hydroxyl and the carbonyl groups in larvicidal activity required further evaluation using various analogs, thus facilitating the syntheses of derivatives or analogs whose hydroxyl and aldehyde groups have been converted into other functionalities.

CHAPTER 4

SYNTHESIS OF ANALOGUES

The proposed synthetic approaches targeted the hydroxyl and the aldehyde groups of 2-hydroxy-4-methoxybenzaldehyde (**59**) owing to their higher reactivity as compared to the methoxy group. All reactions were performed according to the strategies highlighted in section 2.3 (Scheme 11).



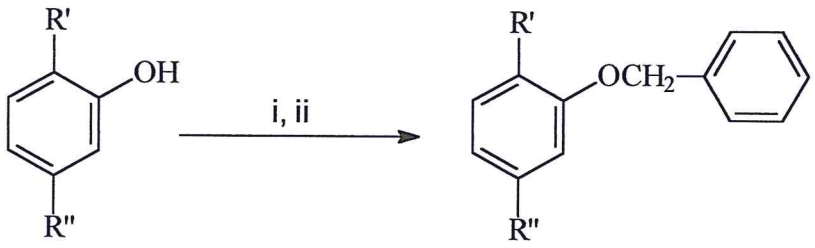
Reagents/conditions: i. $PhCH_2Cl$, K_2CO_3, reflux; ii. KCN/NaCN, reflux; iii. $PhCH_2Cl$, Pyridine, 50 ^0C; iv. CH_3MgBr, R.T; v. R=H: Ph_3P, $BrCH_2COPh$; R=OCH_3: $COPhOCH_3$, NaOH, 25 ^0C; R=CH_3: $COPhCH_3$, NaOH, 25 ^0C

Scheme 11: Synthetic routes

This chapter discusses the synthetic methods explored during the present study and structural confirmation using various spectrometric methods of the compounds obtained. Scheme 11 summarizes the synthetic reactions attempted and the reagents/conditions employed.

4.1 Benzyl ethers

Since the corresponding phenols are commercially available, direct benzylation of the compounds was carried out yielding the benzyl ethers; 2-benzyloxy-4-methoxybenzaldehyde (**75**), 2-benzyloxybenzaldehyde (**82**) and benzylphenyl ether (**83**) as described in section 2.3.1 and subjected to larvicidal bio-assays. These compounds were prepared from **59**, **69**, and **67**, respectively, and benzyl chloride in the presence of K_2CO_3 and ethanol (Scheme 12). Recrystalization from 1:4 ethanol:water mixture afforded white crystalline substances with melting point 54-56 , 42-43, and 34-35 ^{0}C, respectively.



[**59**. R'=CHO, R"=OCH_3, **67**. R'=R"=H, **69**. R'=CHO, R"=H]
75. R'=CHO, R"=OCH_3, **82**. R'=CHO, R"=H, **83**. R'=H, R"=H

Reagents/conditions: i. $BrCH_2Ph$ ii. K_2CO_3, reflux

Scheme 12: Benzyl ethers

4.1.1 2-Benzyloxy-4-methoxybenzaldehyde (75)

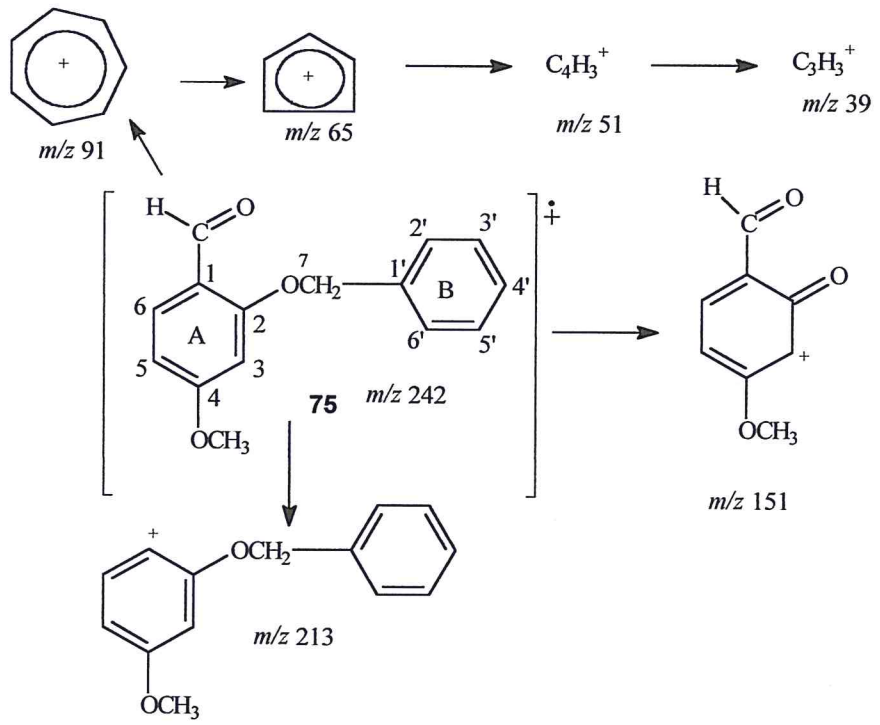
The IR spectrum of compound **75** revealed a C-O asymmetric stretch at 1265 and a symmetric C-H stretch at 2867 cm^{-1}. The aromatic C=C stretches were attributed to the peaks at 1502, 1577 while the C-H stretch appeared at 3020 cm^{-1}. The peaks at 1670, 2830 and 2780 cm^{-1} confirmed the presence of the aldehyde group.

From the ^{1}H NMR spectrum, 7 signals were observed. The proton at δ 10.22 (1H, s) confirmed the presence of the aldehydic group. The doublets at δ 7.66 (1H, *J*=8.8, 1.2 Hz) and δ 6.65 (1H, *J*=8.8 Hz) also confirmed the presence of the AB system in the compound. The other 5 aromatic protons were observed at δ 7.30-7.51 (5H, m) confirming the presence of the phenyl group. The proton *ortho* to the benzyloxy group appeared at δ 6.79 (1H, d, *J*=1.2 Hz). The methylene protons were confirmed by the signal at δ 5.27 (2H, s). The peak at δ 3.83 confirmed the presence of the methoxy

group. The ^{1}H-^{1}H COSY NMR spectrum revealed coupling between the peaks at δ 7.66 and 6.65.

The ^{13}C NMR revealed 13 peaks indicating symmetry in the molecule. The signal at 187.9 ppm confirmed the presence of the carbonyl group. The aromatic signals appeared at δ 166.6, 163.2, 137.1, 130.6, 129.2, 129.2, 128.7, 128.2, 128.2, 119.3, 107.8 and 100.4 ppm confirming the presence of 2 aromatic rings. The methylene carbon was observed at 70.7 ppm while the methoxy carbon appeared at 56.5 ppm. DEPT analysis confirmed the presence of one CH_3, one CH_2 and six CH carbons tallying with the expected product.

EIMS revealed molecular ion peak at *m/z* 242 [$M^{\pm}$], which is consistent with the formula $C_{15}H_{14}O_3$. The base peak at *m/z* 91 (100%) was attributed to $[C_7H_7]^+$. The other peaks were observed at *m/z* 213 $[C_{14}H_{13}O_2]^+$, 151 $[C_8H_7O_3]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$, 39 $[C_3H_3]^+$. The MS can be justified by the fragmentation pattern in scheme 13.



Scheme 13: 2-Benzyloxy-4-methoxybenzaldehyde mass fragmentation

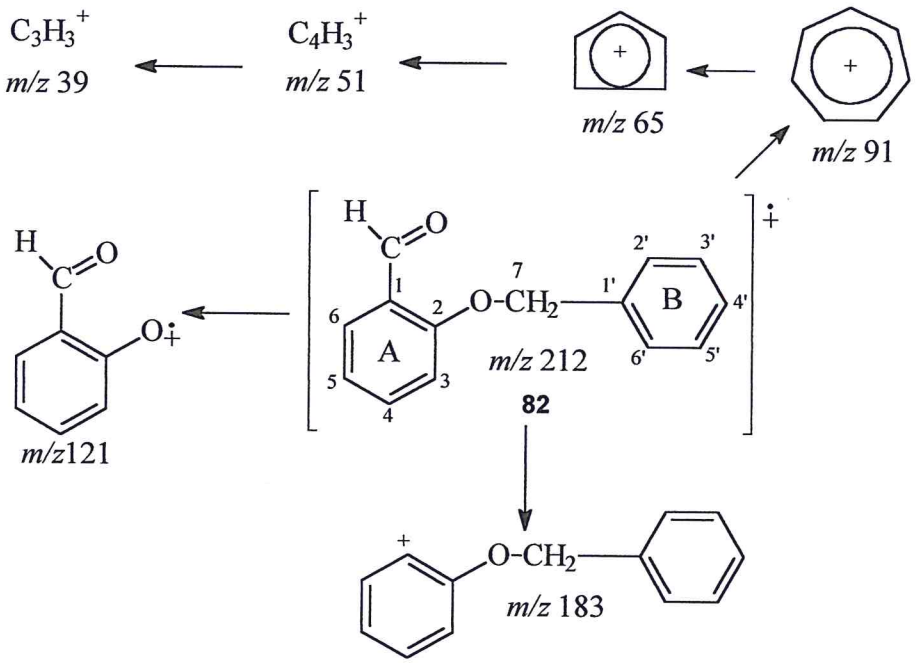
4.1.2 2-Benzyloxybenzaldehyde (82)

The IR spectrum of compound **82** confirmed the presence of C-O-C stretch 1238 and 1161 cm^{-1} while C-H stretch appeared at 2877 cm^{-1}. The aromatic C=C stretches were observed at 1596 and 1500 cm^{-1} while the weak stretch at 2750 cm^{-1} confirmed the presence of the aldehyde group. This is further supported by the peak at 1682 cm^{-1}.

The ^{1}H NMR showed the presence of a singlet at δ 10.41 due to the aldehydic proton. The peaks at δ 7.37 (1H, d, *J*=7.4 Hz), 7.07 (1H, dd, *J*=7.2Hz, 7.4 Hz), 7.61 (1H, d, *J*=8.4 Hz) and 7.67 (1H, dd, *J*=8.4, 8.6 Hz) represents the 4 protons on ring A while the multiplet between δ 7.27-7.42 represents the 5 protons on ring B. The signal at δ 5.27 (2H, s) was assigned to the methylene protons.

From the ^{13}C NMR a carbonyl signal was observed at 189.9 ppm while 10 aromatic signals at; δ 161.3, 137.13, 137.05, 129.2, 129.2, 128.7, 128.5, 128.2, 128.2, 125.2, 121.6 and 114.7 ppm were noted thus confirming the presence of 2 aromatic rings. The methylene signal was observed at 70.5 ppm. Three quaternary carbons were evident from the DEPT spectra with the presence of 11 protonated carbons.

EIMS revealed molecular ion peak at *m/z* 212 [$M^{\pm}$], which is consistent with the formula $C_{14}H_{12}O_2$. The base peak at *m/z* 91 (100%) was attributed to the ion $[C_7H_7]^+$. Other peaks were observed at *m/z* 18 $[C_{13}H_{11}O]^+$, 121 $[C_7H_5O_2]^+$, 91 $[C_7H_7]^+$ 51 $[C_4H_3]^+$ and 39 $[C_3H_3]^+$. The MS can be justified by the fragmentation pattern in scheme 14.



Scheme 14: 2-Benzyloxybenzaldehyde mass fragmentation

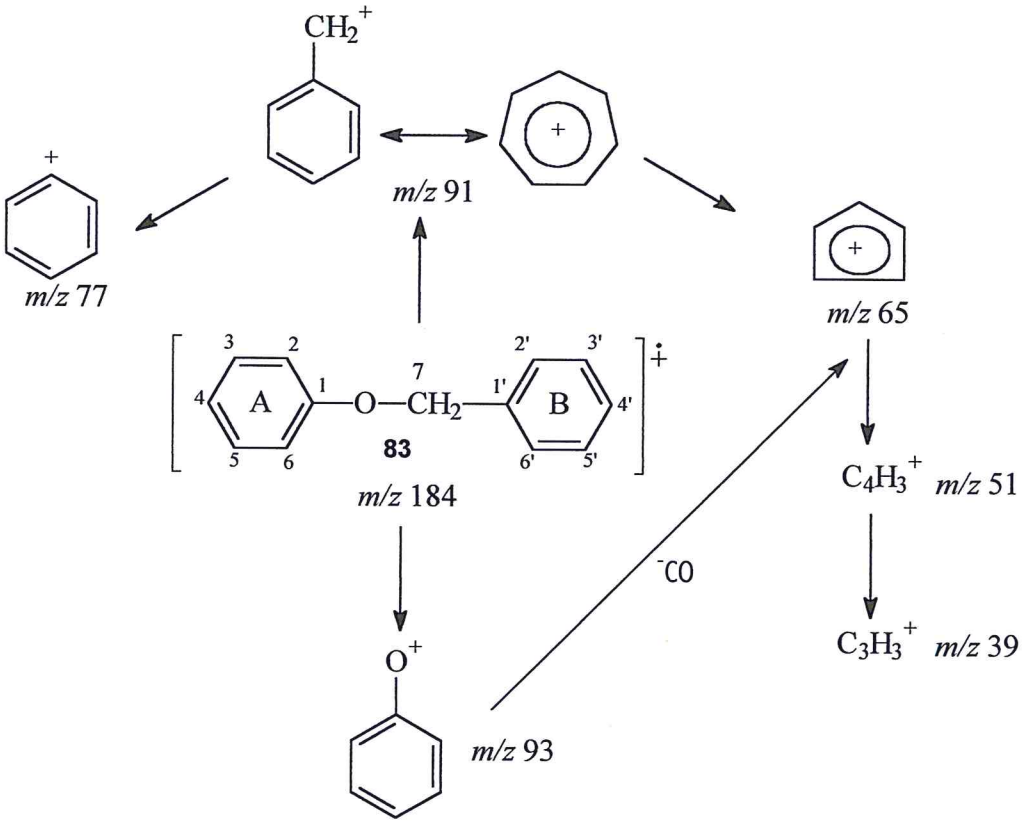
4.1.3 Benzylphenyl ether (83)

The IR spectrum of compound **83** confirmed the presence of C-O and C-H stretch at 1246 and 2862 cm^{-1}, respectively. The aryl C-H stretch was observed at 3037 cm^{-1}.

The ^{1}H NMR spectrum revealed a peak at δ 5.07 (2H, s) distinctive of the methylene protons. The multiplet at δ 6.92 (1H) was assigned to the *para* proton on ring A while the doublet at 6.99 (2H, *J*=8.4 Hz) to the two *ortho* protons of the same ring. The multiplet between δ 7.27-7.42 (7H) represented the remaining 7 protons.

^{13}C NMR spectrum likewise confirmed the presence of several aromatic carbon signals at 159.0, 137.8, 130.2, 130.2, 129.1, 129.1, 128.5, 128.4, 128.4, 121.4, and 115.4, 115.4 ppm. The methylene carbon signal was observed at δ 69.7 ppm. The DEPT spectrum indicated the presence of 11 protonated carbons suggesting the presence of 2 quaternary carbons.

EIMS revealed molecular ion peak at *m/z* 184 [M^+], which is consistent with the formula $C_{13}H_{12}O$. The base peak at *m/z* 91 (100%) was attributed to $[C_7H_7]^+$. The other peaks were observed at *m/z* 92 $[C_7H_3]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$, 39 $[C_3H_3]^+$. The MS can be justified by the fragmentation pattern in scheme 15.

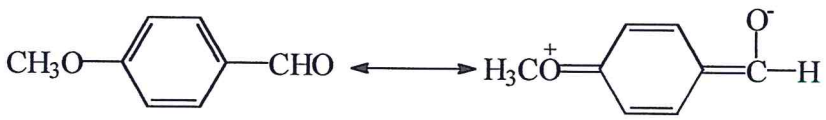


Scheme 15: Benzyl phenyl ether mass fragmentation

4.2 The benzoin reaction

This reaction, which aimed to prepare 2, 2'- dihydroxy-4, 4'-dimethoxybenzoin (**76**) using the normal benzoin synthesis procedures, did not yield the expected product after final work-up. Several minute fractions were obtained from column chromatography and attempts to characterize the products were futile giving non-interpretable spectroscopic information.

Certain substituted aldehydes do not undergoes self-condensation under cyanide catalysis (Miyashita *et al.*, 1996). This may be due to the lack of adequate electrophilicity on the carbonyl as a result of resonance stabilization for example (**84**).

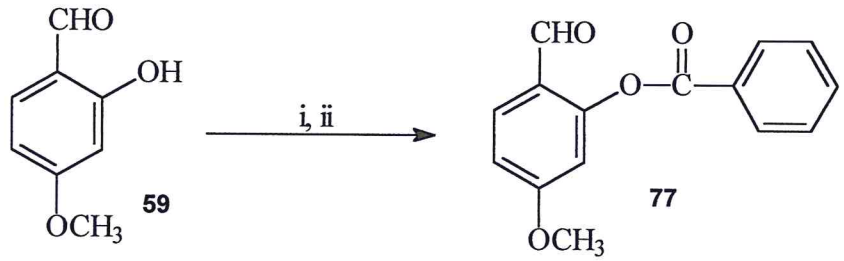


74 **84**

It is suggested that the electron donating functional groups may have affected the distribution of charges around the carbonyl group thus rendering the site less electrophillic. However, it is assumed that other more powerful catalysts may be used to overcome this problem. For instance, thiazolium salts have been applied in the synthesis of substituted benzaldehydes with good yields (Miyashita *et al.*, 1996).

4.3 2-Benzoyloxy-4-methoxybenzaldehyde (77)

Benzoylation of the phenolic hydroxyl was achieved with benzoyl bromide in the presence of pyridine (Schotten-Baumann conditions) (scheme 16) and the product recrystalized in 1:4 methanol:water mixture. The melting point was determined as 76-77 ^{0}C.



59 **77**

Reagents/conditions: i. $BrCOC_6H_5$, ii. Pyridine, 50 ^{0}C

Scheme 16: Benzoylation of 2-hydroxy-4-methoxybenzaldehyde

The IR spectrum of **77** confirmed the presence of C-O-C(O) stretches at 1096 and 1241 cm^{-1} while the aromatic C=C stretch appeared at 1566 and 1509 cm^{-1}. The aldehydic C-H and C=O stretches appeared at 2767 and 1693 cm^{-1}, respectively.

The ^{1}HNMR revealed two singlets at δ 10.04 (1H) and 3.87 (3H) characteristic of the aldehydic and methoxy protons respectively. The signals at δ 7.88 (1H, d, *J*=8.6Hz) and 6.92 (1H, dd, *J*=8.6, 2.1 Hz) are supportive of the AB system in ring A. The doublet at δ 6.80 (1H,d, *J*=2.1Hz) was assigned the proton *ortho* to the methoxy and the benzoyl groups on ring A. The doublet at δ 8.21 (2H, *J*=7.2 Hz) was assigned to the 2 *ortho* protons on ring B while the peak at 7.52 (2H, dd) to the two *meta* protons of the same ring. The para proton of ring B was assigned the peak at 7.66 (1H, dd, *J*=7.2, 7.5 Hz).

^{13}C NMR showed 12 aromatic signals at δ 165.2, 164.8, 134.0, 132.0, 130.3, 130.3, 130.1, 128.7, 128.7, 121.8, 112.6 and 108.6 ppm confirming the presence of 2 aromatic rings. The signal at δ 187.2 ppm confirmed the presence of the aldehyde. The peak at 154.0 ppm confirmed the ester moiety while that at δ 55.9 ppm confirmed the presence of the methoxy group. DEPT analysis showed the presence of 10 protonated carbons suggesting the presence of 5 quaternary carbons.

EIMS revealed molecular ion peak at *m/z* 256 [M^+], which is consistent with the formula $C_{15}H_{12}O_4$. The base peak at *m/z* 77 (100%) was attributed to the ion $[C_6H_5]^+$. Other peaks were observed at *m/z* 151 $[C_8H_7O_3]^+$, 122 $[C_7H_6O_2]^+$, 105 $[C_7H_5O]^+$, 51 $[C_4H_3]^+$ and 39 $[C_3H_3]^+$. The MS can be justified by the fragmentation pattern in scheme 17.

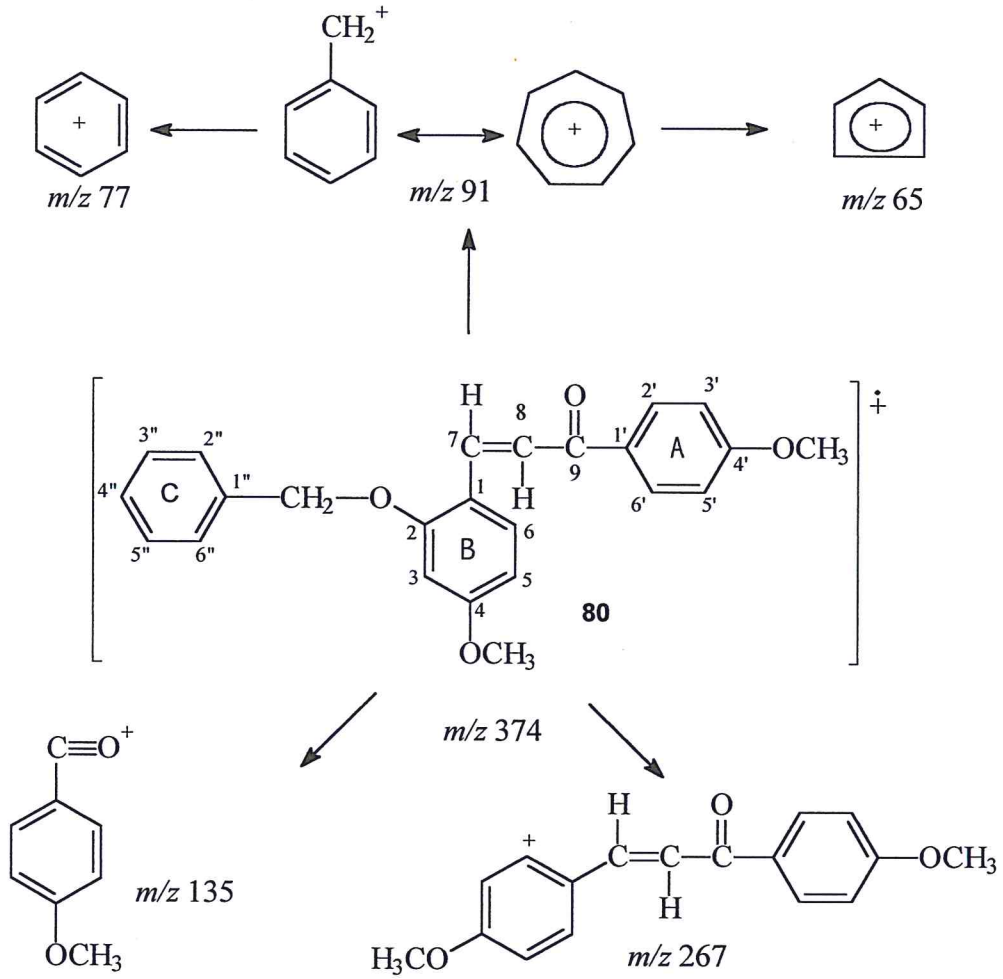
4.5.1 2-Benzyloxy-4, 4'-dimethoxychalcone (80)

The IR spectrum revealed the presence of a conjugated alkene group by the presence of peaks at 1649 cm^{-1} and 3049 cm^{-1} for C=C and C-H stretch, respectively. The asymmetric and symmetric C-O-C stretches were observed at 1249 and 1050 cm^{-1}, respectively. The aromatic system was supported by the peaks at 3060 and 1598 cm^{-1} attributed to the aryl C-H and C=C stretch.

The ^{1}H NMR of **80** showed 12 signals. The singlet at δ 6.79 (1H) was assigned the proton *ortho* to both the benzyl and methoxy groups on ring A. One of the AB system protons was assigned the peak at δ 6.65 (1H, d, *J*=9.0 Hz) while the other was assigned the multiplet between 7.81-7.96 (2H) in which the *para* proton of ring C is to be found. The two doublets at δ 7.50 (2H, d, *J*=10.2 Hz) and 7.90 (2H, d, *J*=10.2 Hz) were assigned the *ortho* and *meta* protons of ring B respectively. The α, β-protons in the system were assigned the 2 doublets at 7.84 (1H, *J*=14.4 Hz) and 7.49 (1H, *J*=14.4 Hz), respectively suggesting a *trans* geometry. The doublet at δ 7.00 (2H, *J*=8.6 Hz) was assigned the 2 *ortho* protons on ring C while the multiplet between 7.44-7.53 (2H) was assigned the two *meta* protons on the same ring.

The ^{13}C NMR showed 18 aromatic signals at δ 162.8, 162.6, 159.1, 136.4, 131.5, 130.4, 130.7, 130.4, 128.6, 128.6, 128.12, 128.07, 128.07, 116.2, 113.8, 113.8, 106.4 and 99.5 ppm. From DEPT analysis, the two peaks at δ 55.5 and 55.4 ppm supported the presence of the 2 methoxy groups while the signal at 187.3 confirmed the conjugated ketone. The α, β carbon signals appeared at δ 119.4 and 138.5 ppm, respectively. The methylene signal was observed at 70.0 ppm.

EIMS revealed molecular ion peak at *m/z* 374 [M^+], which is consistent with the formula $C_{24}H_{22}O_4$. The base peak at *m/z* 91 (100%) was attributed to the ion $[C_7H_7]^+$. Other peaks were observed at *m/z* 267 $[C_{17}H_{15}O_3]^+$, 135 $[C_8H_7O_2]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^+$. The MS can be justified by the fragmentation pattern in scheme 22.



Scheme 22: 2-Benzyloxy-4, 4'-dimethoxychalcone mass fragmentation

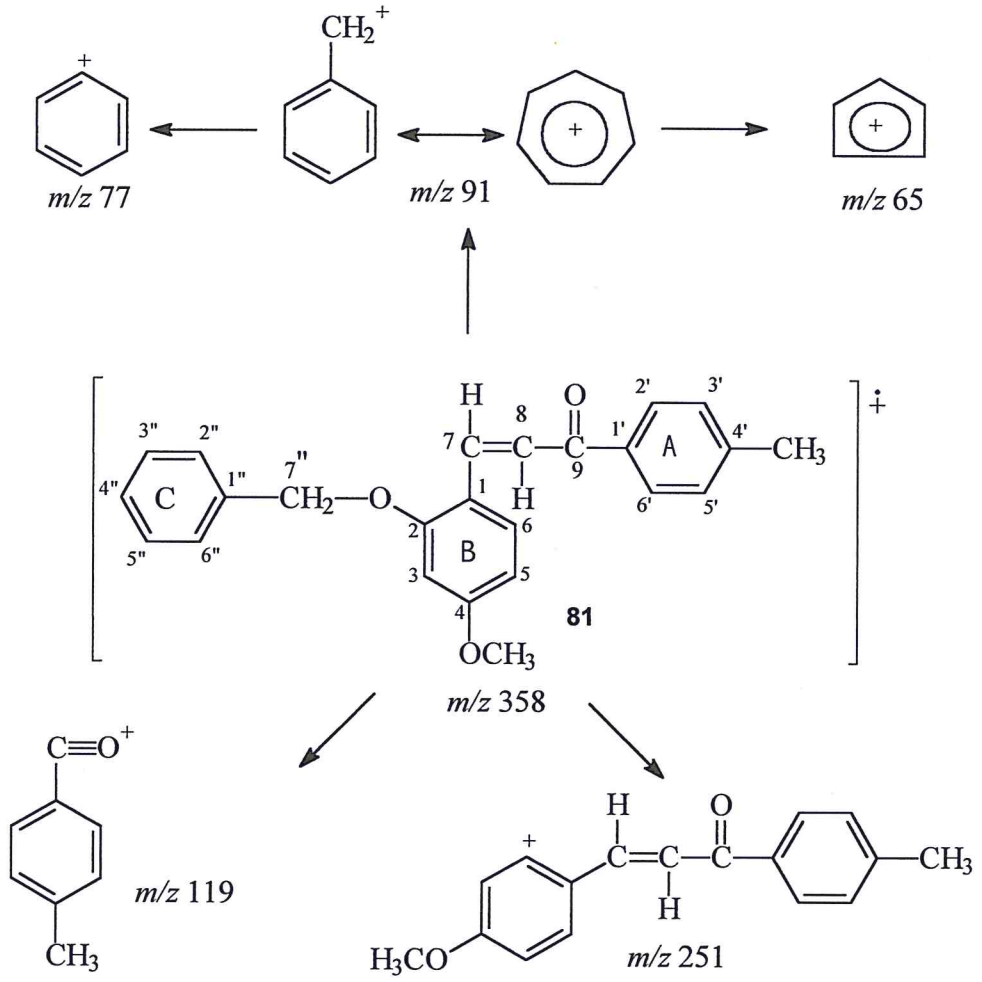
4.5.2 2-Benzyloxy-4-methoxy-4'-methylchalcone (81)

The IR spectrum revealed the presence of an alkene group by peaks at 1651 and 3050 cm^{-1} for C=C and C-H stretch, respectively. The asymmetric and symmetric C-O-C stretches were observed at 1257 and 1049 cm^{-1}, respectively. The aromatic system was supported by the peaks at 3028, 3000, 2950, 2850 and 1598 cm^{-1} attributed to the aryl and aliphatic C-H together with C-C stretch, respectively.

This compound gave similar ^{13}C and ^{1}H NMR spectrum to 2-benzyloxy-4, 4'-dimethoxychalcone (**80**). However, the presence of a methyl group was confirmed by the

peak at δ 2.38 in the ^{1}H and δ 21.2 ppm in ^{13}C NMR spectra of **81** instead of a methoxy group at δ 3.83 and 55.5 ppm, respectively, in **80**.

EIMS revealed molecular ion peak at *m/z* 358 $[M^{+}]$, which is consistent with the formula $C_{24}H_{22}O_3$ for 2-Benzyloxy-4-methoxy-4'-methylchalcone (**81**). The base peak at *m/z* 91 (100%) was attributed to $[C_7H_7]^{+}$. Other peaks were observed at *m/z* 251 $[C_{17}H_{15}O_2]^{+}$, 119 $[C_8H_7O]^{+}$, 77 $[C_6H_5]^{+}$, 65 $[C_5H_5]^{+}$. The MS can be justified by the fragmentation pattern in scheme 23.



Scheme 23: 2-Benzyloxy-4-methoxy-4'-methylchalcone mass fragmentation

In summary a good number of the targeted routes afforded the expected products in good yield (Table 5). The melting points of the compounds obtained are also summarized in table 5.

Table 6: Summary of yields and melting points of synthesized compounds.

Compound	Yield (%)	mp (^{0}C)
75	78	54-56
77	76	76-77
78	65	46-48
80	82	112-112.5
81	78	133-134
82	82	42-43
83	81	34-35

These compounds were then subjected to larvicidal bioassay to establish the effect of the new functional groups on activity.

CHAPTER 5

STRUCTURAL ACTIVITY RELATIONSHIP STUDIES

The synthetic derivatives obtained were subjected to larvicidal assays and their LD values which are summarized in table 6 obtained by probit analysis (Busvine, 1971).

Table 7: Summary of LD values for synthetic analogues

Compound	Duration (hr)	LD (mg/ml)			
		25	50	75	90
75	24	0.007	0.011	0.018	0.029
	48	0.007	0.010	0.016	0.024
	72	0.006	0.009	0.015	0.022
77	24	0.028	0.028	0.040	0.057
	48	0.016	0.023	0.035	0.049
	72	0.013	0.019	0.027	0.036
78	24	0.062	0.075	0.092	0.109
	48	0.058	0.071	0.086	0.102
	72	0.058	0.68	0.079	0.091
80	24	0.044	0.059	0.080	0.106
	48	0.042	0.052	0.078	0.102
	72	0.039	0.048	0.072	0.098
81	24	0.415	0.633	0.738	1.248
	48	0.405	0.613	0.696	1.202
	72	0.400	0.591	0.659	0.925
82	24	0.0027	0.0048	0.0085	0.0143
	48	0.0026	0.0040	0.0065	0.0097
	72	0.0021	0.0035	0.0058	0.0092
83	24	0.0006	0.0012	0.0025	0.0047
	48	0.0006	0.0011	0.0022	0.0039
	72	0.0005	0.0009	0,0018	0.0026

To evaluate the influence of the hydroxyl group on the larvicidal activity **59** was benzylated. The product 2-benzyloxy-4-methoxybenzaldehyde (**75**) showed an improved activity (LD_{50} 0.011 mg/ml), being twice as effective as **59**. It was suspected that the activity of the benzyl ether prepared from **59** was profoundly boosted by the presence of the *ortho* benzyl substituent. This prompted the preparation of a benzyl ether of salicaldehyde (**69**), which gave 2-benzyloxybenzaldehyde (**82**). Compound **82** was assayed and displayed improved activity (LD_{50} 0.0048 mg/ml) compared to **69** (LD_{50} 0.009 mg/ml). Once again here, benzylation was shown to double the activity. These two observations confirmed the little role played by methoxyl group in the larvicidal activity of these type of compounds.

To determine whether the increment was due to a mutual effect of the carbonyl and the benzyl group, phenol, **67** (LD_{50} 0.054 mg/ml) was also benzylated to give benzyl phenyl ether (**83**), which showed the highest activity (LD_{50} of 0.0012 mg/ml) being 45 times higher than phenol, 4 times 2-benzyloxybenzaldehyde (**82**) (LD_{50} 0.0048 mg/ml), 8 times salicaldehyde (**69**) (LD_{50} 0.009 mg/ml), 10 times 2-benzyloxy-4-methoxybenzaldehyde (**75**) (LD_{50} 0.011 mg/ml) and 20 times 2-hydroxy-4-methoxybenzaldehyde (**59**) (LD_{50} 0.022 mg/ml). This confirmed the antagonistic effect of the aldehyde group.

To determine the role of the benzyl group in **75** it became necessary to change to benzoyl group and evaluate the effect that it would have on the activity of the parent compound. Esterification of **59** with benzoyl bromide yielded 2-benzoyloxy-4-methoxybenzaldehyde (**77**). Unlike the benzyl ether derivatives, **77** revealed a diminishing activity (LD_{50} of 0.028 mg/ml), which was slightly lower than **59** (LD_{50} 0.022 mg/ml) and about 2.5 times lower than **75** (LD_{50} 0.011 mg/ml).

From the preliminary SAR the carbonyl group presents itself as an equally important group as the hydroxyl group in eliciting larvicidal activity. To elaborate this, a Grignard reaction was carried out on **75** giving the secondary alcohol (**78**). The compound showed a relatively lower larvicidal activity (LD_{50} 0.075 mg/ml) than **75**.

To further evaluate the role of the carbonyl group in **59**, two chalcones, **80** and **81** were obtained by a Claisen-Schmidt type reaction on **75**. They were also assayed against *An. gambiae* larvae. The two compounds showed substantial decrease in activity (LD_{50} 0.059 and 0.633 mg/ml for **80** and **81**, respectively). The large difference in activity between these two compounds may be attributed to the *para*-methoxy and methyl substituents on the additional ring in **80** and **81**, respectively. Probably, the *p*-methoxy group has a potentiating effect on the activity contrary to the observations in the simple benzene derivatives.

These results suggest that the benzyl group has a potentiating effect on the larvicidal activity of the ether as compared to the phenols. The effect of the benzyl ether is dependent on the presence of other functional groups on the ring. For instance, *ortho*-substitution of **83** with the carbonyl group lowered the activity as demonstrated by **82**, which had an activity 4 times lower. Even worse is the effect of the *meta*-methoxy substitution giving **75**, which saw the activity, falling to half that of **82** and one ninth that of **83**.

The results obtained for **77** suggest that an ester moiety exerts a negative effect on the larvicidal activity of phenols. The carbonyl group of the ester may be responsible for the negative effect, when compared to the ethers.

The most important group responsible for the activity of **59** is the carbonyl. This was confirmed by the low activities of **78** and the chalcones (**80** and **81**), which despite having the highly activating benzyl group had their activities lowered. It is also apparent that despite the hydroxyl being an activating group there are other functional groups such as the benzyl group that may enhance the activity. Like in the disubstituted compounds, it appears that the methoxy group lowers the activity of **59**. However, the chalcone derivative with a *para*-methoxy substituent gave higher activity than the one with a *para*-methyl group suggesting the possibility of there existing other groups that can further enhance the activity of the chalcones.

The phenyl ether groups have been used in various classes of insecticides to enhance their activities. For instance Ferenc and Julius (1972) reported the enhancement of growth regulatory activity of juvenile hormone analogs by synthesizing various geranyl phenyl ethers. An example is the phenylgeranyl ether [1-(4'-ethylphenoxy-6,7-epoxy-3,7-dimethyl-2-octene] (**54**), which displayed high morphogenic activity against *Cx. pipiens* larvae (ED_{50} of 10^{-7} mg/ml). Other phenyl ether-based insecticides include phenoxyphenoxy carbamate (**51**) (Karrer and Farooq, 1981), permethrin (**39**), cypermethrin (**40**), fenvalerate (**41**) and deltamethrin (Elliot, 1989; Nisha and Kalyanasundaran, 1992). The same phenomenon is being displayed by the three phenyl ether derivatives synthesized in this study. It is encouraging that such simple compounds can be potent larvicides against *An. gambiae*. Efforts to improve the activity of this compound through further structure activity studies are underway.

CHAPTER 6

6.1 CONCLUSIONS

Natural products of plant origin are a good source of lead bio-active substances, which may serve as models for the synthesis of practical insecticides. 2-Hydroxy-4-methoxybenzaldehyde from a natural source served as a good chemical lead by exhibiting larvicidal activity against mosquito larvae.

The closely related compounds vanillin and 2-hydroxy-4-methoxysalicylic acid elicited very low or no activity. Salicaldehyde (**69**), which is a simpler derivative, had a higher larvicidal activity (LD_{50} 0.009 mg/ml (9 ppm) within 24 hrs) than 2-hydroxy-4-methoxybenzaldehyde.

It is evident that the carbonyl and hydroxyl groups are important functional groups for the larvicidal activity of 2-hydroxy-4-methoxybenzaldehyde (**59**). The relative positions of these two substituents have a role to play towards the larvicidal activity of the compounds studied. The optimal arrangement was found when the two groups are *ortho* to each other.

Simple synthetic transformations were used to produce more active compounds than the parent compounds in the study. For instance the single step benzylation of compounds with a hydroxyl group resulted in derivatives with higher larvicidal activity. These included 2-benzyloxy-4-methoxybenzaldehyde (**75**), 2-benzyloxybenzaldehyde (**82**) and benzylphenyl ether (**83**) (LD_{50} 0.011 (11 ppm), 0.0048 (4.8 ppm) and 0.0012 mg/ml (1.2 ppm) , respectively, within 24 hours).

Benzylphenyl ether (**83**) was the simplest of all the synthetic derivatives prepared and yet the most active compound in the study. This observation suggests that the benzyloxy group has a greater potentiating effect than the carbonyl and the hydroxyl groups. The observed activity of this compound conforms to what has been reported. Apparently phenyl ether moiety is an inherent part of most IGRs and larvicides such as phenoxyphenoxy carbamate (**51**), 3-[4-(3-methylphenoxy)phenoxy]propionadoxime-O-

isopropylether (**50**) (LD_{50} 1.8 ppm), phenylgeranyl ether (**54**) (LD_{50} 0.0001 ppm) and (4-phenoxyphenoxy)acetaldoxime O-ether (**49**).

6.2 RECOMMENDATIONS

The active compounds should be tested against other species of anopheline and culicine larvae.

Field trials should be done for the most active compounds to find out their stability, toxicity and applicability in real life situations. This will also be necessary to assess the frequency of application and therefore affordability.

The compounds should be tested for their efficacy against the adult stages of anopheline and culicine mosquitoes to see if they can be used in the treatment of clothing such as bednets and curtains.

The compounds should be tested against other medical and veterinary vectors, agricultural and domestic pests to ascertain the spectrum of activity and their suitability as general-purpose insecticides.

The toxicity of these compounds to beneficial insects like bees, fish and other aquatic organisms should also be investigated to establish their ecological safety.

Similarly, mammalian toxicity should also be evaluated and environmental persistence also examined.

Further structure activity studies should be conducted on other functional groups to determine whether there exist other more potent derivatives and analogues. A quantitative structure activity relationship (QSAR) study should be done to establish which physical and chemical parameters influence the larvicidal activity of these compounds and further optimization of their larvicidal activity done.

Long lasting assays should be carried out to determine whether the active compounds can act as insect growth regulators. These may require treatment with sub-lethal doses and monitoring the larvae upto adult and their progeny upto F_4 generation.

CHAPTER 7

EXPERIMENTAL

7.1 General procedures for synthesis

Glassware: All recyclable glassware were soaked in chromic acid overnight, washed thoroughly in hot water and soap, rinsed with acetone and oven dried at 110° C overnight.

All solvents were analytical grade from Sigma Aldrich or Merck-BDH and were used as supplied.

Reagents of over 95% purity were used as supplied unless stated otherwise.
Diethyl ether was first passed through alumina packed column followed by distillation and stored under 5-8 mesh molecular sieves.

Chromatography: Analytical thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates of 5 x 10 cm and 0.20 mm film thickness. The plates were first visualized under UV light on both long (365 nm) and short wave lengths (254 nm) then sprayed with 25% sulphuric acid in methanol and oven dried.

Column chromatography was done using silica gel 60 (0.040 – 0.063 mm, 230 – 400 mesh) (Merck). Elution was done using different solvent systems as indicated in the relevant sections. The eluent fractions from chromatography were analyzed by TLC, fractions with same R_f values combined and solvent evaporated *in vacuo*.

Melting points of the pure recrystalized compounds were determined on Sanyo Gallenkamp electronic melting point apparatus and are uncorrected.

Infrared (IR) spectra were obtained using analytical grade KBr from Shimadzu Fourier transform spectrophotometer. Absorption frequencies (γ_{max}) were recorded in wave numbers cm^{-1} and (%) transmittance (T).

Ultraviolet (UV) Spectra were obtained using a Shimadzu double beam spectrophotometer (UV-180). Absorbance maxima (λ_{max}) were recorded in nanometers (nm).

Nuclear magnetic resonance (NMR) spectra were recorded on Varian Mercury Gemini 200 MHz spectrometer. ^{1}H and ^{13}C spectra were recorded at 200 and 50 MHz, respectively. Samples were dissolved in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide $(CD_3)SO_2$. Chemical shifts were reported as δ values or parts per million (ppm) relative to TMS. ^{13}C NMR spectra were recorded in the proton noise decoupled (PND) mode and the multiplicities determined from the DEPT experiments. The multiplicities of the NMR peaks are indicated by: s= singlet, d=doublet, t= triplet, q=quartet, quin=quintet, sex=sextet, m=multiplet, br=broad.

Electron impact mass spectra (EIMS) were obtained from a Fission VG Platform II spectrometer at 70 eV.

2-Benzyloxy-4-methoxybenzaldehyde (75)

A mixture of 2-hydroxy-4-methoxybenzaldehyde (0.46 g, 3 mmol), benzyl chloride (0.38 g, 1.5 mmol), potassium carbonate (0.21 g, 3.6 mmol) and ethanol (25 ml) was stirred under reflux for 5 hrs. The mixture developed a deep green color and later became brown. The mixture was cooled to room temperature, potassium chloride removed by filtration and washed with ethanol. The filtrate was evaporated *in vacuo* and purified by column chromatography to give 0.39 g (78%) of the product (R_f 0.33, 8:2 hexane:EtOAc, SiO_2). Alternatively, after refluxing for 5 hrs the mixture was cooled overnight in a refrigerator forming long needle like crystals. The crystals were separated by filtration under suction, washed in cold ethanol giving white needle-like crystals in 83% with an R_f of 0.33. Found: mp 54-56 ^{0}C; IR γ_{max} (KBr), 3020, 2867, 2830, 2780,1670,1577,1502 cm^{-1}; λ_{max} (DMSO) 324 nm; ^{1}H NMR (200 MHz, $(CD_3)_2SO$) δ 10.22 (s, 1H, -CHO), 7.66 (dd, 1H, J=8.8, 1.2 Hz, H-5), 7.30-7.51 (m, 5H, C_6H_5), 6.79 (d, 1H, J=1.2 Hz, H-3), 6.65 (d, 1H, J=8.8 Hz, H-6), 5.27 (s, 2H, H-7), 3.83 (s, 3H,4-OCH_3); ^{13}C NMR (200 MHz, $(CD_3)_2SO$) δ 187.9 (s, CHO), 166.6 (s, C-4), 163.2 (s, C-2), 137.1 (s, C-1'), 130.6 (d, C-6), 129.2 (d, C-2',6'), 128.7 (d, C-4'), 128.2 (d, C-3',5'),

119.3 (s, C-1), 107.8 (d, C-5), 100.4 (d, C-3), 70.7 (t, C-7), 56.5 (q, -OCH_3) ppm; EIMS (m/z) 242 [M^+] (1%), 213 (20), 151 (24), 91 (100), 77 (8), 65 (82), 51 (26), 39 (30).

2-Benzyloxybenzaldehyde (82)

Benzyl chloride (2.06 g, 16 mmol), 2-hydroxybenzaldehyde (**69**) (2 g, 16 mmol), potassium carbonate (1.5 g, 10 mmol) and ethanol (25 ml) were mixed and stirred under reflux 5 hrs. The mixture developed a deep green color and later became brown. The mixture was cooled to room temperature, potassium chloride removed by filtration and washed with ethanol. The filtrate was evaporated *in vacuo* to give 1.74g (82%) white needle-like crystals (R_f 0.53, 8:2, hexane:EtOAc, SiO_2). Found: mp 42-43 ^{0}C ; IR γ_{max} (KBr), 3059, 2877, 2750, 1681,1596, 1500, 1238, 1161 cm^{-1}; λ_{max} (DMSO) 324 nm; ^{1}H NMR (200 MHz, $(CD_3)_2SO$) δ 10.41 (s, 1H, -CHO), 7.67 (dd, 1H, J=8.4, 8.6 Hz, H-5), 7.61 (d, J=8.4 Hz, H-6), 7.37 (d, 1H, J=7.4 Hz, H-3), 7.27-7.42 (m, 5H, H-2', H-3', H-4', H-5', H-6'), 7.07 (dd, 1H, J=7.2, 7.4 Hz, H-4), 5.27 (s, 2H, OCH_2); ^{13}C NMR (200 MHz, $(CD_3)_2SO$) δ 189.9 (d, -CHO), 161.3 (s, C-2), 137.13 (d, C-1'), 137.05 (s, C-6), 129.2 (d, C-2', C-6'), 128.7 (d, C-4), 128.5 (d, C-4'), 128.2 (d, C-3', 5'), 125.2 (s, C-1), 121.6 (s, C-5), 114.7 (d, C-3), 70.5 (t, C-7) ppm; EIMS (m/z) ; 212 [M^+] (9%), 183 (23), 121 (58), 92 (48), 91 (100), 77 (10), 65 (87), 51 (21) 39 (51).

Benzylphenyl ether (83)

Hydroxybenzaldehyde (**67**) (0.46 g, 5 mmol), benzyl chloride (0.38 g, 5 mmol), potassium carbonate (0.21 g, 1.5 mmol) and ethanol (25 ml) were mixed and stirred under reflux for 5 hrs. The mixture developed a deep green color and later became brown. The mixture was cooled to room temperature, potassium chloride removed by filtration and washed with ethanol. The filtrate was evaporated *in vacuo* to give 0.68g (81%) of white needle-like crystals (R_f 0.78, 8:2, hexane: EtOAc, SiO_2). Found: mp 34-35 ^{0}C; IR γ_{max} (KBr); 3035, 2862, 1245 cm^{-1}; λ_{max} (DMSO) 277 nm; ^{1}H NMR (200 MHz, $(CD_3)_2SO$); δ 7.27-7.42 (m, 7H, H-2', H-3', H-4', H-5', H-6', H-3, H-5), 6.99 (d, 2 H, J=8.4 Hz, H-2, H-6), 6.92 (m, 1H, H-4), 5.07 (s, 2H, H-7); ^{13}CNMR (200 MHz, $(CD_3)_2SO$) δ 159.0 (s, C-1), 137.8 (s, C-1'), 130.2 (d, C-3, 5), 129.1 (d, C-2', 6'), 128.5 (d, C-4'), 128.4 (d, C-3', 5'), 121.4 (d, C-4), 115.4 (d, C-2, 6), 69.7 (t, C-7) ppm; EIMS (m/z) 184 [M^+] (29%), 92 (32), 91 (100), 77 (14), 65 (89), 51 (37), 39 (73).

2-Benzoyloxy-4-methoxybenzaldehyde (77)

Benzoyl bromide (1.5 g, 6 mmol) was added drop wise to a stirred solution of 2-hydroxy-4-methoxybenzaldehyde (**59**) (1 g, 6.6 mmol) in 20 ml of anhydrous pyridine over a period of 10 minutes. The solution was warmed to 50 ^{0}C and stirred for 40 min, poured into a mixture of 20 g ice and 50 ml of 1M HCl and stirred until a fine crystalline suspension was formed. The crude product was collected by filtration, washed with ice-acid water and recrystalized from 80% MeOH to give 1.95g (78.3%) of white crystalline product (R_f 0.32, 8:2 hexane: EtOAc, SiO_2). Found: mp 76-77 ^{0}C ; IR γ_{max} (KBr) 2767, 1693, 1566, 1500, 1265, 1217, 1132 cm^{-1}; λ_{max} (DMSO) 304 nm; ^{1}H NMR (200 MHz $(CD_3)_2SO$) δ 10.04 (s, 1H, -CHO), 8.21 (d, 2H, J=7.2 Hz, H-2', H-6'), 7.88 (d, 1H, J=8.6Hz, H-6), 7.66 (dd, H, J=7.2, 7.5 Hz, H-4'), 7.52 (dd, 2H, J=7.0, 7.5 Hz, H-3', H-5'), 6.92 (dd, 1H, J=8.6, 2.1 Hz, H-5), 6.80 (d, 1H, J=2.1 Hz, H-3), 3.87 (s, 3H, 4-OCH_3); ^{13}C NMR (200 MHz, $(CD_3)_2SO$) δ 187.2 (s, -CHO), 165.2 (s, C-4), 164.8 (s, C-2), 154.0 (s, C-7), 134.0 (s, C-4'), 132.0 (d, C-6), 130.3 (d, C-2', 6'), 130.1 (d, C-1'), 128.7 (d, C-3', 5'), 121.8 (C-1), 112.6 (d, C-5), 108.6 (d, C-3), 55.9 (q, OCH_3) ppm; EIMS (m/z) 256 [M^+] (1%), 151 (16), 122 (86), 105 (97), 77 (100), 51 (62).

Benzyloxy-4-methoxy(1-methyl) benzyl alcohol (78)

2-Benzyloxy-4-methoxybenzaldehyde(75) (0.05 g, 2 mmol) was dissolved in anhydrous ether (10 ml) and put in a dropping funnel. Methyl magnesium bromide (1.4 M solution) (1.43 ml, 2 mmol) was added to a three-neck flask in which a stream of dry nitrogen was flowing and the 2-benzyloxy-4-methoxybenzaldehyde solution added slowly while vigorously stirring. The mixture was stirred for 4 hrs, 5 ml mixture of distilled water/HCl (50:50) added and the organic layer separated, dried with $CaSO_4$ and evaporated *in vacuo*. Analysis of the concentrate by TLC revealed two spots (R_f 0.30 and 0.33, 8:2 hexane: EtOAc, SiO_2). These were separated by column chromatography using hexane/ethyl acetate. The product (R_f 0.30) was recovered in 65% (0.03g) yield. Found: mp 46-48 ^{O}C; IR γ_{max} (KBr), 3284, 2934, 2831,1504, 1255,1195, 1082, 1014 cm^{-1}; λ_{max} (DMSO) 305 nm; ^{1}H NMR (200 MHz, $(CD_3)_2SO$), δ 7.40-7.43 (m, 5H, H-2', H-3', H-4', H-5', H-6'), 7.34 (d, 1H, J=8.4 Hz, H-6), 6.58 (s, 1H, H-3), 6.52 (d, 1H, J=8.4 Hz, H-5), 5.11 (s, 2H, -OCH_2-), 5.01 (q, 1H, J=5.6 Hz, H-8), 4.92 (br, 1H, OH), 3.71 (s, 3H, 4-OCH_3), 1.26 (d, 3H, J=5.6 Hz, -CH_3); ^{13}C NMR (200 MHz, $(CD_3)_2SO$) δ 158.6 (s, C-2),

154.8, (s, C-4), 137.1 (s, C-1'), 128.2 (d, C-2', 6'), 127.8 (d, C-1), 127.5 (d, C-6), 127.0 (d, C-3', 5'), 125.9 (d, C-4'), 104.5 (d, C-5), 99.0 (d, C-3), 69.0 (t, C-7), 62.2 (d, C-8), 55.0 (q, $-OCH_3$), 24.9 (q, C-9) ppm; EIMS (m/z) 258 [M^+] (4%), 240 (8), 150 (14), 91 (100), 77 (8), 65 (16).

2-Benzyloxy-4, 4'-dimethoxychalcone (80)

Sodium hydroxide (0.5 g, 12.5 mmol), 5 ml of distilled water and 5 ml of ethanol were placed in a 50 ml round bottom flask equipped with a magnetic stirrer. The flask was immersed in an ice bath and 0.60 g (4 mmol) of *p*-methoxyacetophenone dissolved in ethanol 10 ml added while stirring followed by drop wise addition of 2-benzyloxy-4-methoxybenzaldehyde (1 g, 4 mmol). The temperature of the mixture was maintained at 25^0C and stirring continued for 4 hrs. The stirrer was removed and the reaction mixture refrigerated overnight. The product was filtered under suction, washed with 40 ml of cold distilled water followed by ice-cold ethanol and recrystalized from boiling ethanol to give pale 1.2g (82%) of yellow crystals of **80** (R_f 0.29, 8:2 hexane: EtOAc, SiO_2). Found: mp 112-112.5^{0}C; IR γ_{max} (KBr), 3350, 3060, 3049, 3000, 1649, 1598, 1249, 1050 cm^{-1}. λ_{max} (DMSO) 376 nm; ^{1}H NMR (200 MHz, $(CD_3)_2SO$) δ 7.81-7.96 (m, 2H, H-4", H-5), 7.90 (d, 2H, J=10.2 Hz, H-3', H-5'), 7.84 (d, 1H, J=14.4 Hz, H-7), 7.50 (d, 2H, J=10.2 Hz, H-2', H-6'), 7.49 (d, 1H, J=14.4 Hz, H-8), 7.44-7.53 (m, 2H, H-3", H-5"), 7.00 (d, 1H, J=8.6 Hz, H-2" H-6"), 6.79 (s, 1H, H-3), 6.65 (d, 1H, J=9.0 Hz, H-6), 5.23 (s, 2H, OCH_2), 3.85 (s, 3H, 4-OCH_3), 3.83 (s, 3H, 4'-OCH_3); ^{13}C NMR (200 MHz $CD_3)_2SO$) δ 187.3 (C, C-9), 162.8 (s, C-4'), 162.6 (s, C-4), 159.1 (s, C-2), 138.5 (d, C-7), 136.5 (s, C-1"), 131.5 (d, C-1), 130.7 (s, C-6), 130.4 (d, C-2', 6'), 128.6 (d, C-2", 6"), 128.13 (d, C-4"), 128.07 (d, C-3", C-5"), 119.4 (d, C-8), 116.2 (s, C-1'), 113.8 (d, C-3', C-5'), 106.4 (d, C-5), 99.5 (d, C-3), 70.0 (t, -C-7"), 55.5 (t, 4-OCH_3), 55.4 (q, 4'-OCH_3) ppm; EIMS (m/z) 374 [M^+] (3%), 267 (40), 135 (81), 91 (100), 77 (22), 65 (17).

2-Benzyloxy-4-methoxy-4'-methylchalcone (81)

A mixture of sodium hydroxide (0.3 g, 7.5 mmol), distilled water (5 ml) and ethanol (5 ml) were placed in a 50 ml round bottom flask equipped with a magnetic stirrer. The flask was immersed in an ice bath and 0.16 g (1.1 mmol) of *p*-methylacetophenone dissolved in ethanol 10 ml added while stirring followed by drop wise addition of 2-

benzyloxy-4-methoxybenzaldehyde (0.3 g, 1.2 mmol). The temperature of the mixture was maintained at 25^0C and stirring continued for 4 hours. The stirrer was removed and the reaction mixture refrigerated for 18 hours. Pale yellow needle-like crystals (R_f 0.42, 8:2 hexane: EtOAc, SiO_2) were recrystalized from boiling ethanol in 78% (0.36g) yield. Found: mp 133-134^{0}C; IR γ_{max}(KBr), 3350, 3050, 3028, 3000, 2950, 2850, 1651, 1598, 1257, 1049 cm^{-1}; λ_{max} (DMSO) 374 nm; ^{1}H NMR (200 MHz $(CD_3)_2SO$) δ 7.71-7.93 (m, 5H, H-5, H-7, H-2', H-6', H-4"), 7.47-7.53 (m, 2H, H-3", H-5"), 7.49 (d, 1H, J=10.8 Hz, H-3', H-5'), 7.48 (d, 1H, J=16.4, H-8), 7.29 (d, 1H, J=8.0 Hz, H-2", H-6"), 6.78 (s, 1H, H-3), 6.65 (d, 1H, J=8.4 Hz, H-6), 5.22 (s, 2H, -OCH_2-), 3.83 (s, 3H, 4-OCH_3), 2.38 (s, 3H, 4' -CH_3); ^{13}C NMR (200 MHz, $(CD_3)_2$ SO) δ 188.1 (s, C-9), 162.5 (s, C-4), 158.9 (s, C-2), 142.8 (s, C-4'), 138.9 (d, C-7), 136.2 (s, C-1") 135.1 (s, C-1'), 131.5 (s, C-1), 129.0 (d, C-2', 6'), 128.4 (d, C-3', C-5'), 128.0 (d, C-2", C-6"), 127.9 (d, C-3", 5", 4"), 119.2 (d, C-8), 115.9 (s, C-1'), 106.3 (d, C-5), 99.4 (d, C-3), 69.9 (t, C-7"), 55.5 (q, 4-OCH_3), 21.2 (q, 4'-CH_3) ppm; MS m/z [M^+1] 359 (1%), 358 (4), 251 (37), 119 (85), 91 (100), 65 (43), 51 (7).

7.2 General procedures for larvicidal bioassay

Preparation of stock solutions: The sample (100 mg) was dissolved in 10.0 ml of acetone. The stock solutions were diluted as appropriate to obtain the required concentration ranges (0.1-100 ppm).

Preliminary bio-assay: Several compounds were screened according to WHO (1996) protocol at 100 ppm to determine whether they are active as larvicides of *An. gambiae*. The required amount of toxicant was added into a beaker containing 20 3rd instar *An. gambiae* larvae in 100 ml of distilled water.

Detailed assays: The same protocol was used on the synthetic and active commercially available compounds. This was done in various concentrations (0.1, 0.08, 0.06, 0.02, 0.01, down to 0.001 mg/ml). Each concentration was run in triplicate and repeated on three different days. After 24 hours of exposure of the larvae under

controlled temperature holding room (38°C) and humidity, mortality readings were recorded. Similar readings were taken after 48 and 72 hours of exposure.

% Mortality was determined using the formula:

% Mortality=[y/x] x100

y=mean death count

X=initial larvae population

Where mortality was observed in the controls, Abbot's (1925) formula was used to correct the percentage mortality:

Corrected % mortality = 100 X (T% - C% / 100% - % C)

T% = the percentage of dead test organisms

C% = the percentage of dead control organisms

The regression equation was obtained using probit analysis involving log dose and probit transformation of dose and % mortality using the probit plane model. A plot of probit transformation versus log dose was obtained using Microsoft Excel® program. The regression equation was used to calculate the expected probits and the lethal doses (LD_{25}, LD_{50}, LD_{75}, LD_{90}) (Busvine, 1971).

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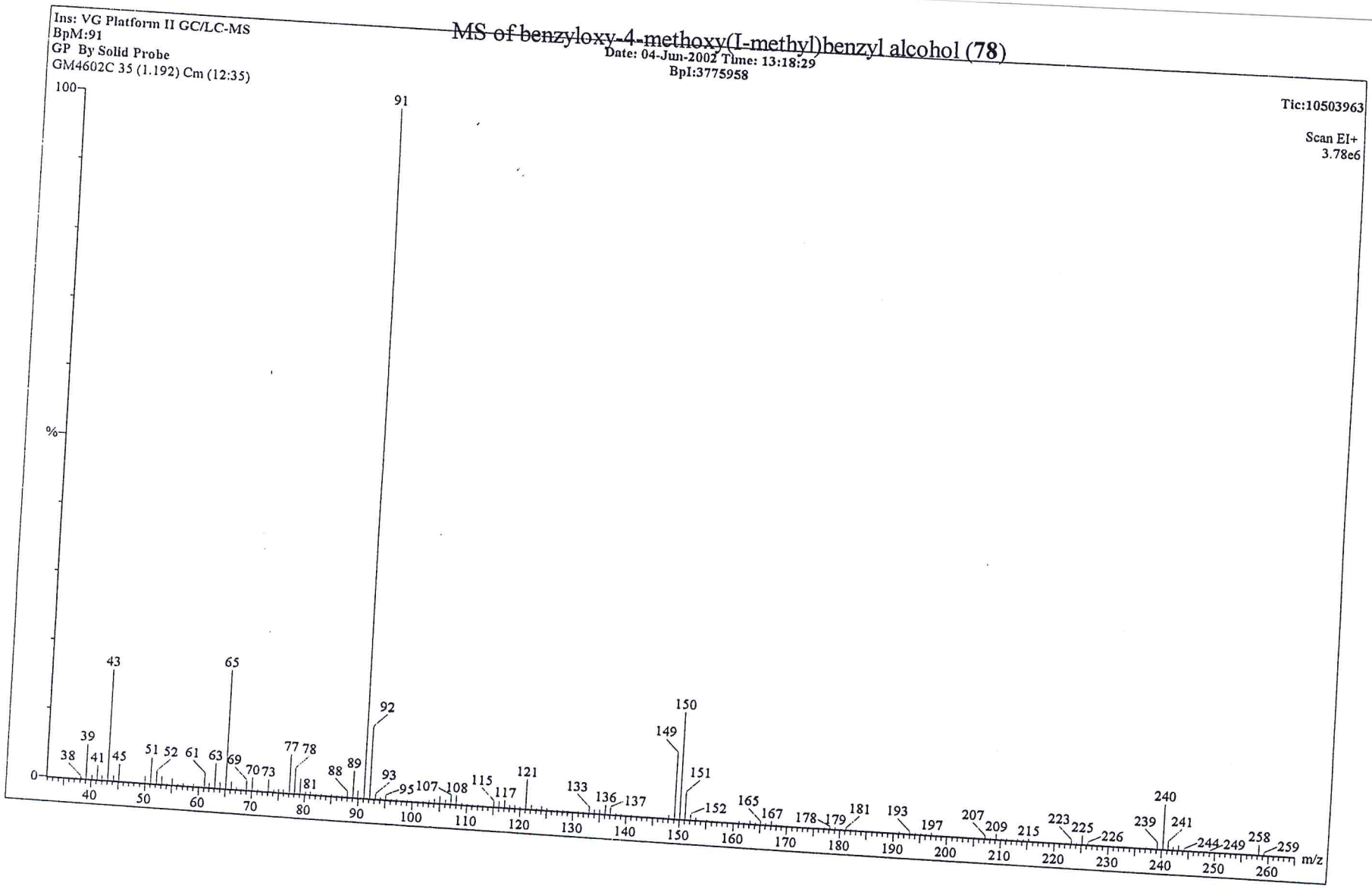
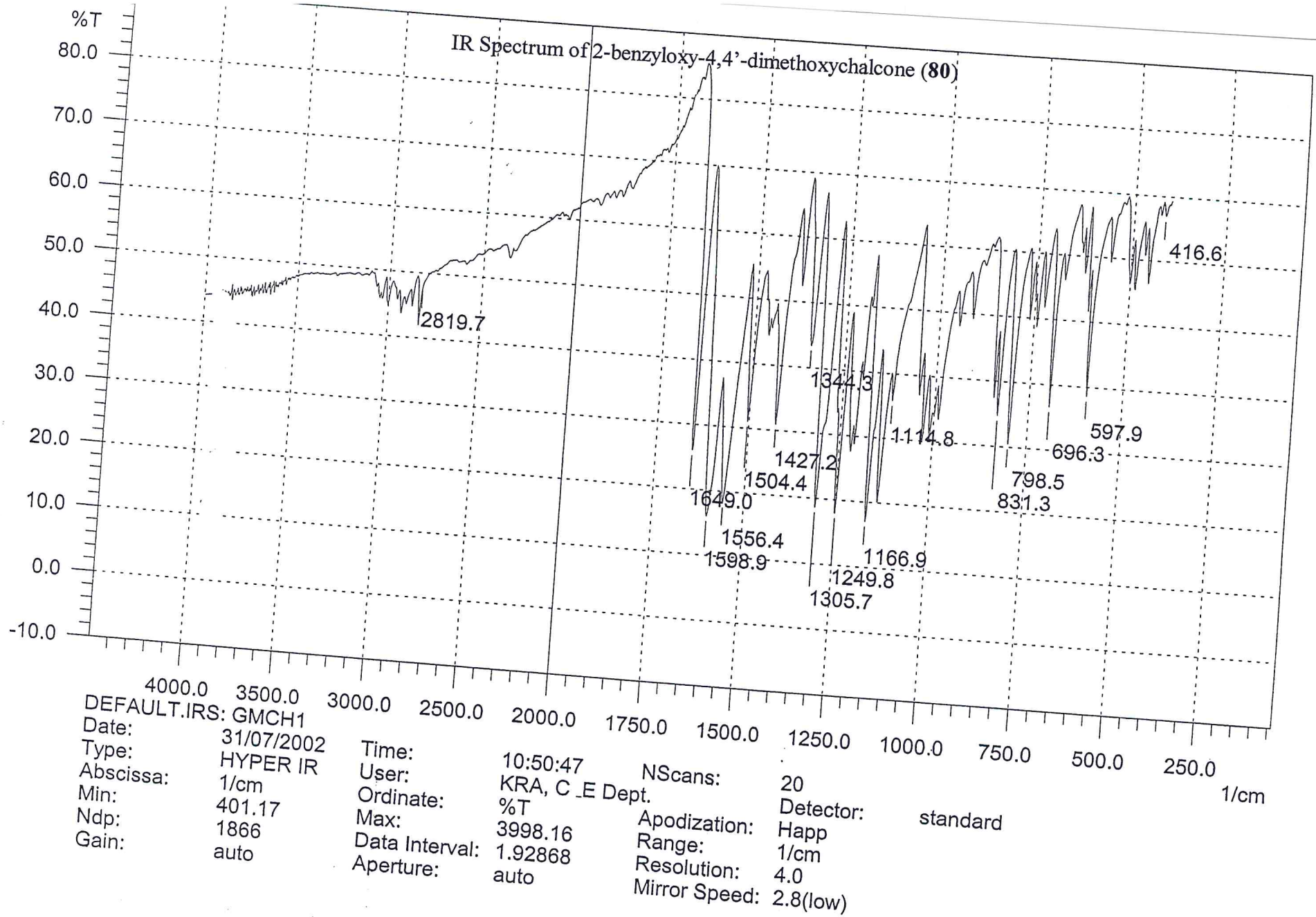
MS of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)




IR Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (80)
%T
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
2819.7
1649.0
1598.9
1556.4
1504.4
1427.2
1344.3
1305.7
1249.8
1166.9
1114.8
831.3
798.5
696.3
597.9
416.6
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
250.0
1/cm
DEFAULT.IRS: GMCH1
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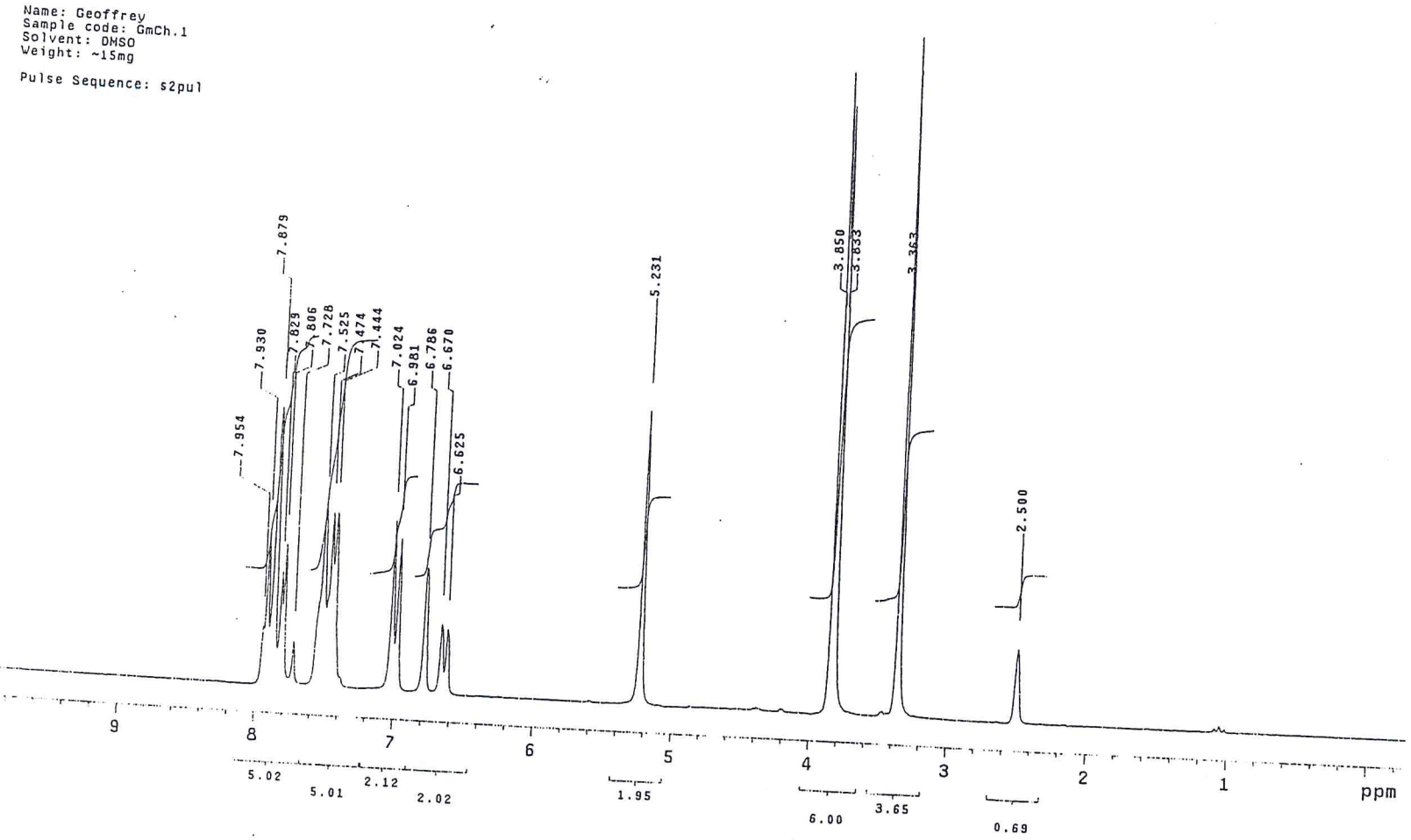
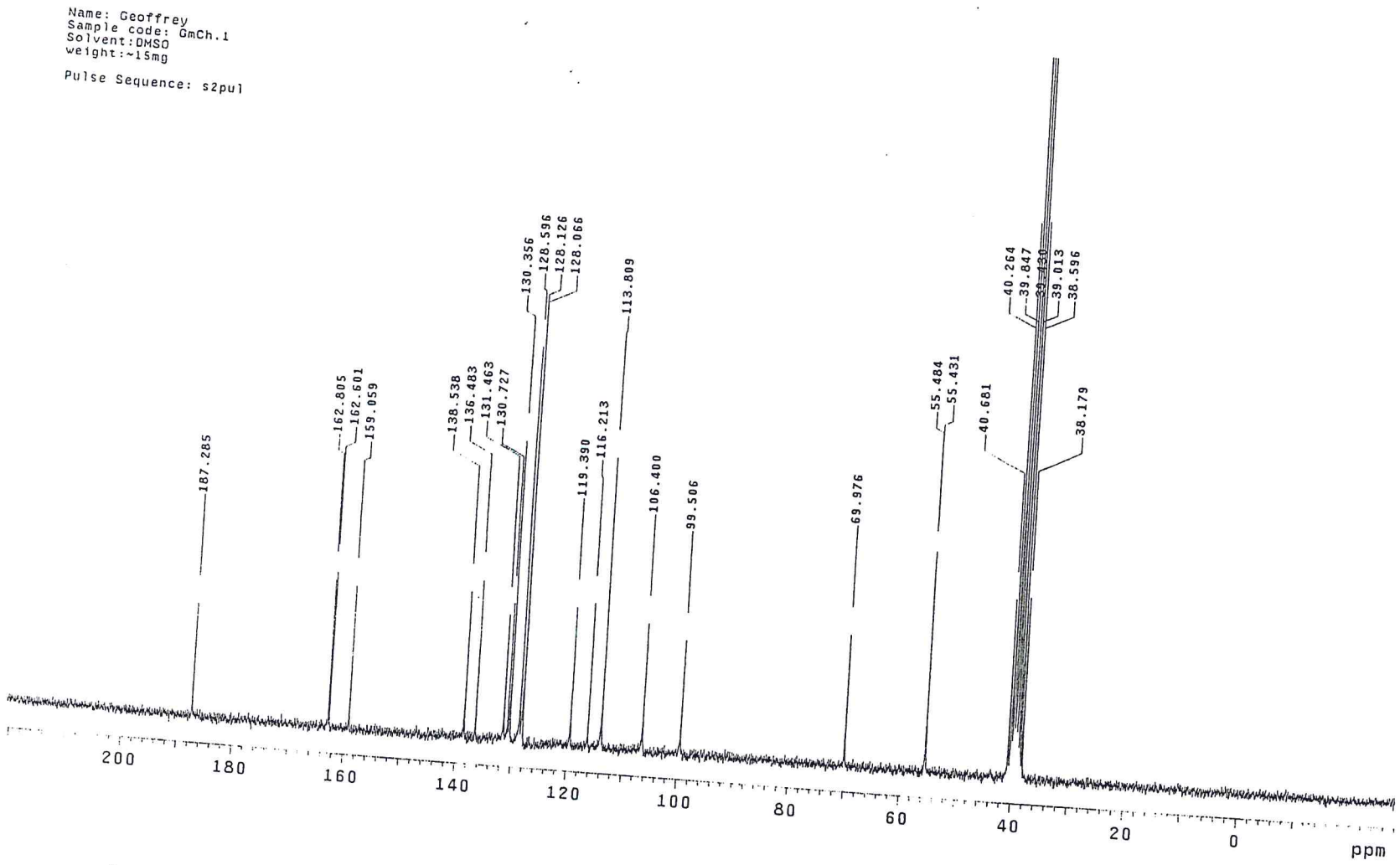
Detector: standard
Abscissa: 1/cm
Ordinate: %T
Min: 401.17
Max: 3998.16
Apodization: Happ
Ndp: 1866
Data Interval: 1.92868
Range: 1/cm
Gain: auto
Aperture: auto
Resolution: 4.0
Mirror Speed: 2.8(low)

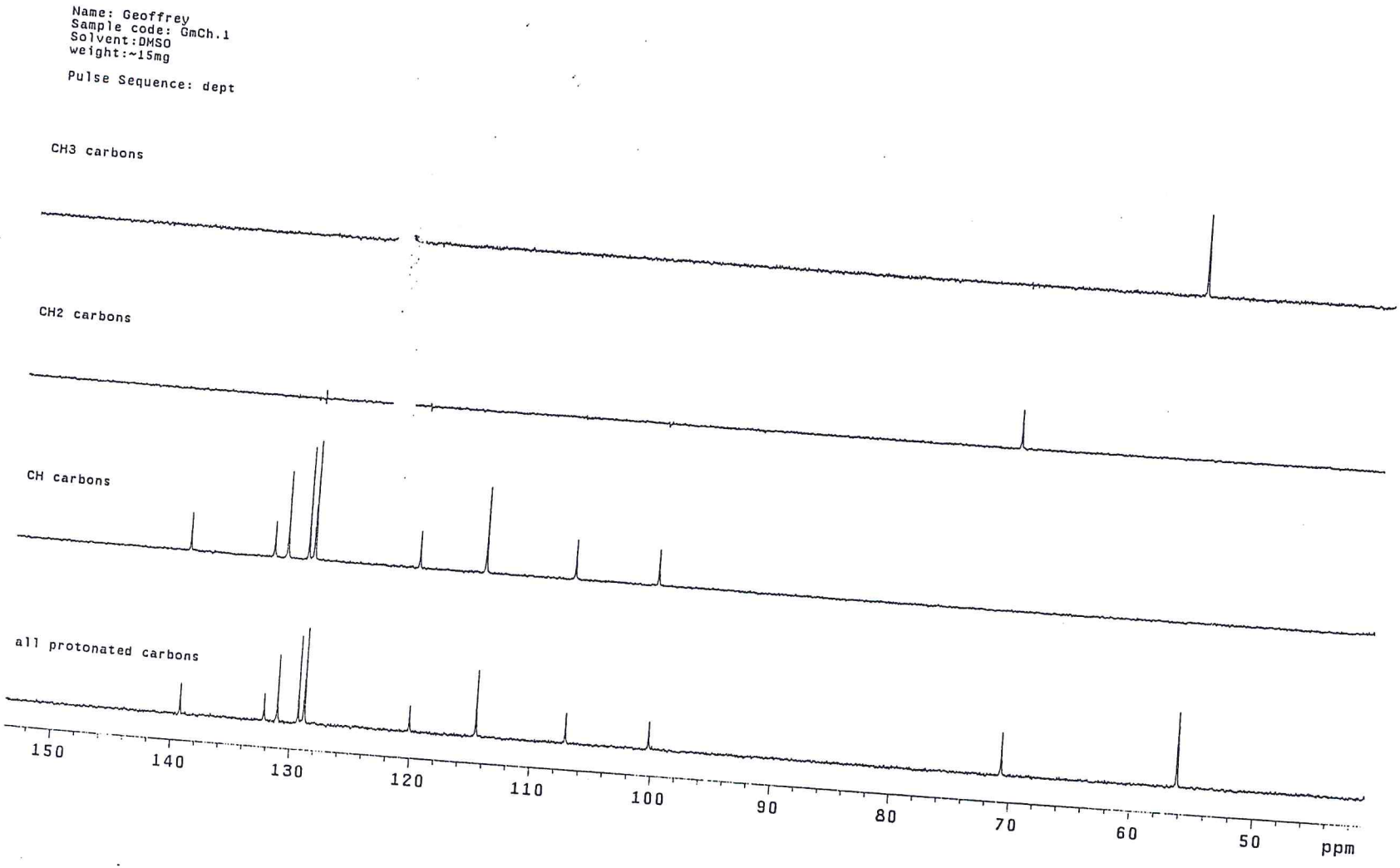
^{1}H NMR Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)



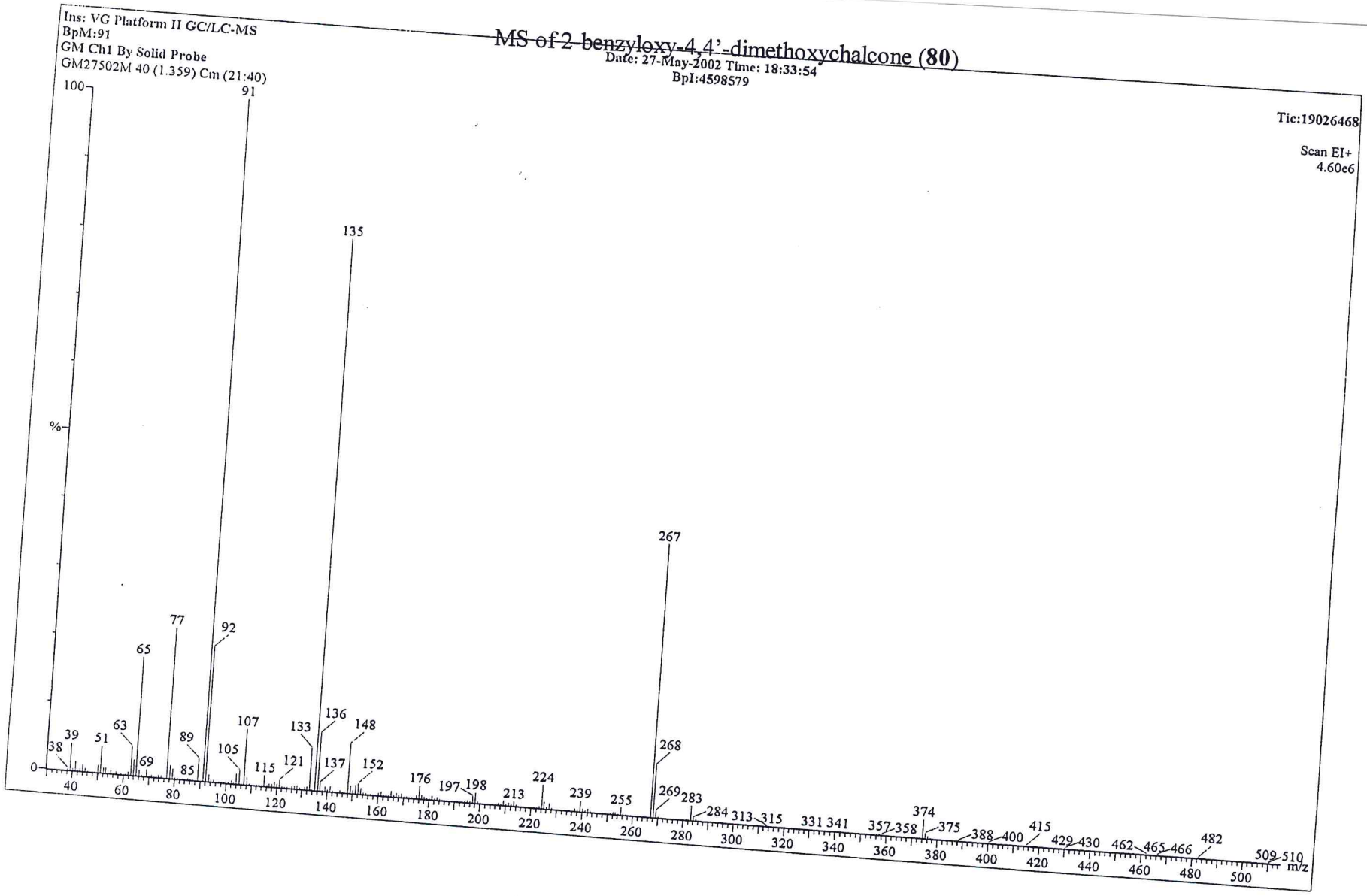
^{13}C NMR Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)

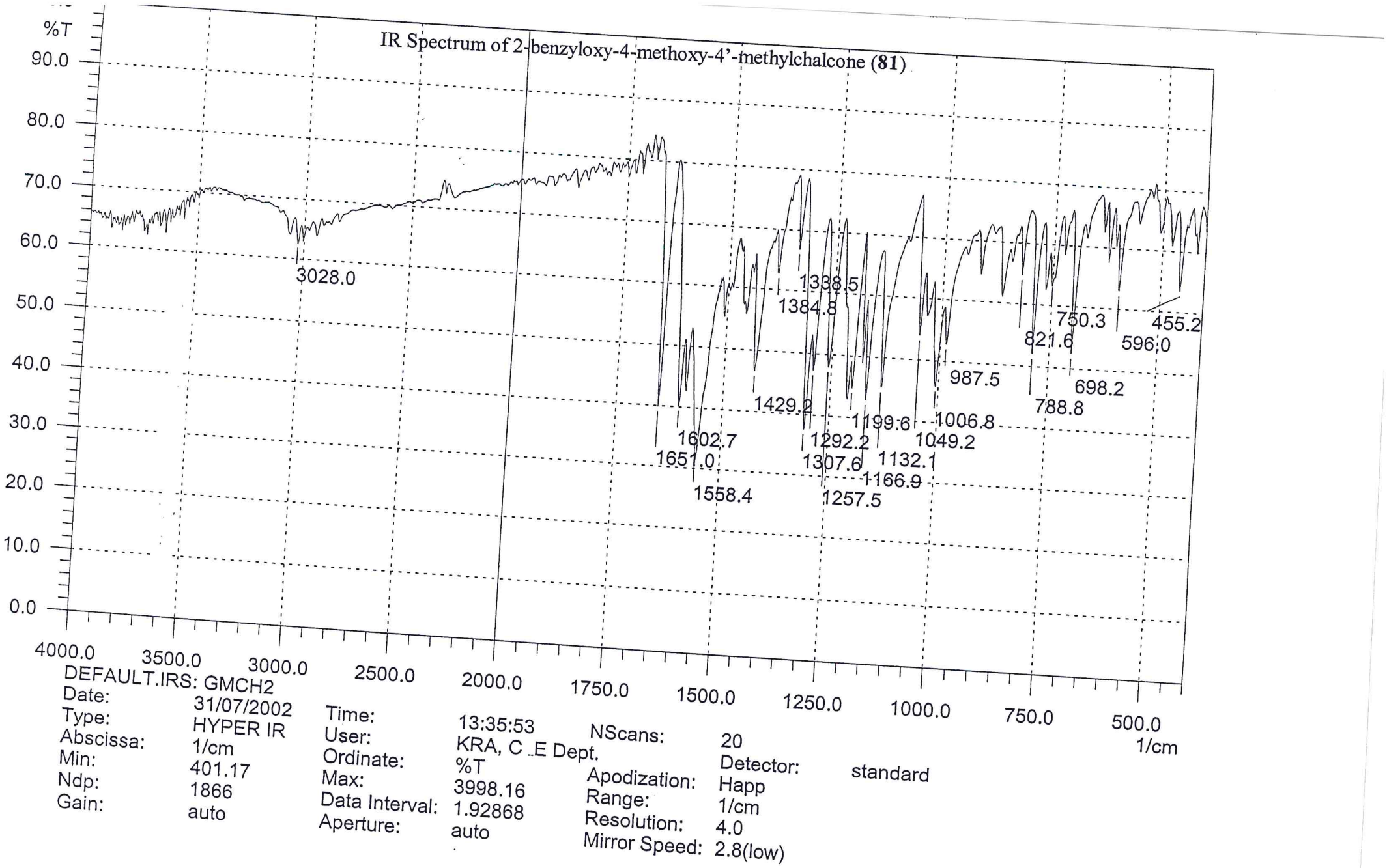


DEPT Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)



MS of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)




IR Spectrum of 2-benzyloxy-4-methoxy-4'-methylchalcone (81)
%T
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80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
3028.0
1651.0
1602.7
1558.4
1429.2
1384.8
1338.5
1307.6
1292.2
1257.5
1199.6
1166.9
1132.1
1049.2
1006.8
987.5
821.6
788.8
750.3
698.2
596.0
455.2
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Max: 3998.16
Range: 1/cm
Ndp: 1866
Data Interval: 1.92868
Resolution: 4.0
Gain: auto
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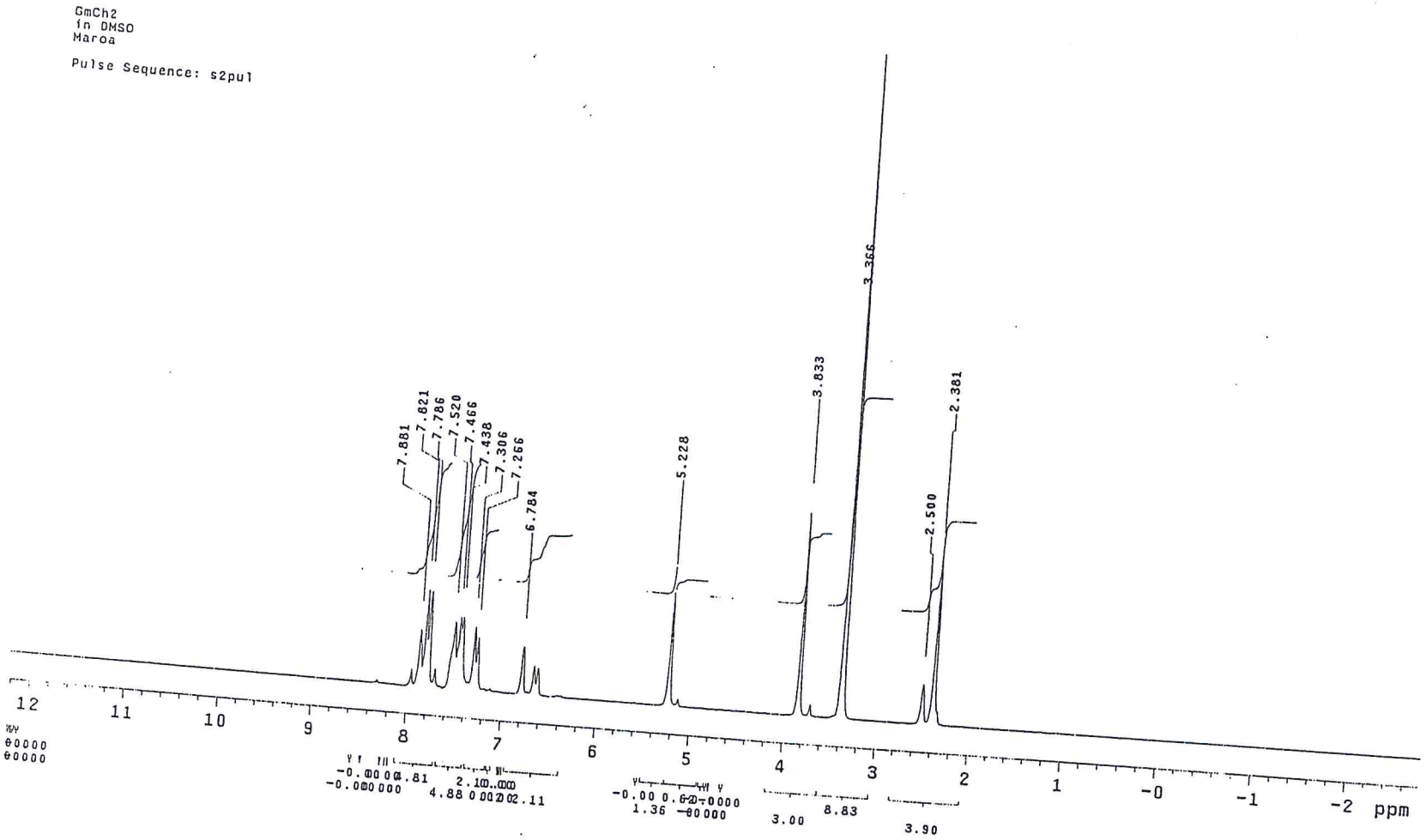
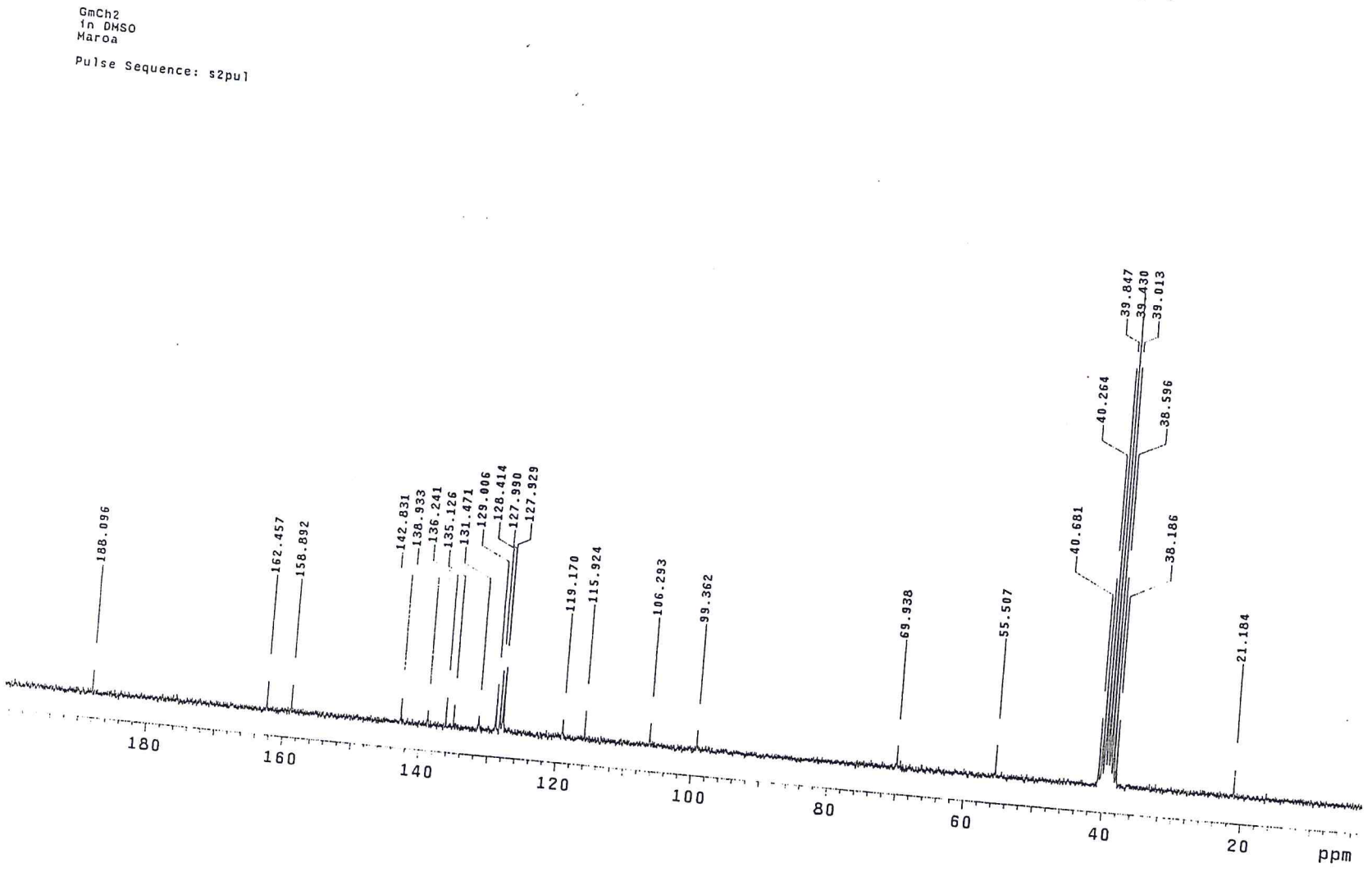
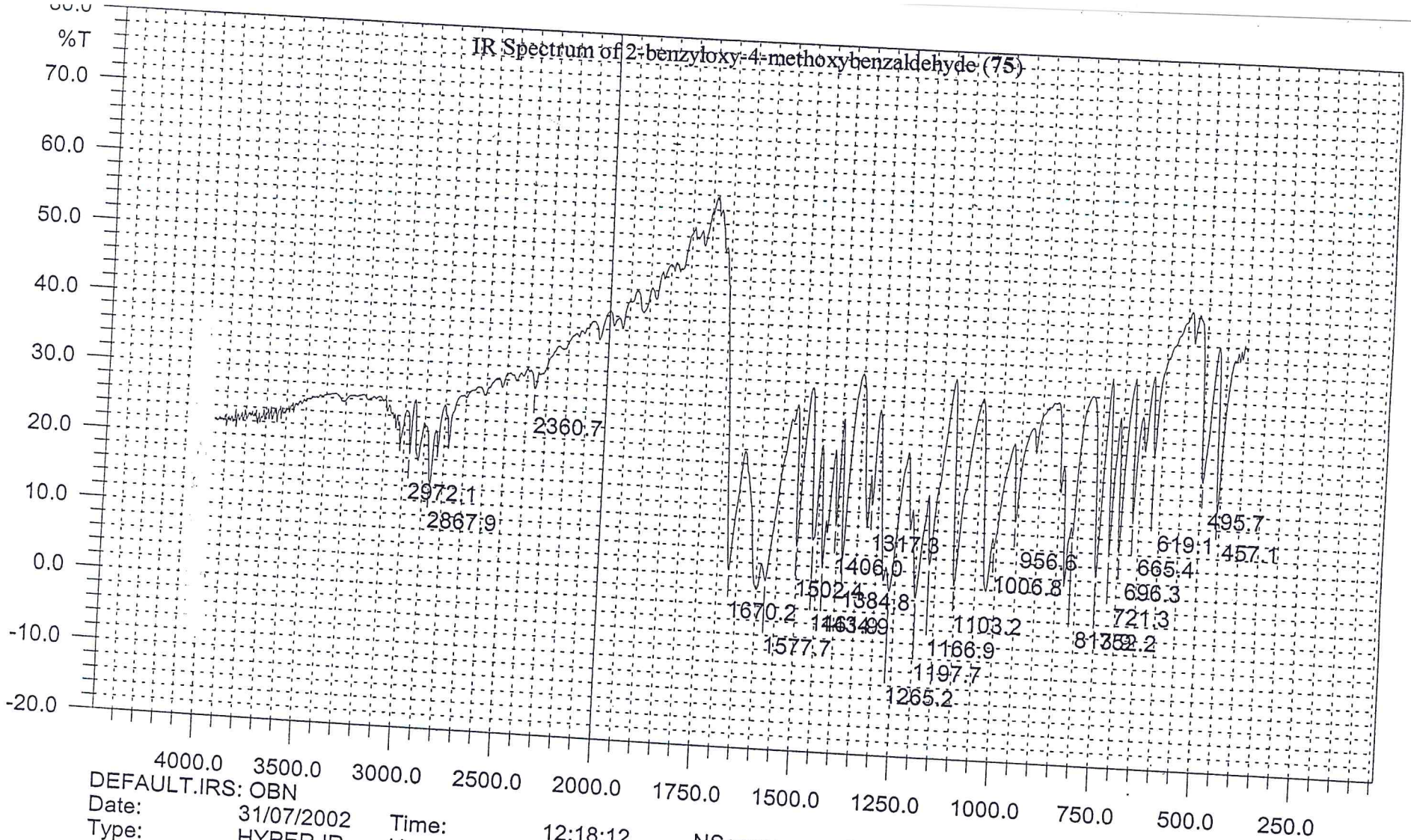
Mirror Speed: 2.8(low)

^{1}H NMR Spectrum of 2-benzyloxy-4-methoxy-4'-methylchalcone (**81**)



^{13}C NMR Spectrum of 2-benzyloxy-4-methoxy-4'-methylchalcone (**81**)





DEFAULT.IRS: OBN

Date:	31/07/2002	Time:	12:18:12	NScans:	20
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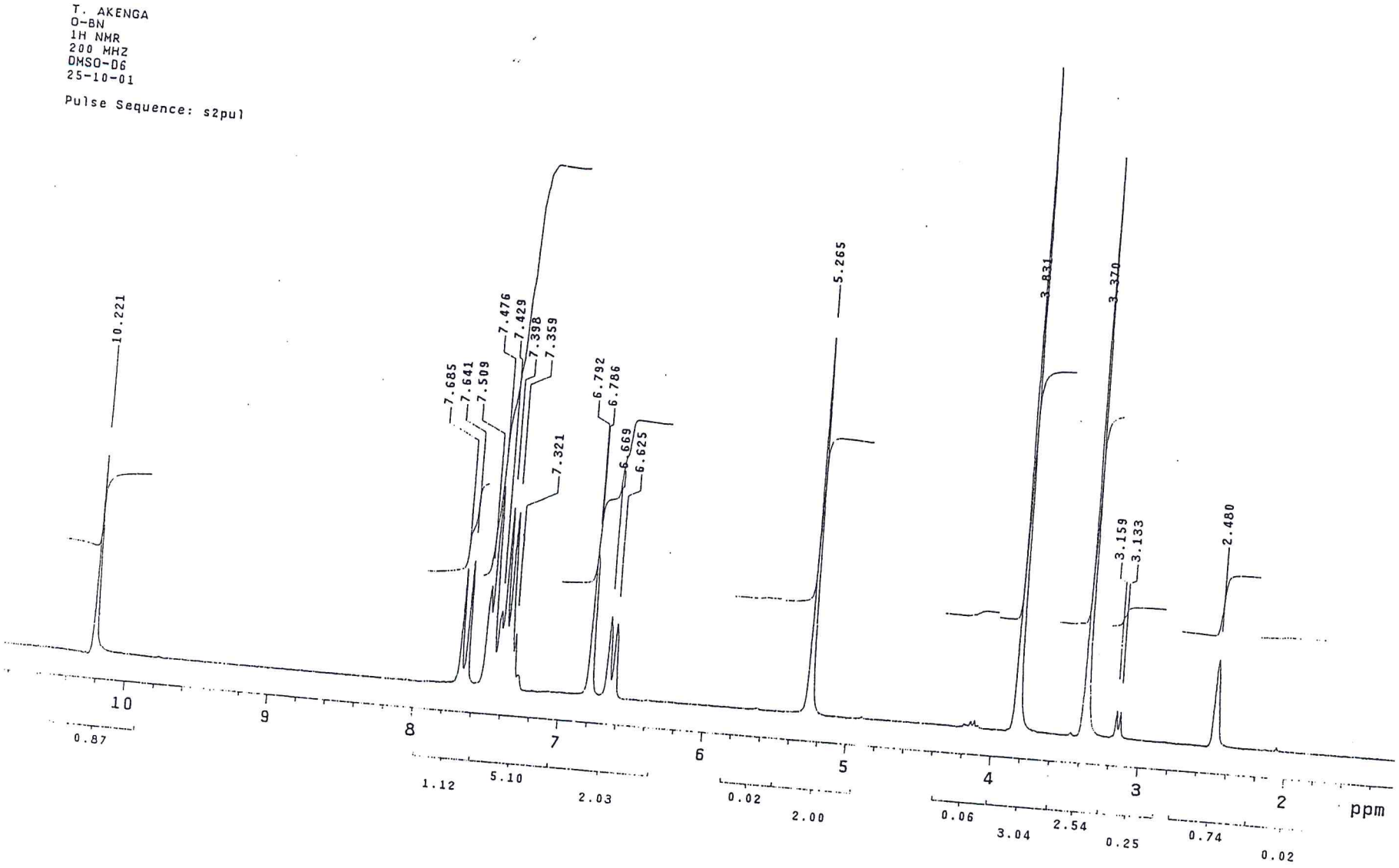
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ
Min:	401.17	Max:	3998.16	Range:	1/cm
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

^{1}H NMR Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)



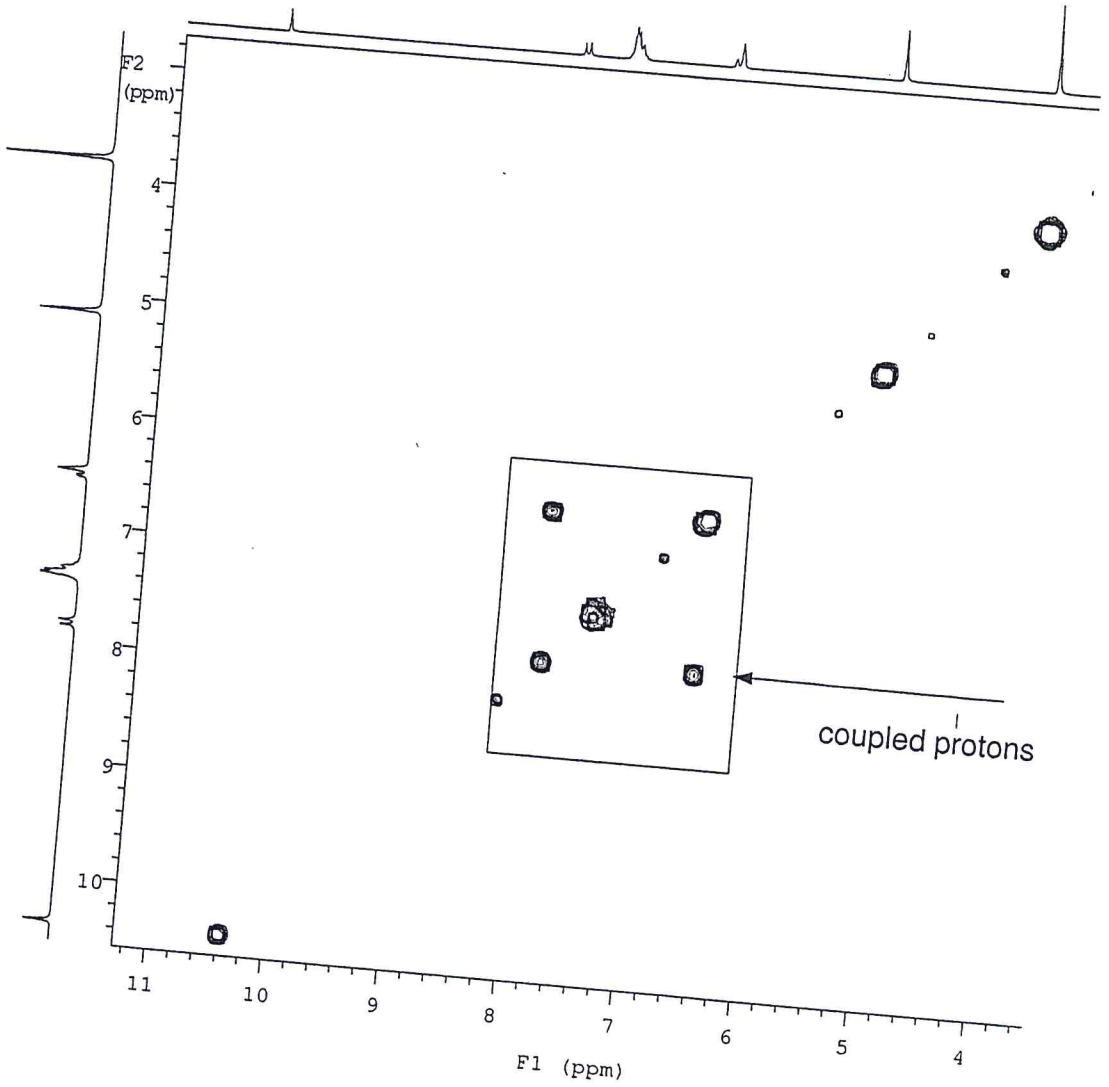
^{1}H-^{1}H Cosy Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)

GMM3
CDCl3
50 mg

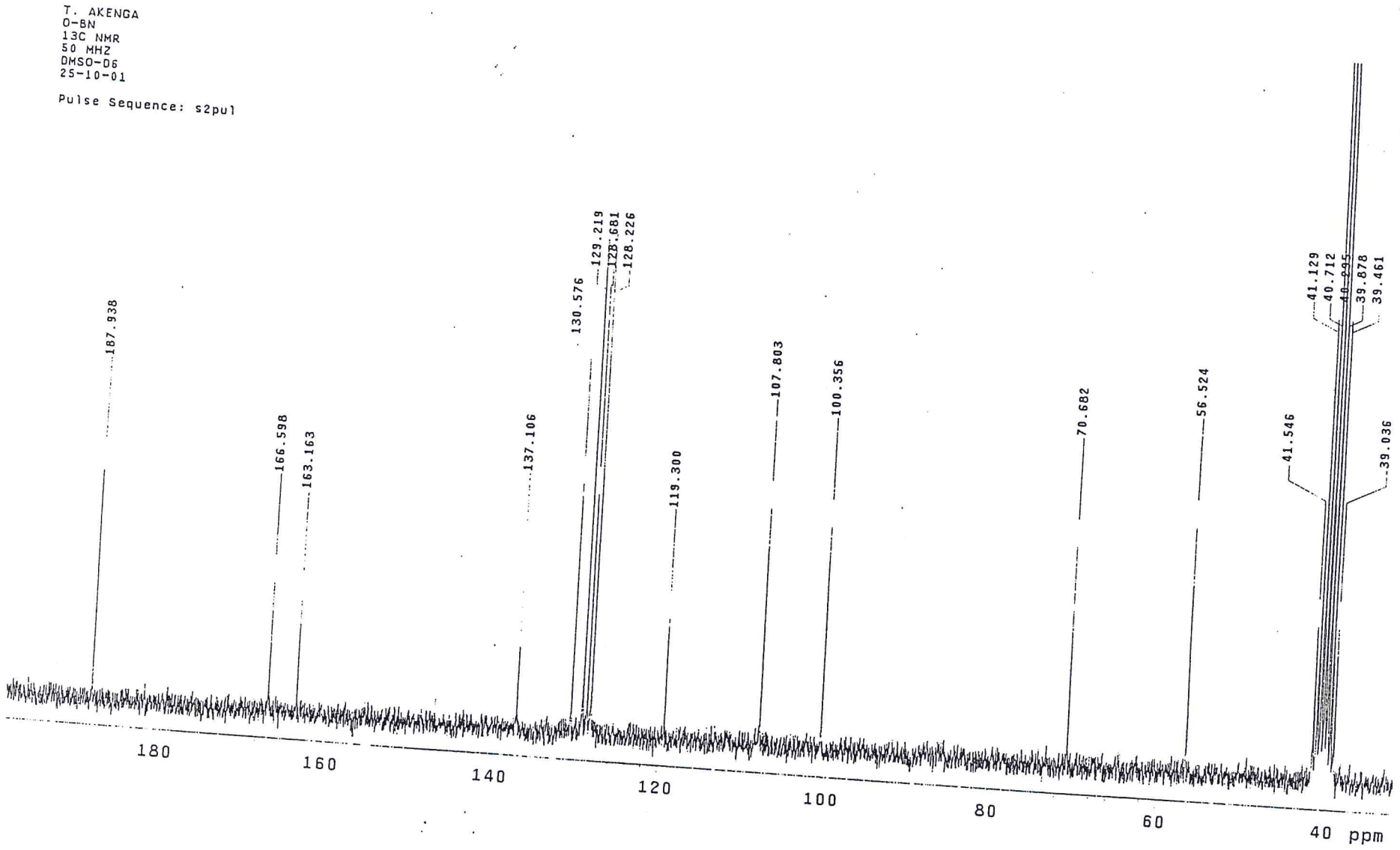
Pulse Sequence: COSY

Solvent: CDCl3
Ambient temperature
File: COSY
Mercury-200 "uonnmr200"

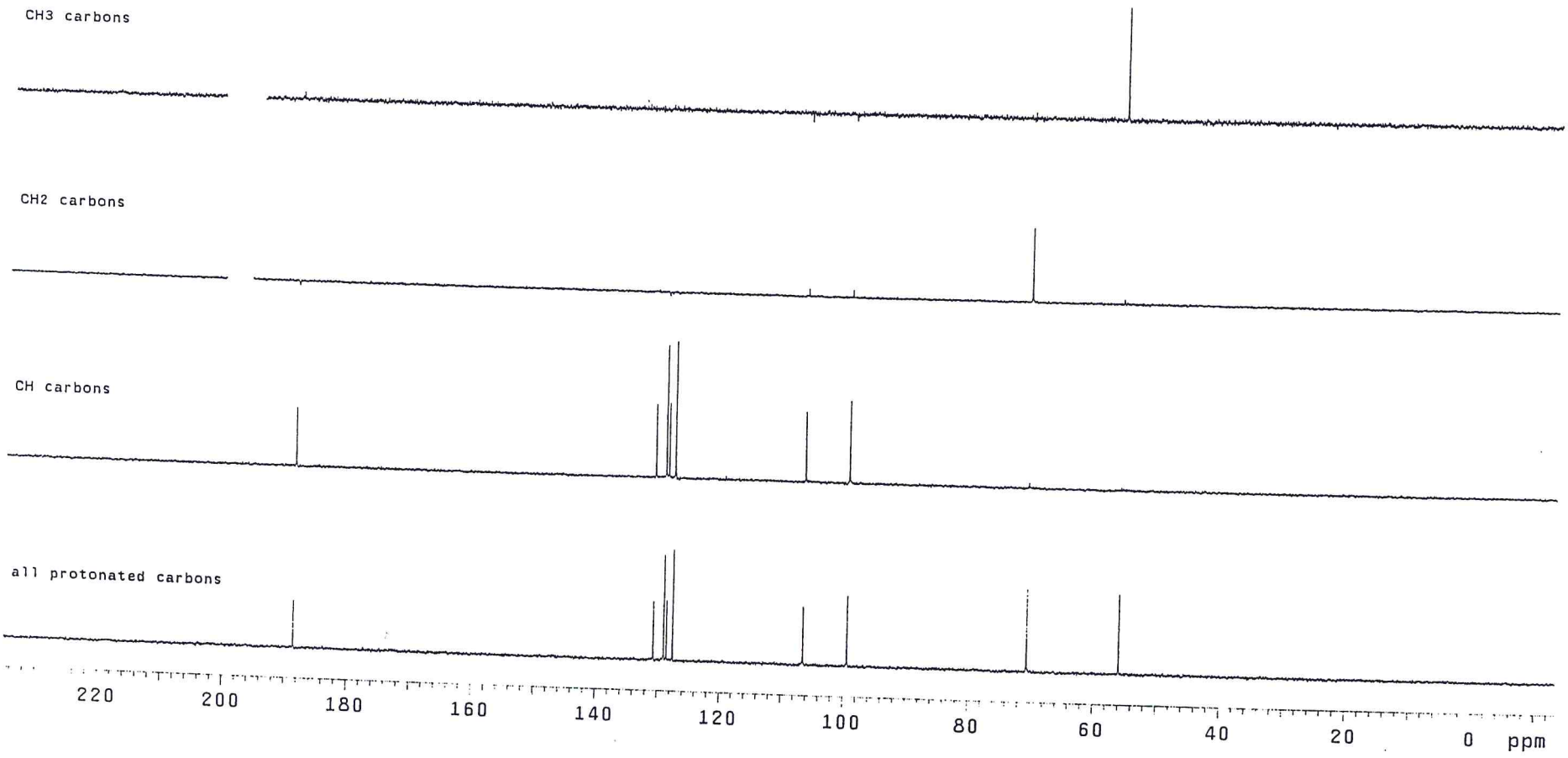
PULSE SEQUENCE: COSY
Relax. delay 1.000 sec
Acq. time 0.160 sec
Width 3200.9 Hz
2D Width 3200.9 Hz
2 repetitions
128 increments
OBSERVE H1, 200.0557687MHz
DATA PROCESSING
Sq. sine bell 0.080 sec
F1 DATA PROCESSING
Sq. sine bell 0.040 sec
FT size 1024 x 1024
Total time 6 min, 36 sec



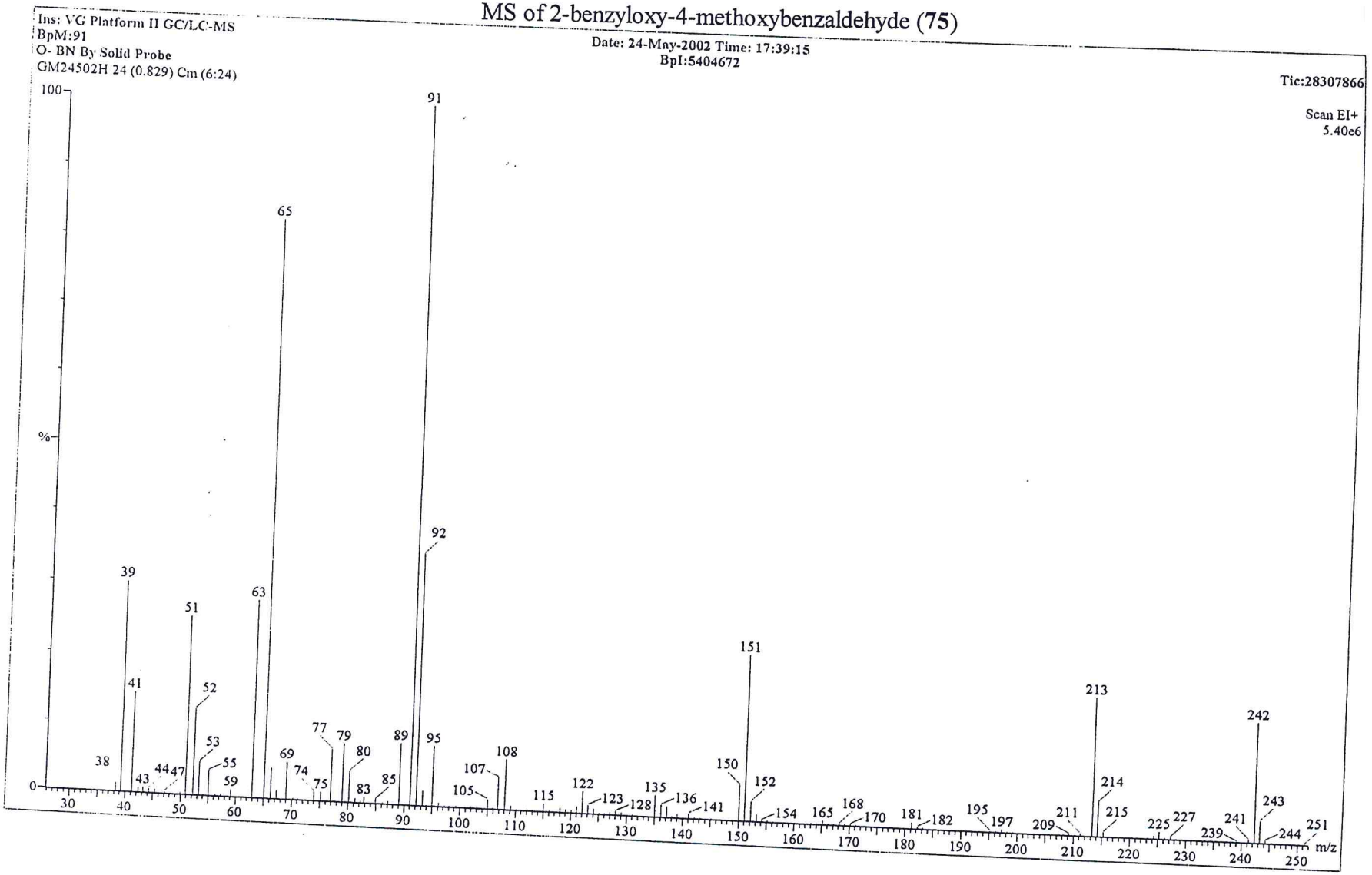
^{13}C NMR Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)



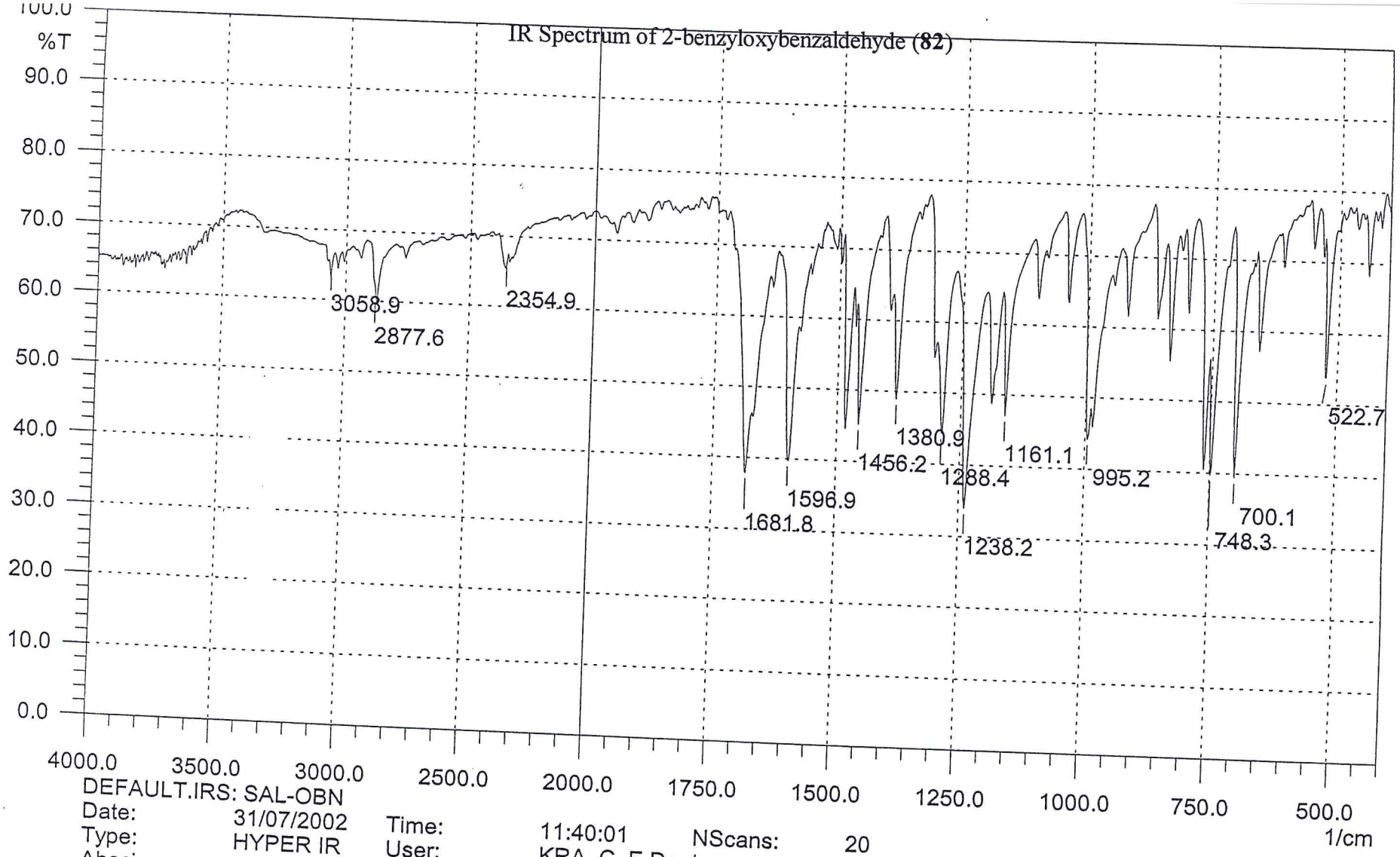
DEPT Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)



MS of 2-benzyloxy-4-methoxybenzaldehyde (75)



IR Spectrum of 2-benzyloxybenzaldehyde (**82**)



DEFAULT.IRS: SAL-OBN

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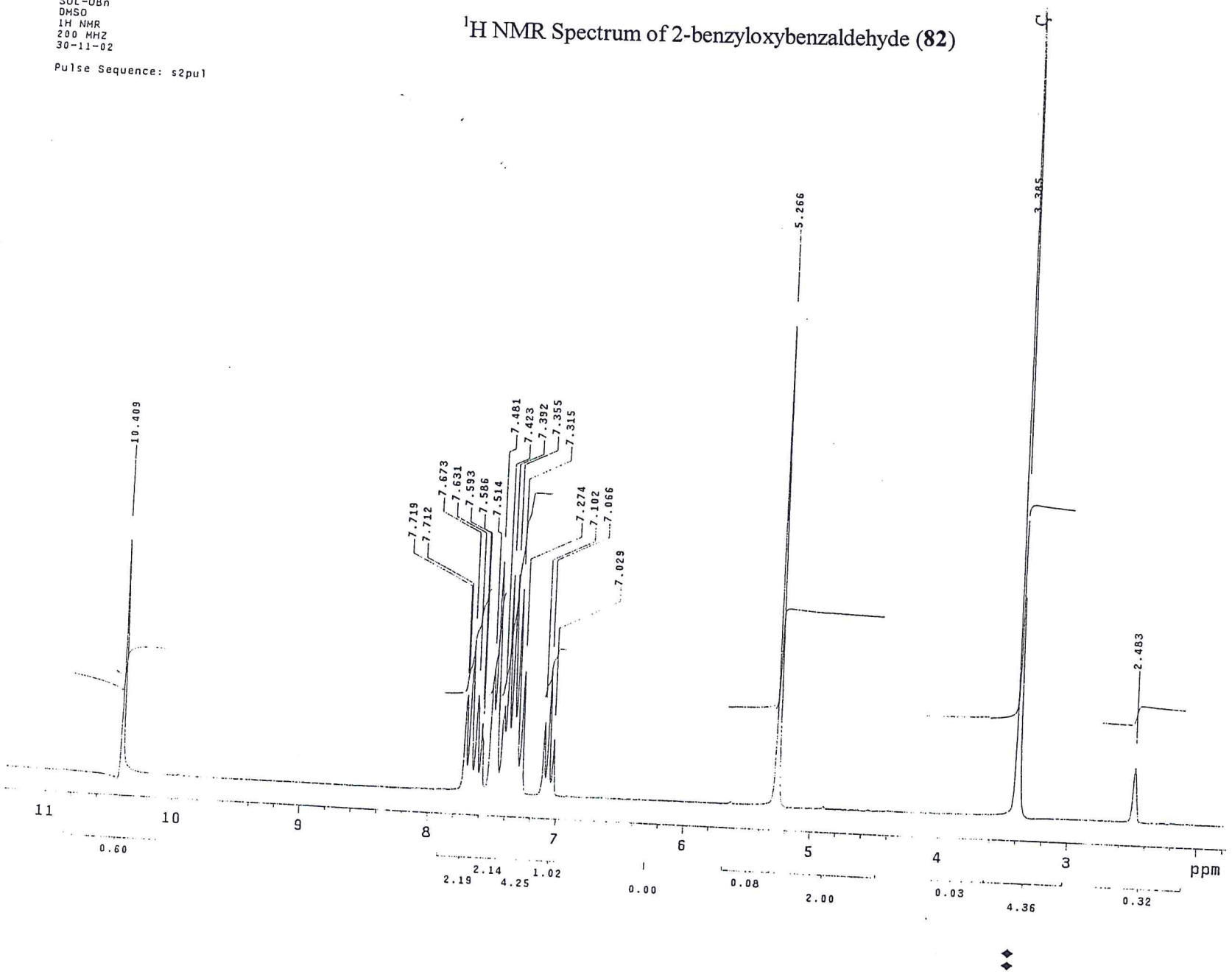
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

DR AKENGA
SOL-OBn
DMSO
1H NMR
200 MHZ
30-11-02

Pulse Sequence: s2pul

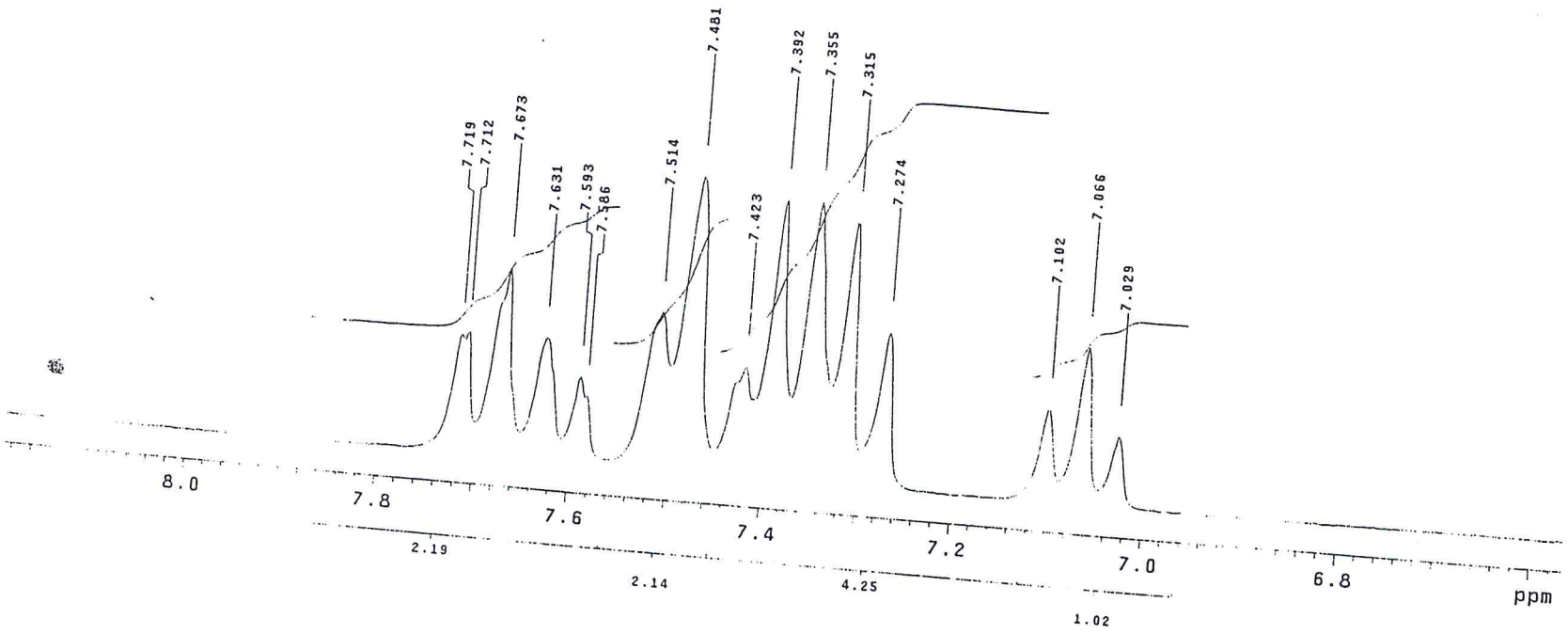
^{1}H NMR Spectrum of 2-benzyloxybenzaldehyde (**82**)



DMSO
1H NMR
200 MHZ
30-11-02

Pulse Sequence: s2pul

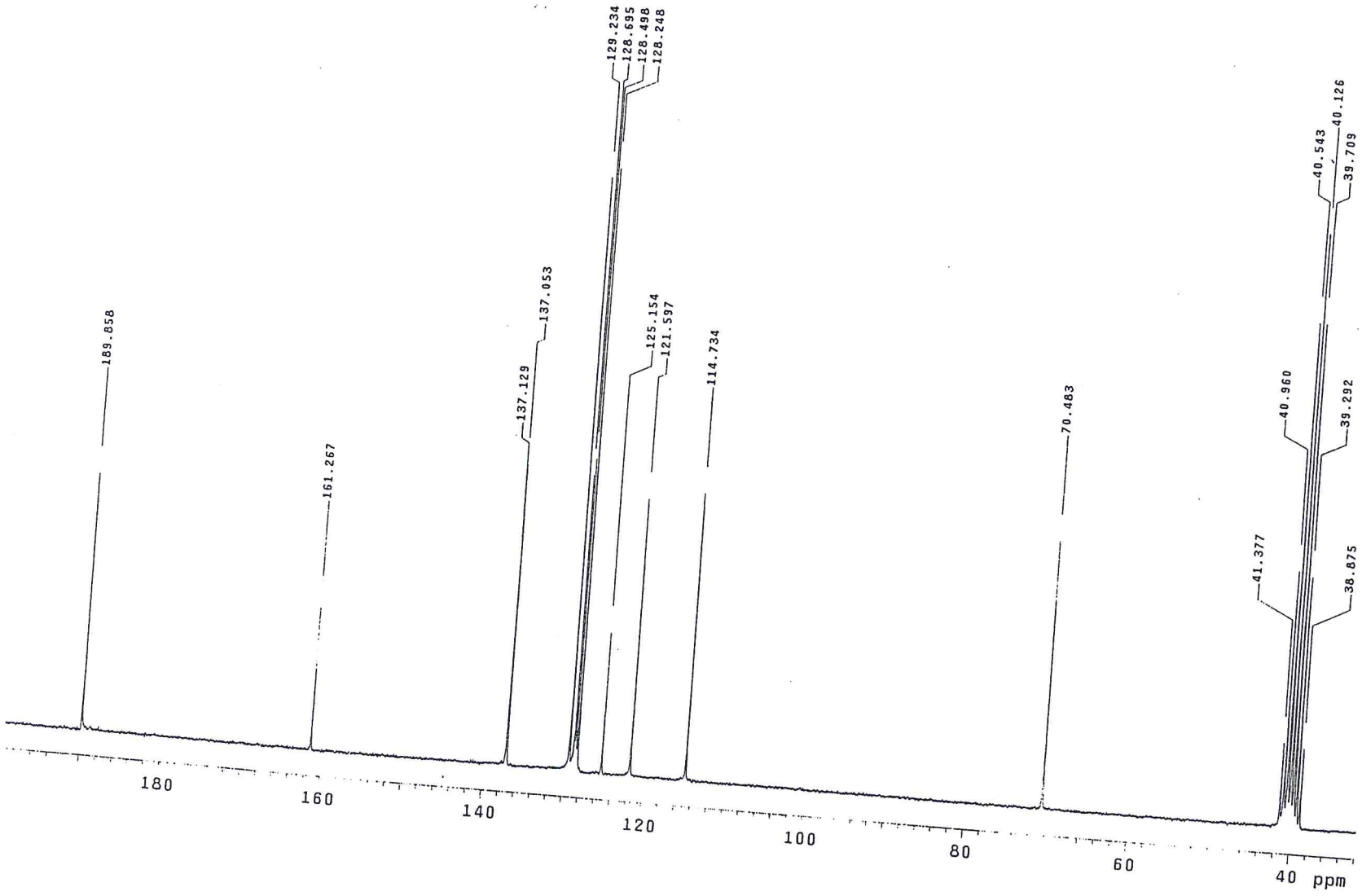
^{1}H NMR Spectrum of 2-benzyloxybenzaldehyde (**82**)



SOL-OBn
DMSO
13C NMR
50 MHZ
02-12-02

Pulse Sequence: s2pul

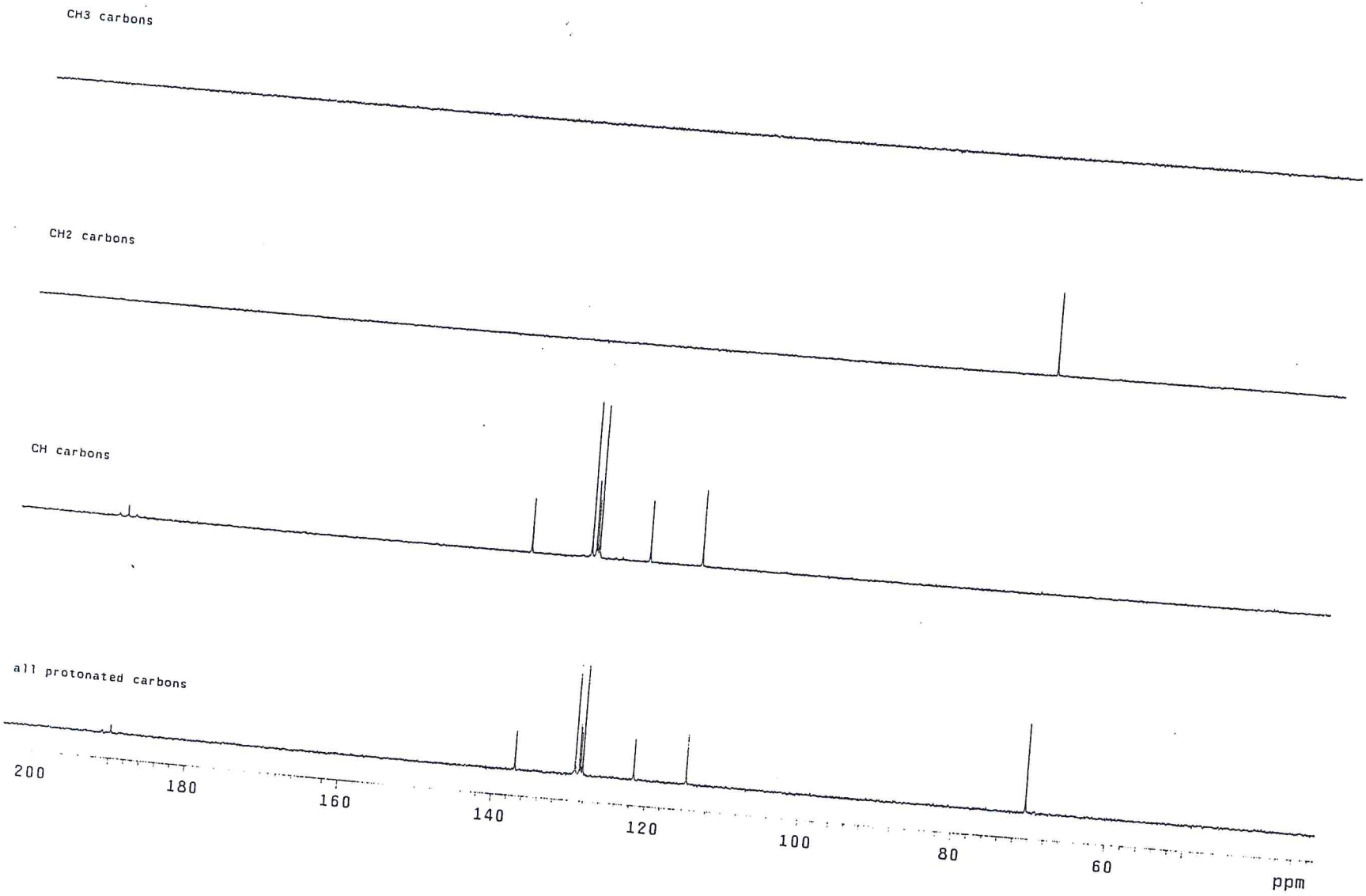
^{13}C NMR Spectrum of 2-benzyloxybenzaldehyde (**82**)



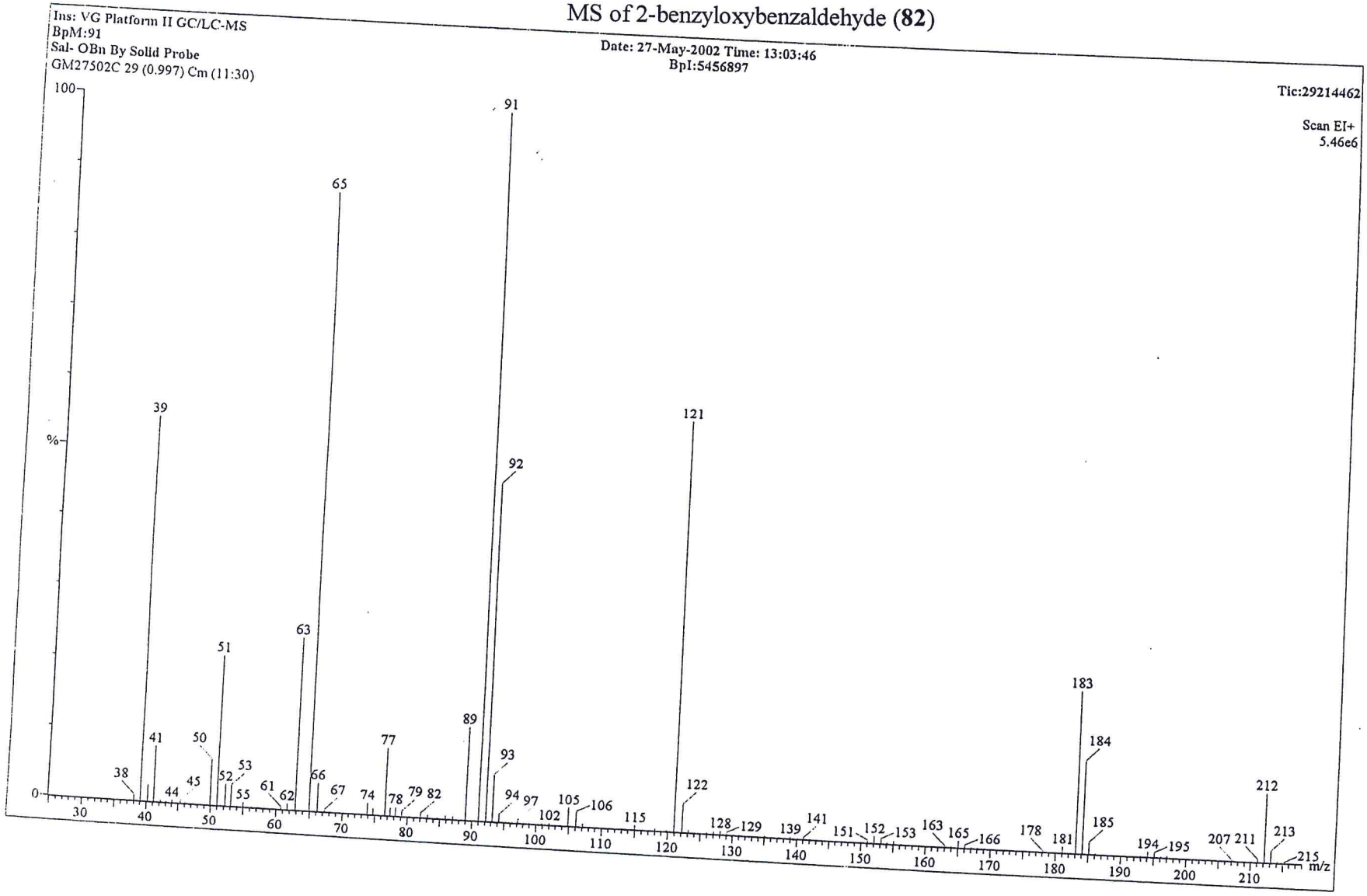
SOL-OBn
DMSO
DEPT
50 MHZ
02-12-02

Pulse Sequence: dept

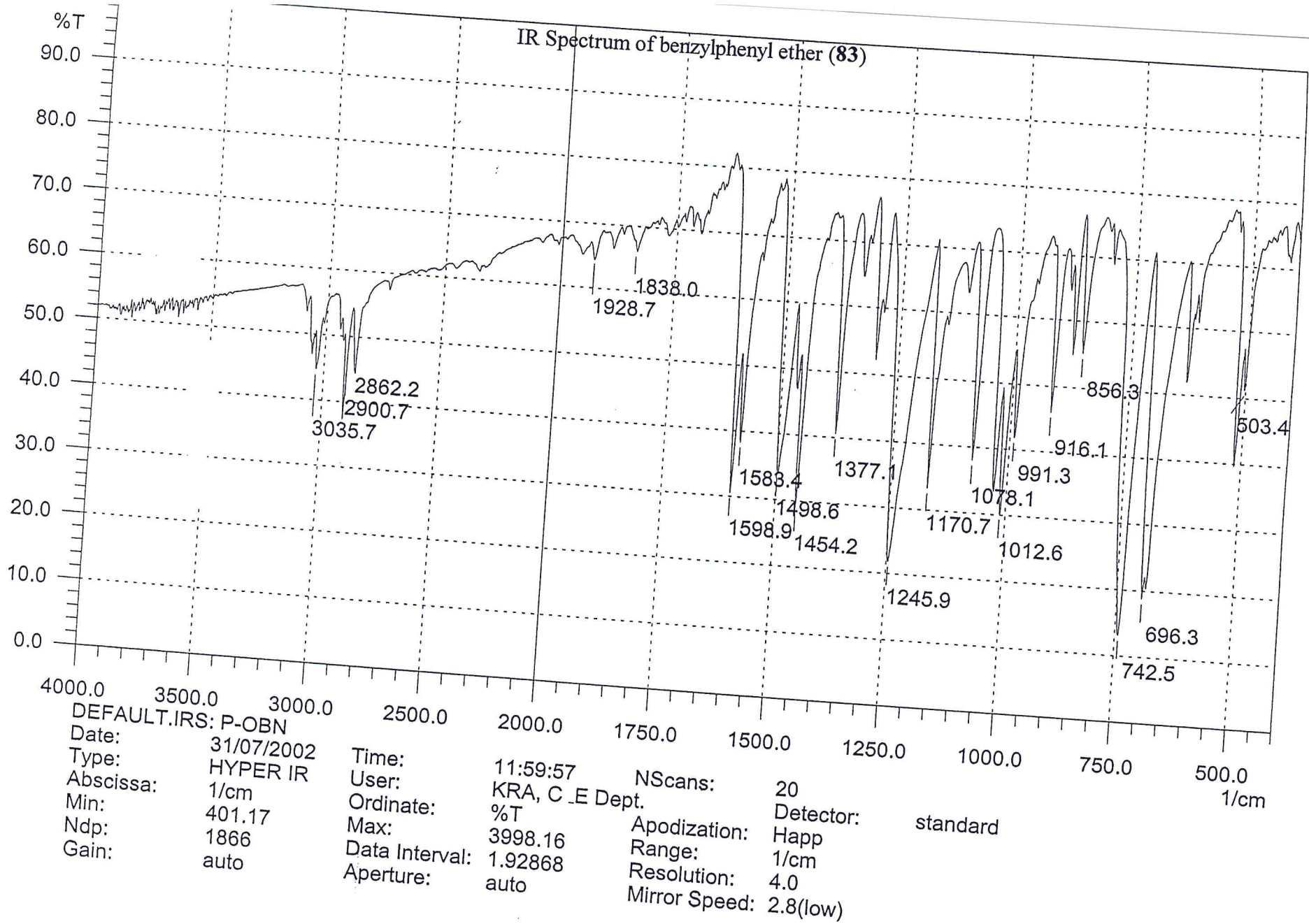
DEPT Spectrum of 2-benzyloxybenzaldehyde (**82**)



MS of 2-benzyloxybenzaldehyde (**82**)



IR Spectrum of benzylphenyl ether (**83**)



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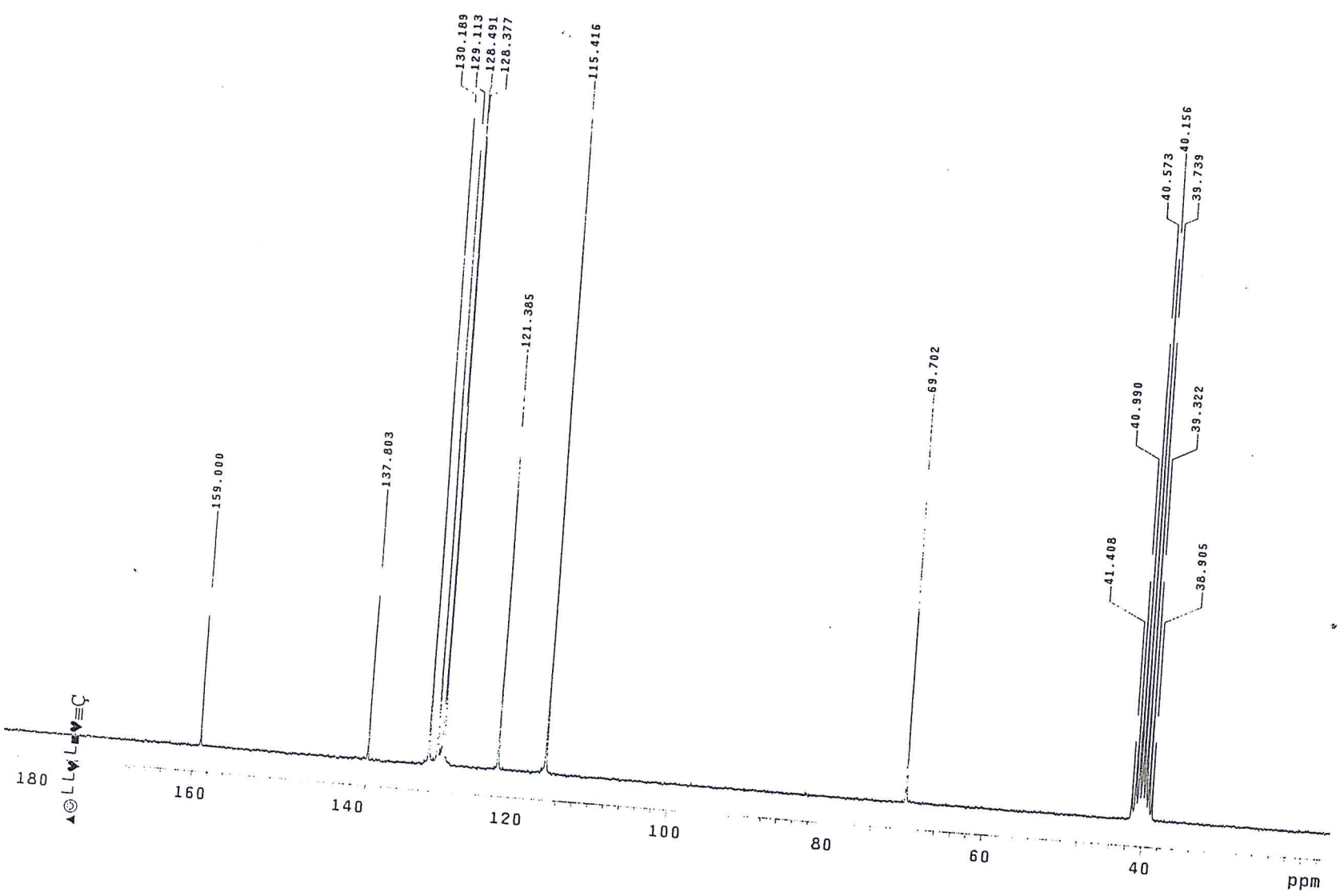
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

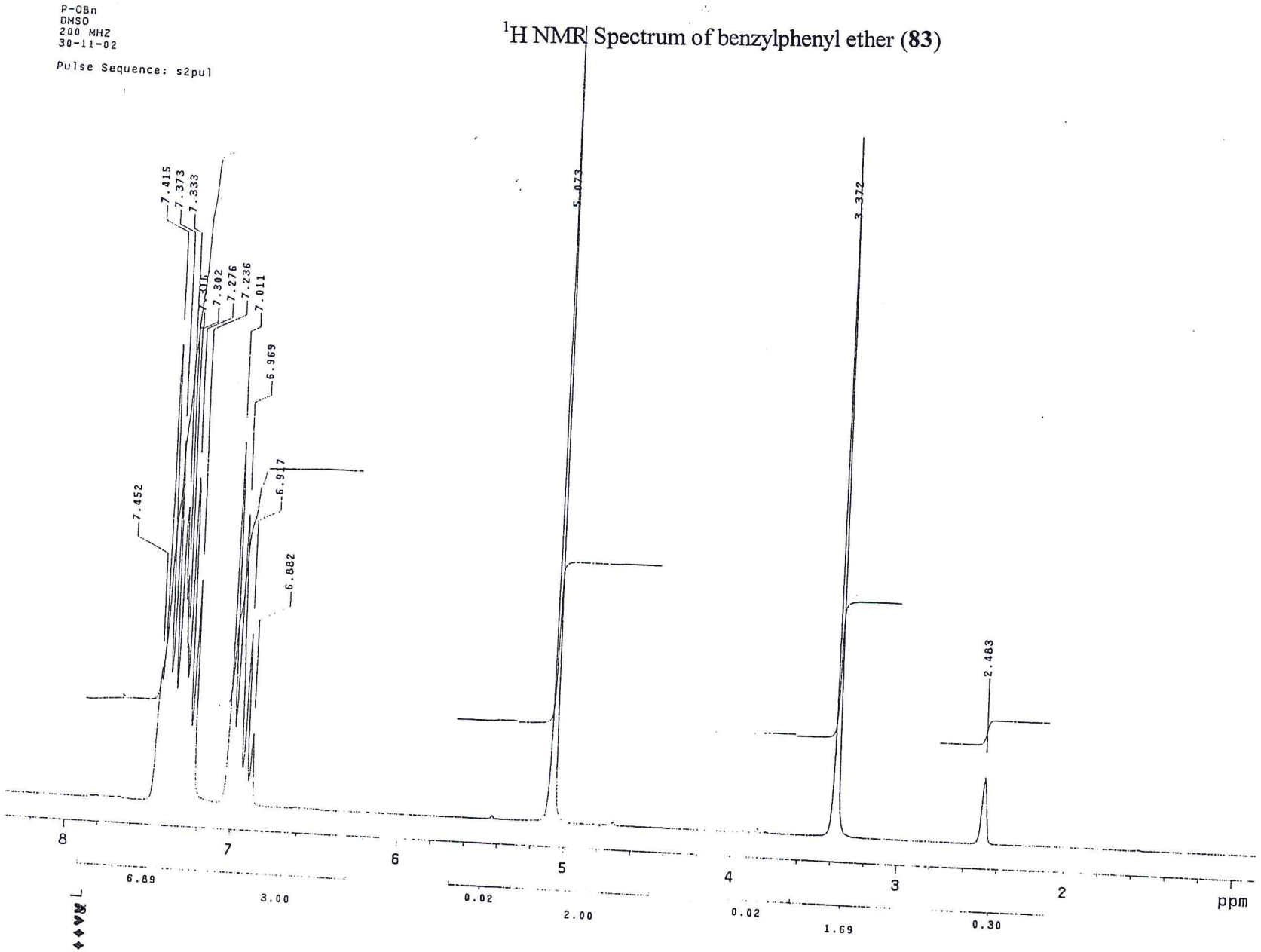
DMSO
13C NMR
50 MHZ
30-11-02

Pulse Sequence: s2pul

^{13}C NMR Spectrum of benzylphenyl ether (**83**)



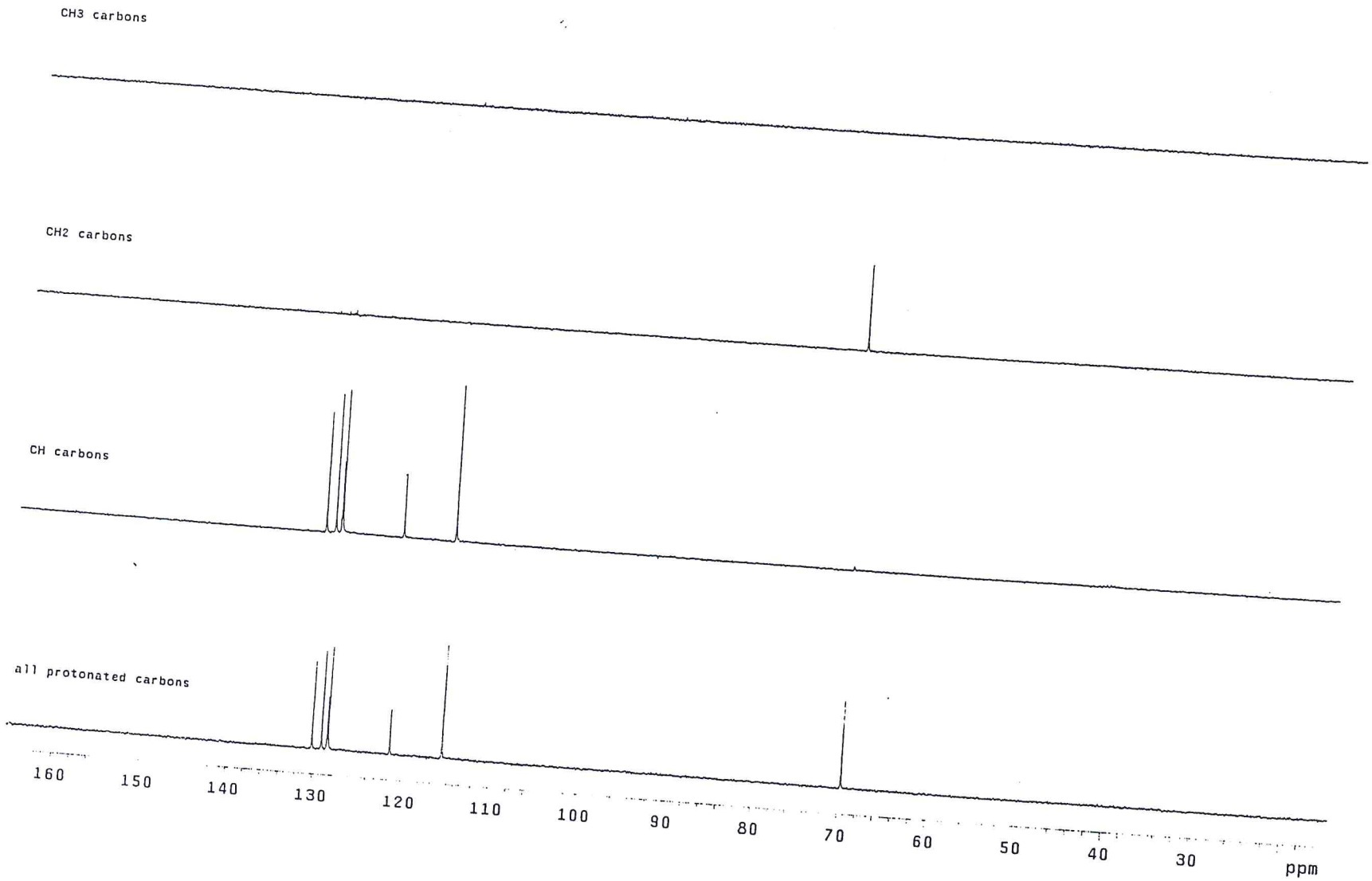
^{1}H NMR Spectrum of benzylphenyl ether (**83**)



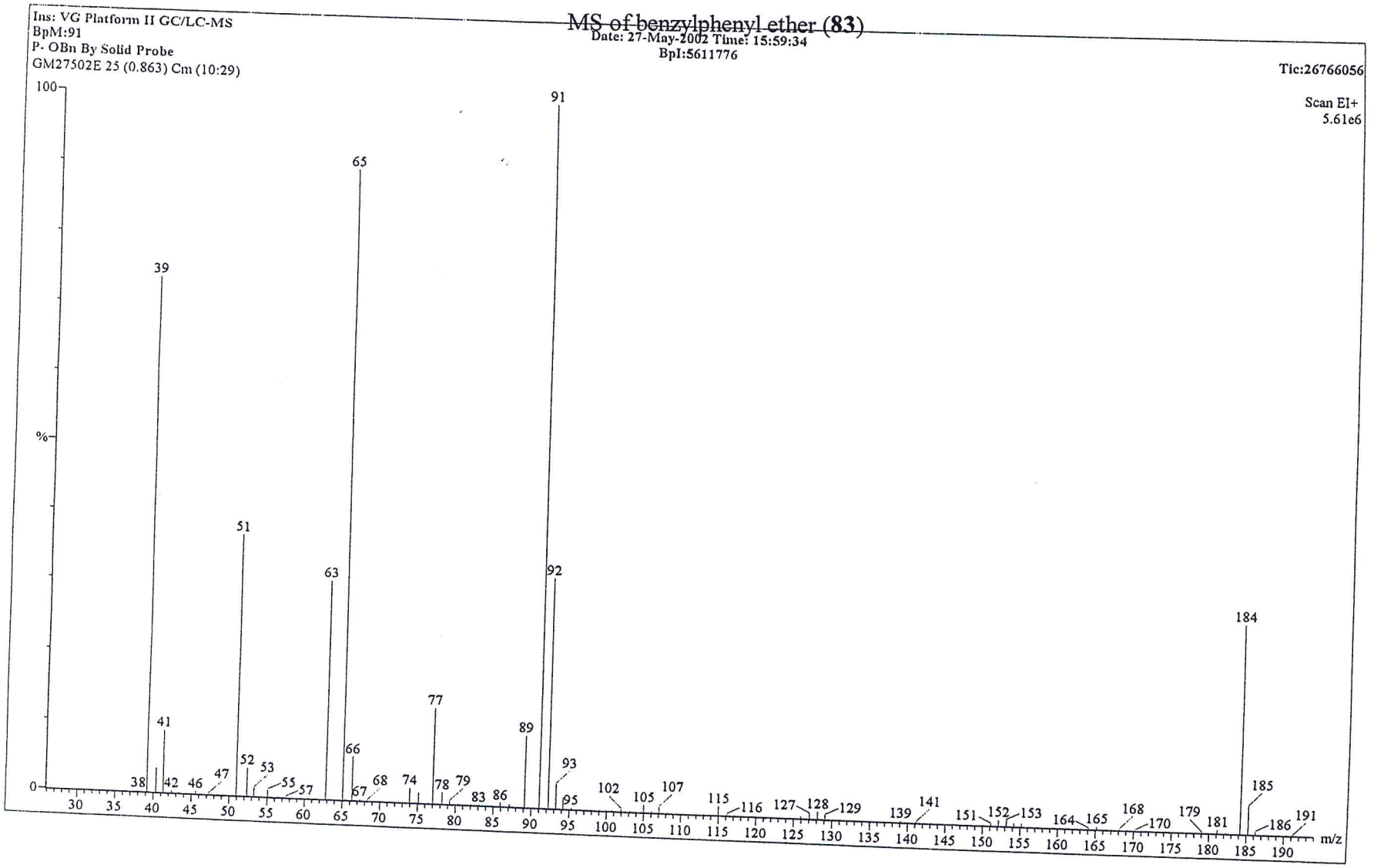
P-OBn
DMSO
DMSO
50 MHZ
30-11-02

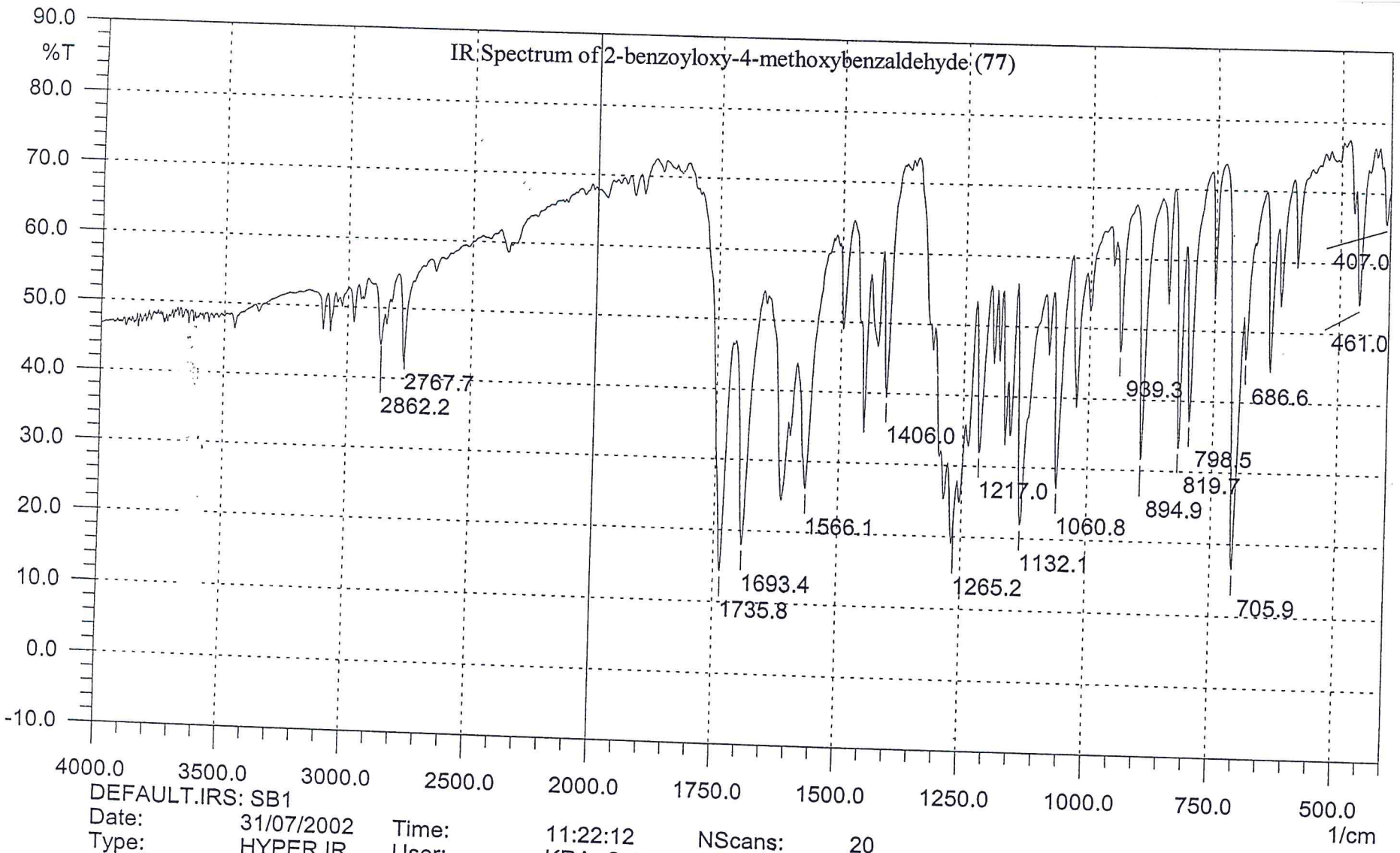
Pulse Sequence: dept

DEPT Spectrum of benzylphenyl ether (**83**)



MS of benzylphenyl ether (**83**)





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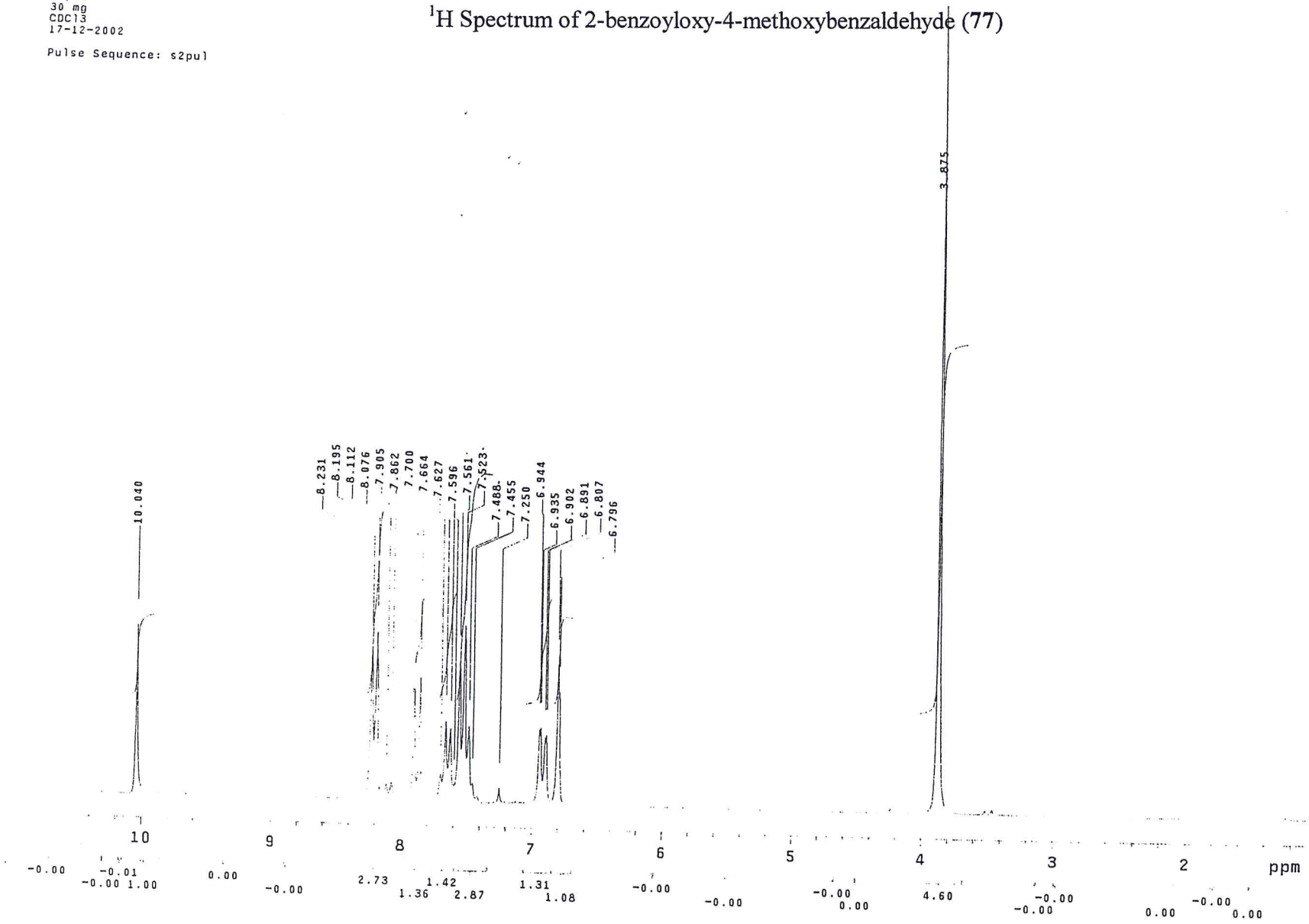
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

G. Mahamga
SB/II
30 mg
CDCl3
17-12-2002

Pulse Sequence: s2pul

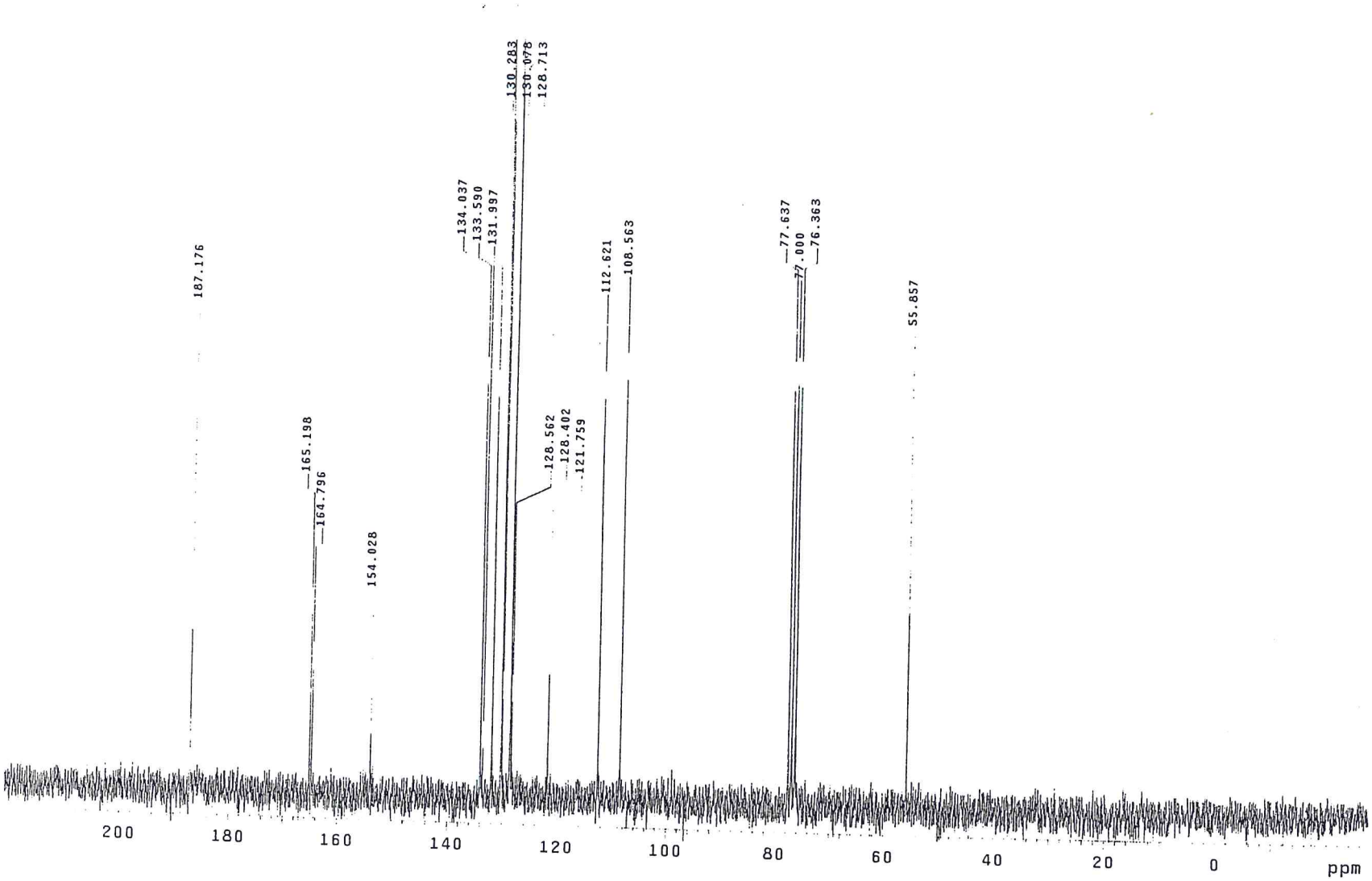
^{1}H Spectrum of 2-benzoyloxy-4-methoxybenzaldehyde (77)



G. Mahamga
SB/II
30 mg
CDCl3
17-12-2002

Pulse Sequence: s2pul

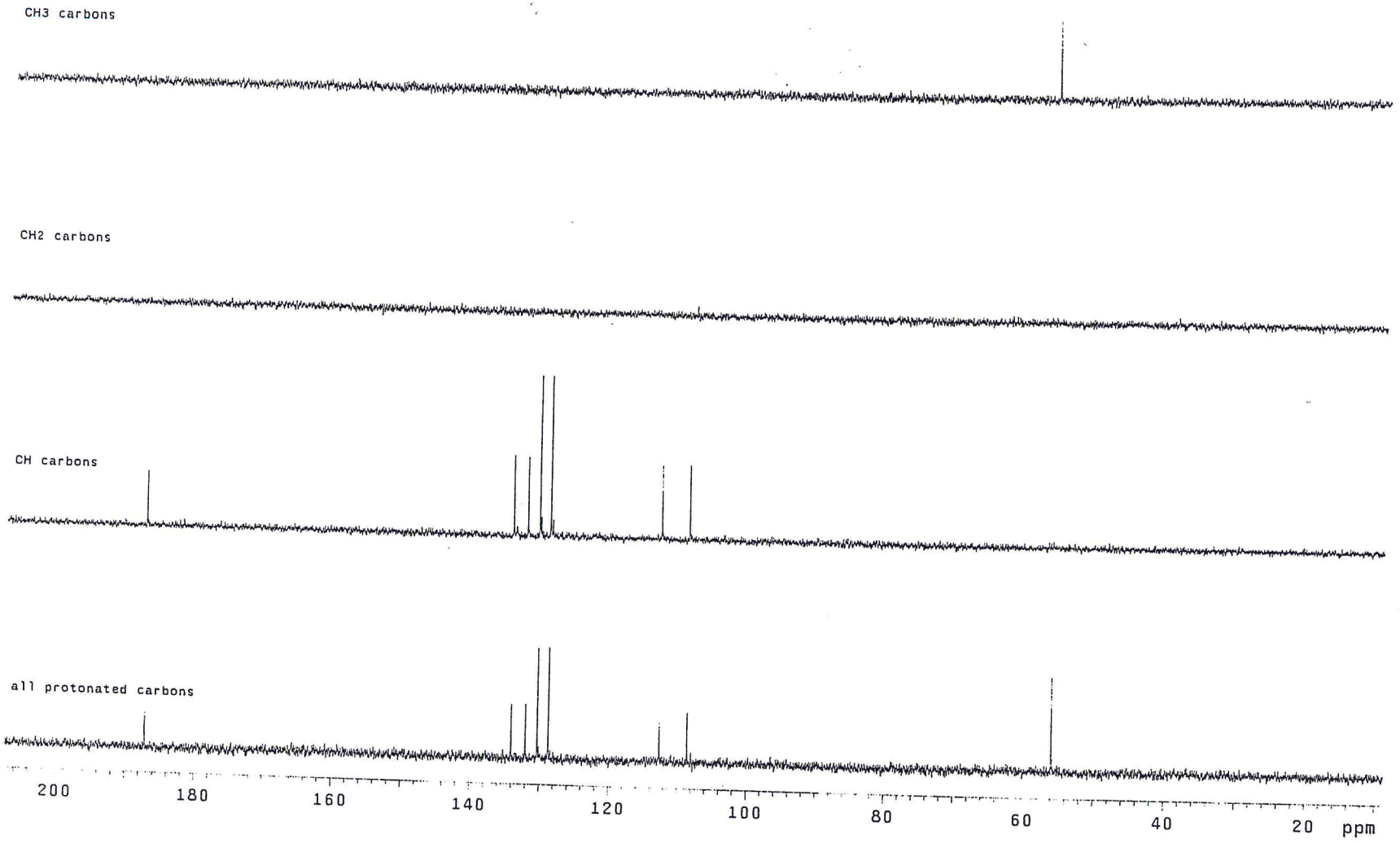
^{13}C NMR Spectrum of 2-benzoyloxy-4-methoxybenzaldehyde (77)



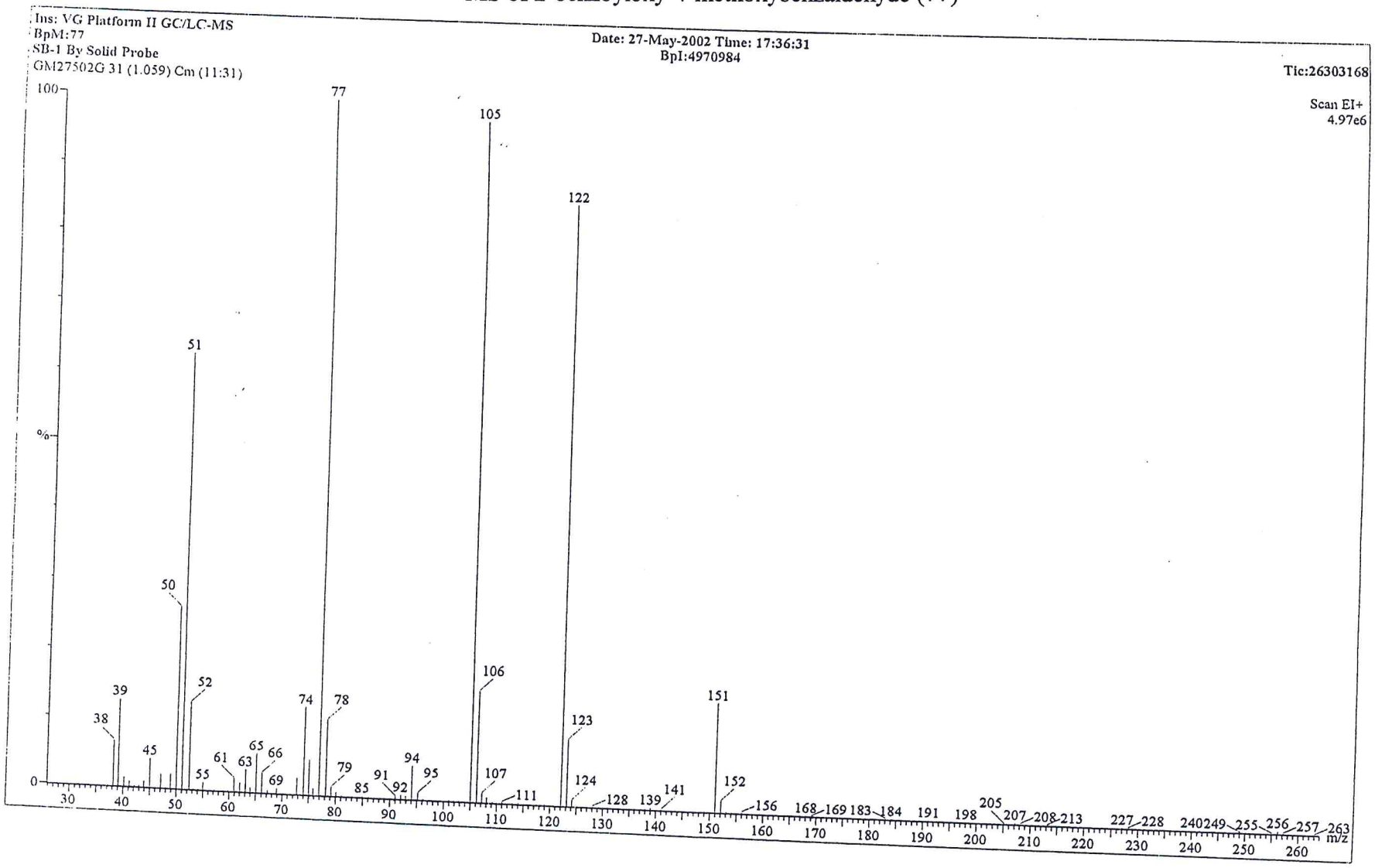
G. Mahanga
SB/II
30 mg
CDCl3
17-12-2002

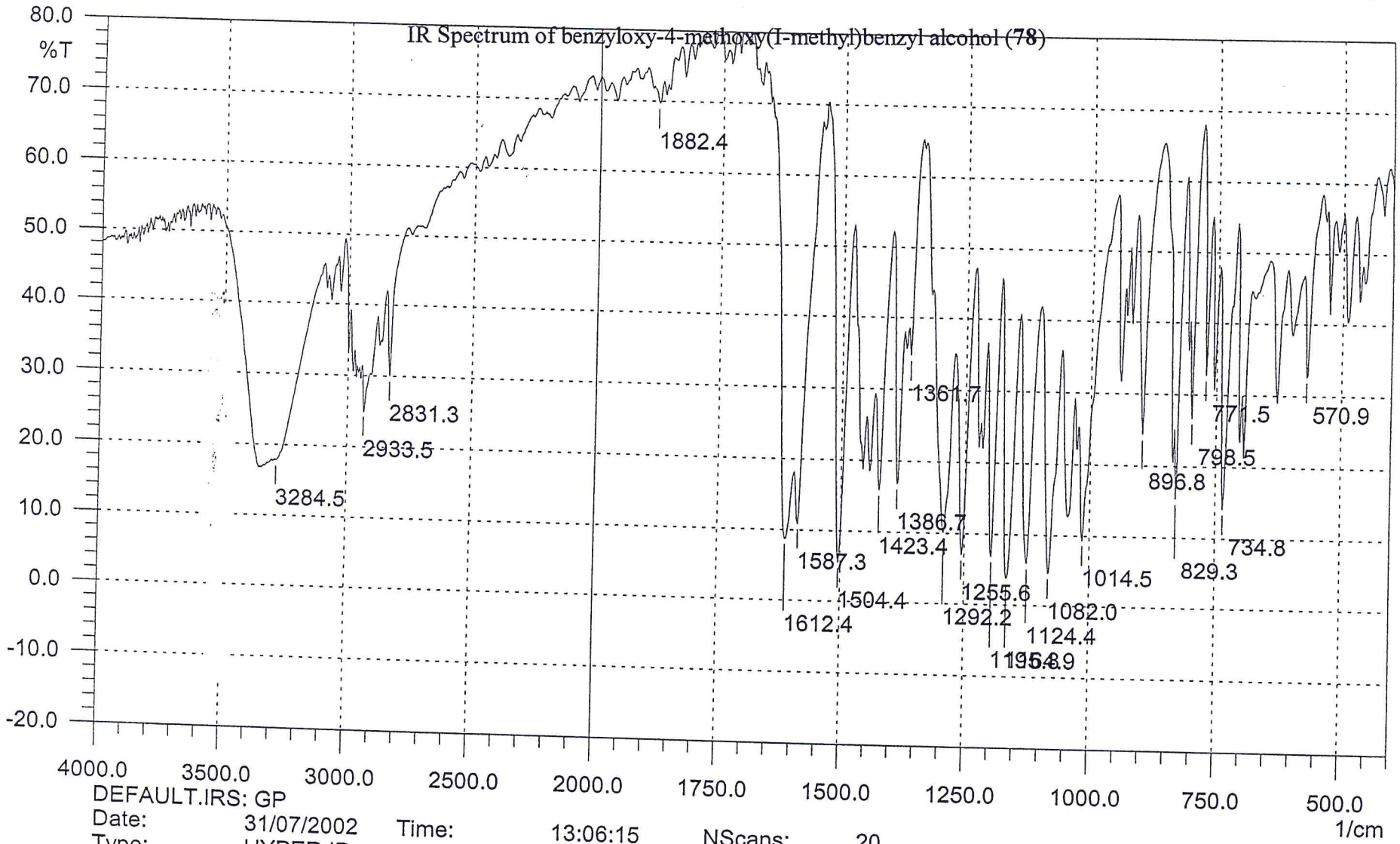
Pulse Sequence: dept

DEPT Spectrum of 2-benzoyloxy-4-methoxybenzaldehyde (77)



MS of 2-benzoyloxy-4-methoxybenzaldehyde (77)





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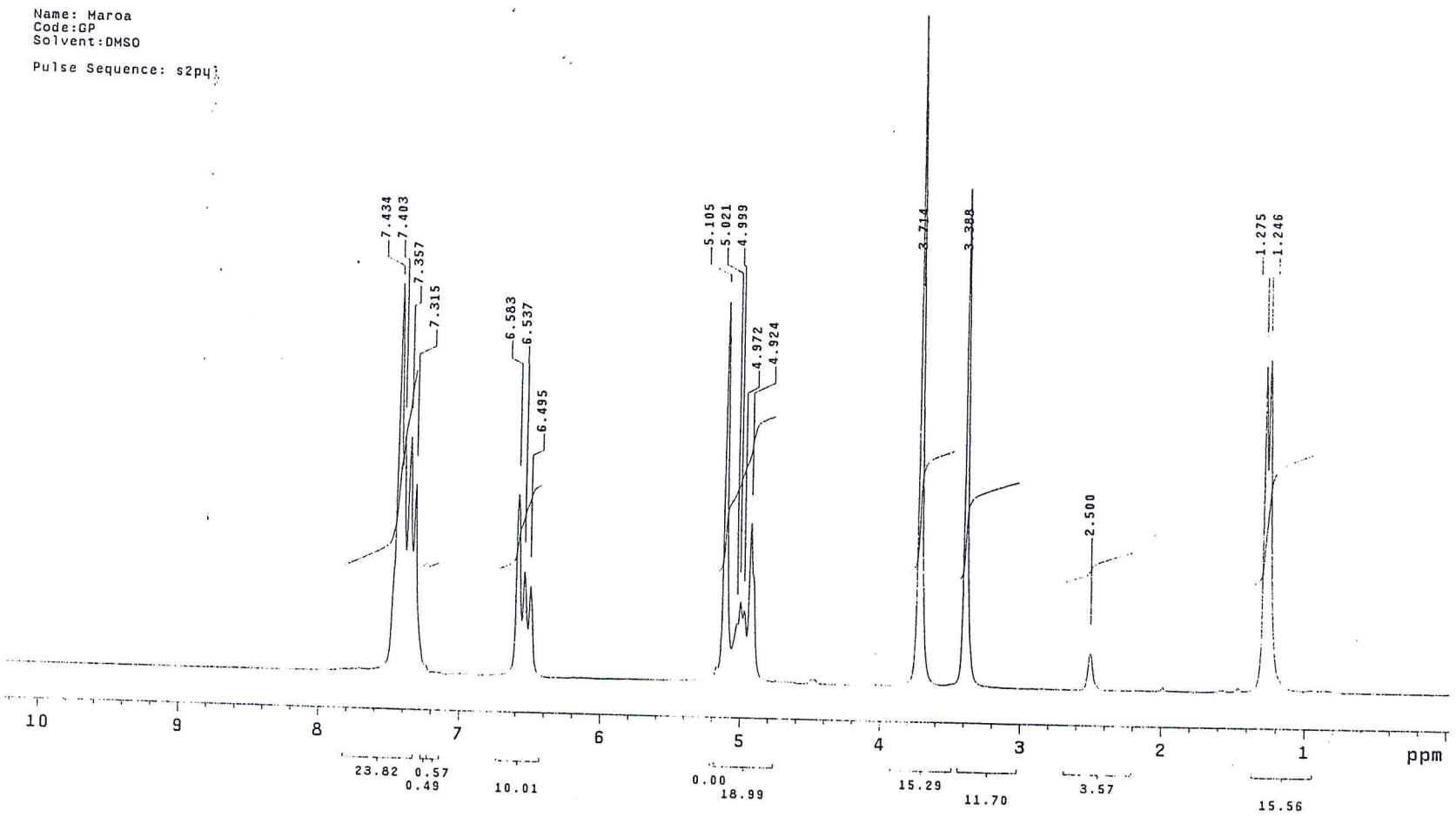
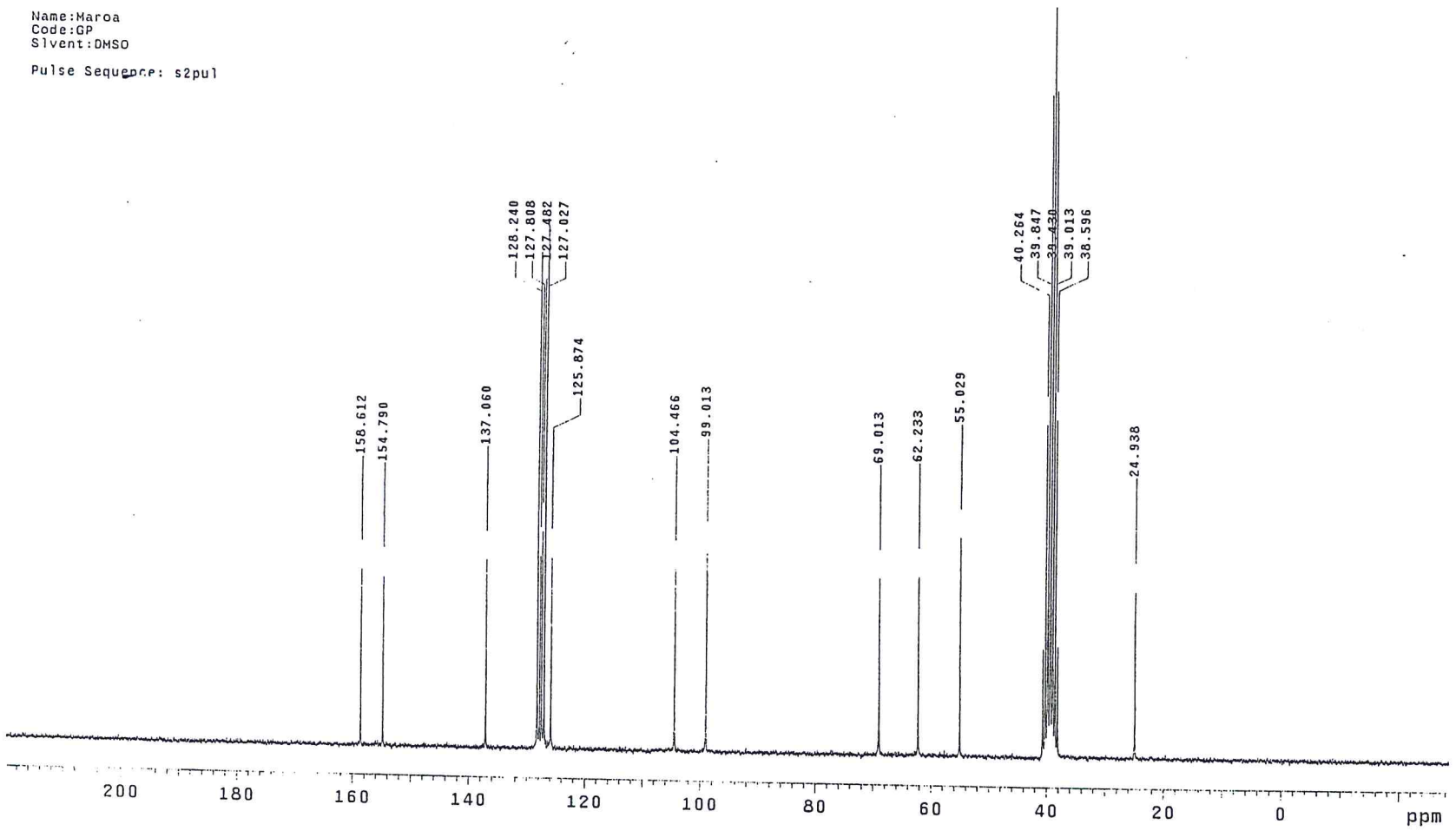
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

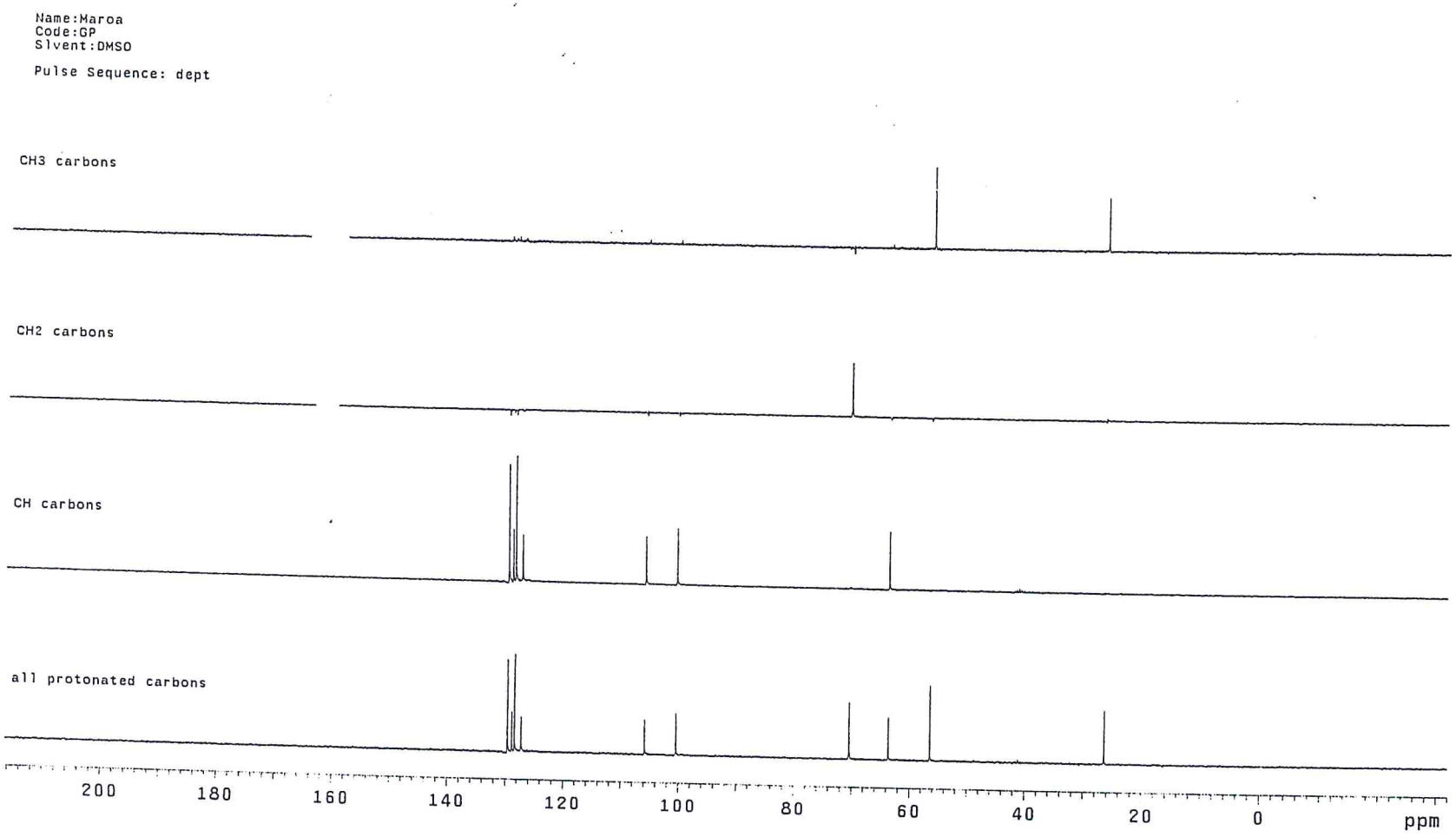
^{1}H NMR Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)



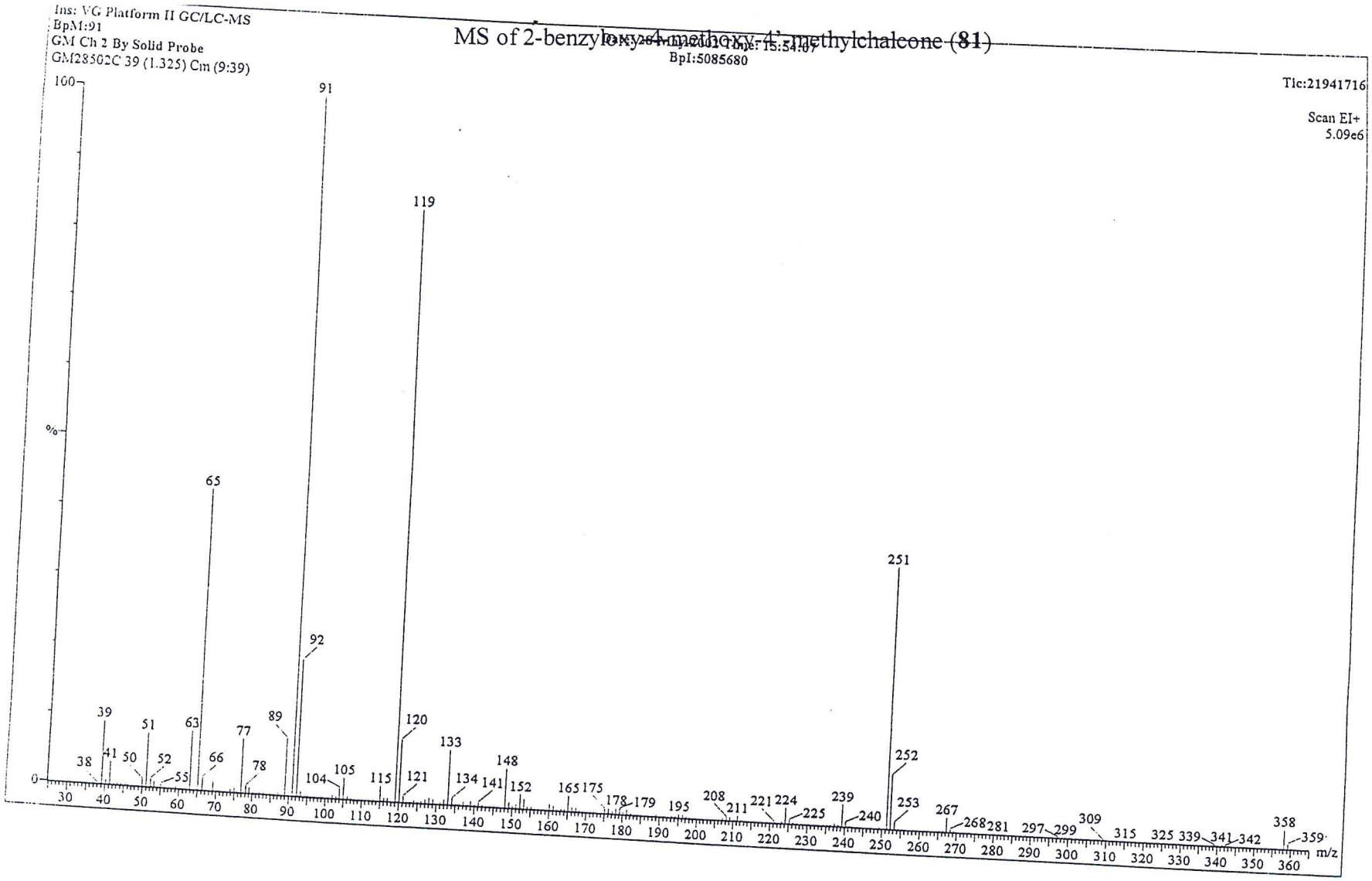
^{13}C NMR Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)



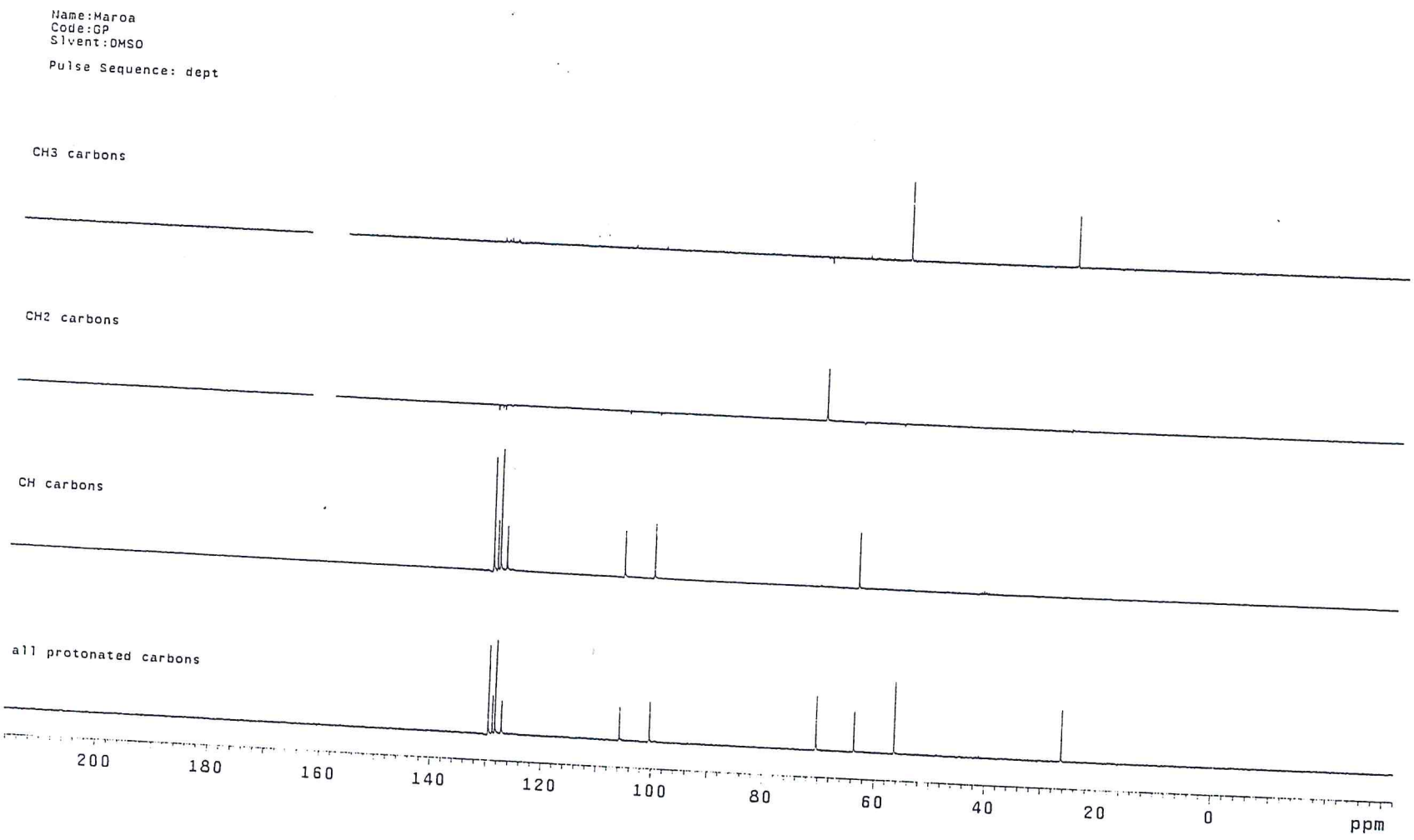
DEPT Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)



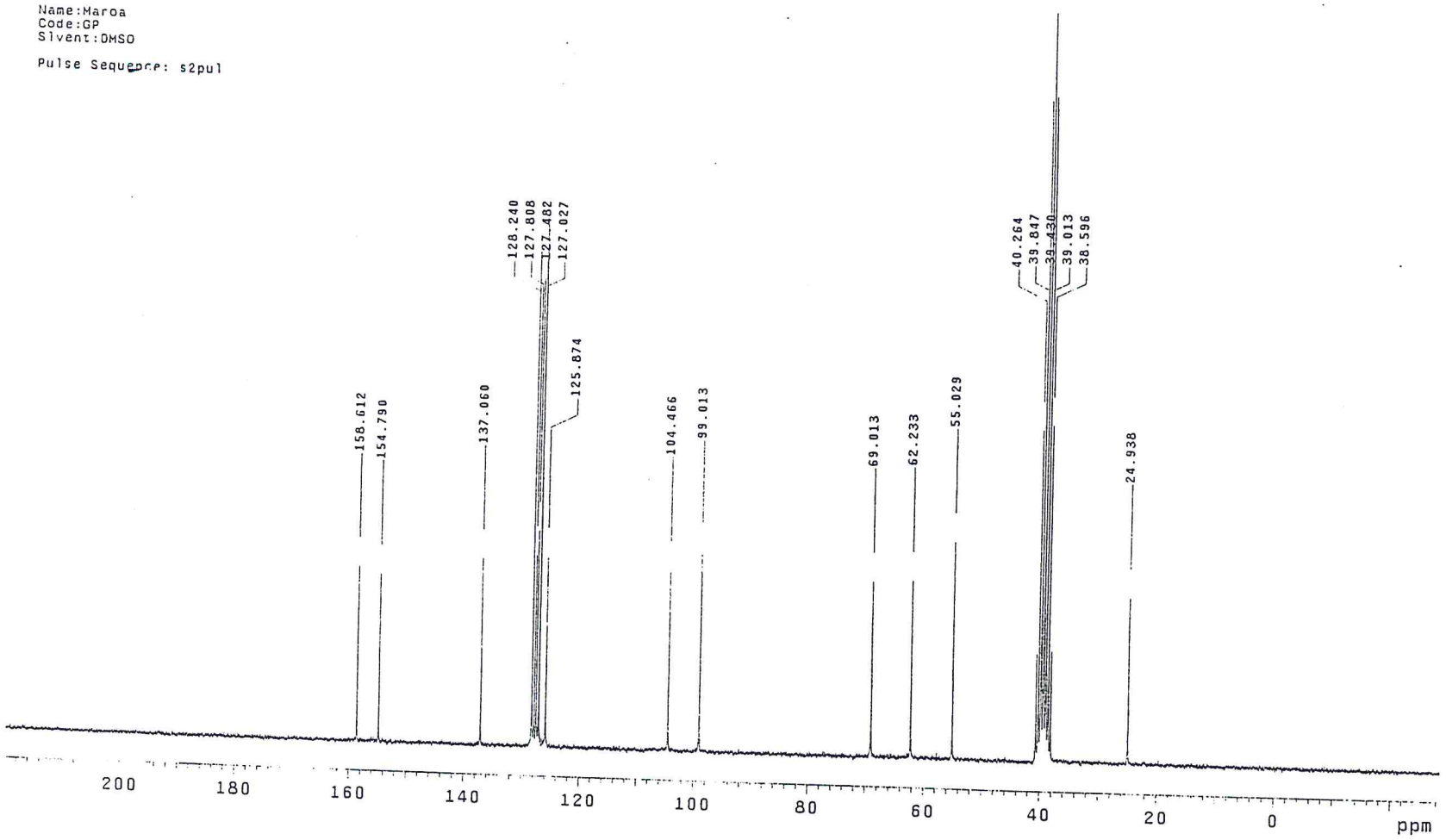
MS of 2-benzyloxy-4-methoxy-4'-methylchalcone (81)



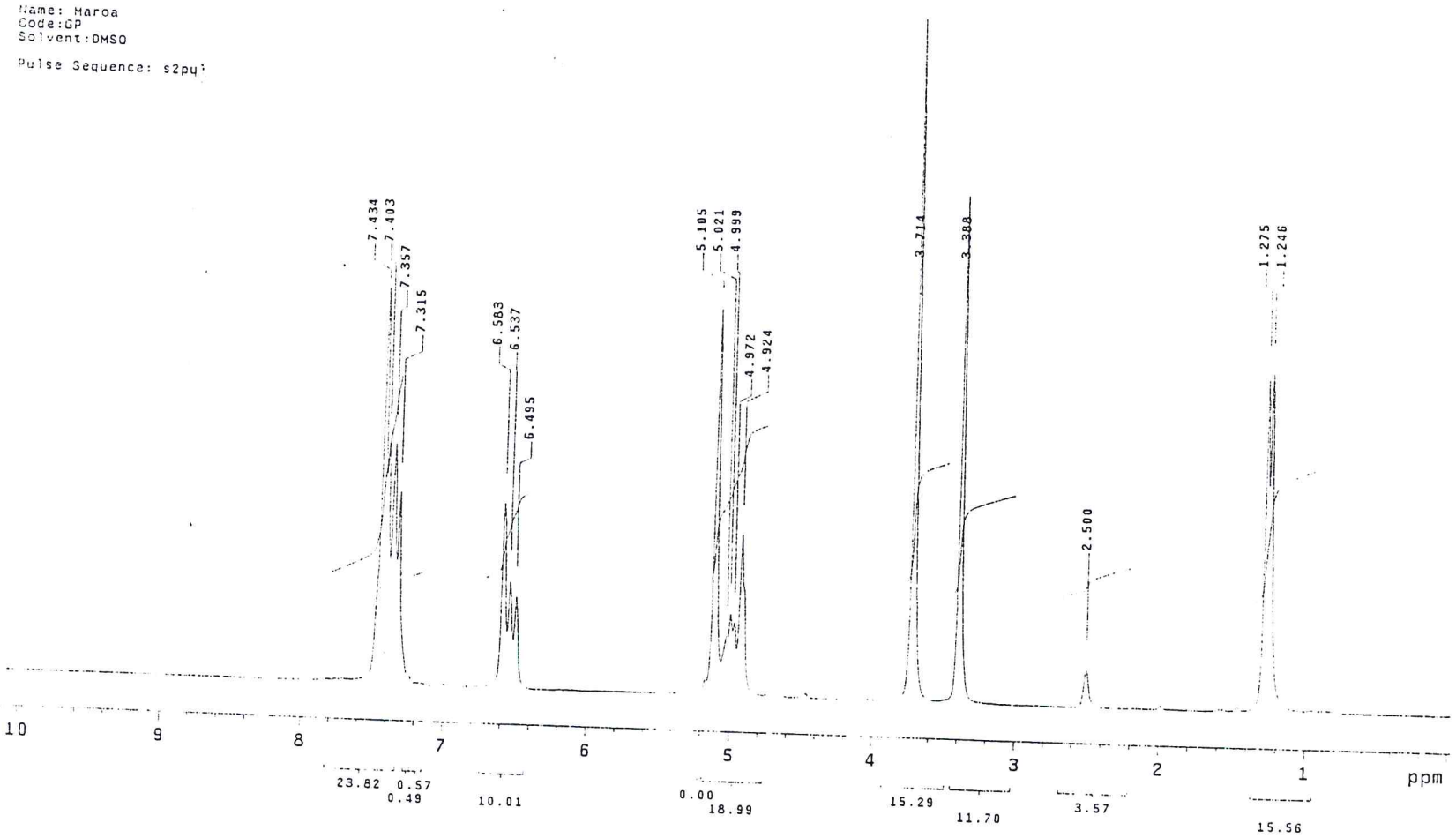
DEPT Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)

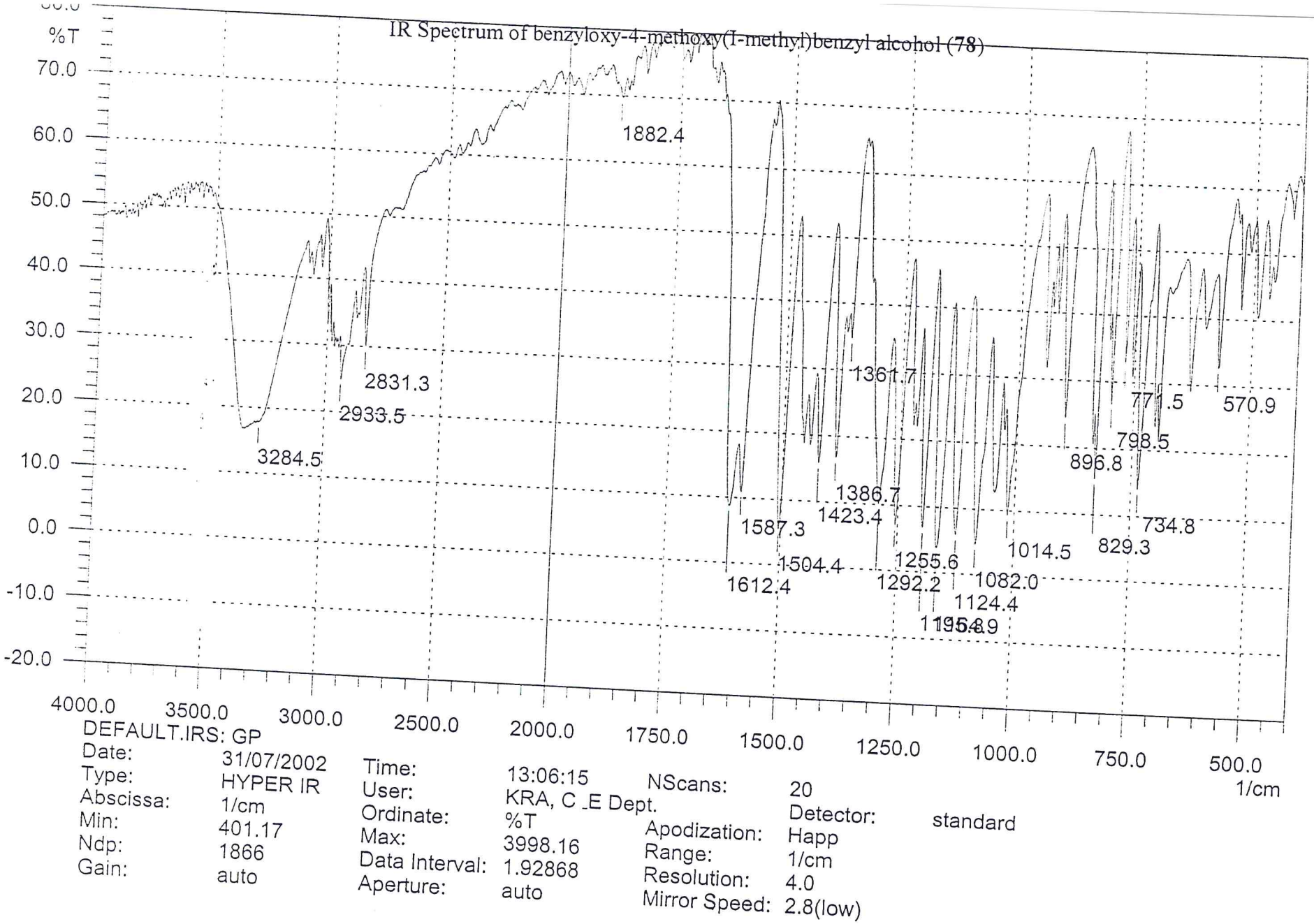


^{13}C NMR Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)

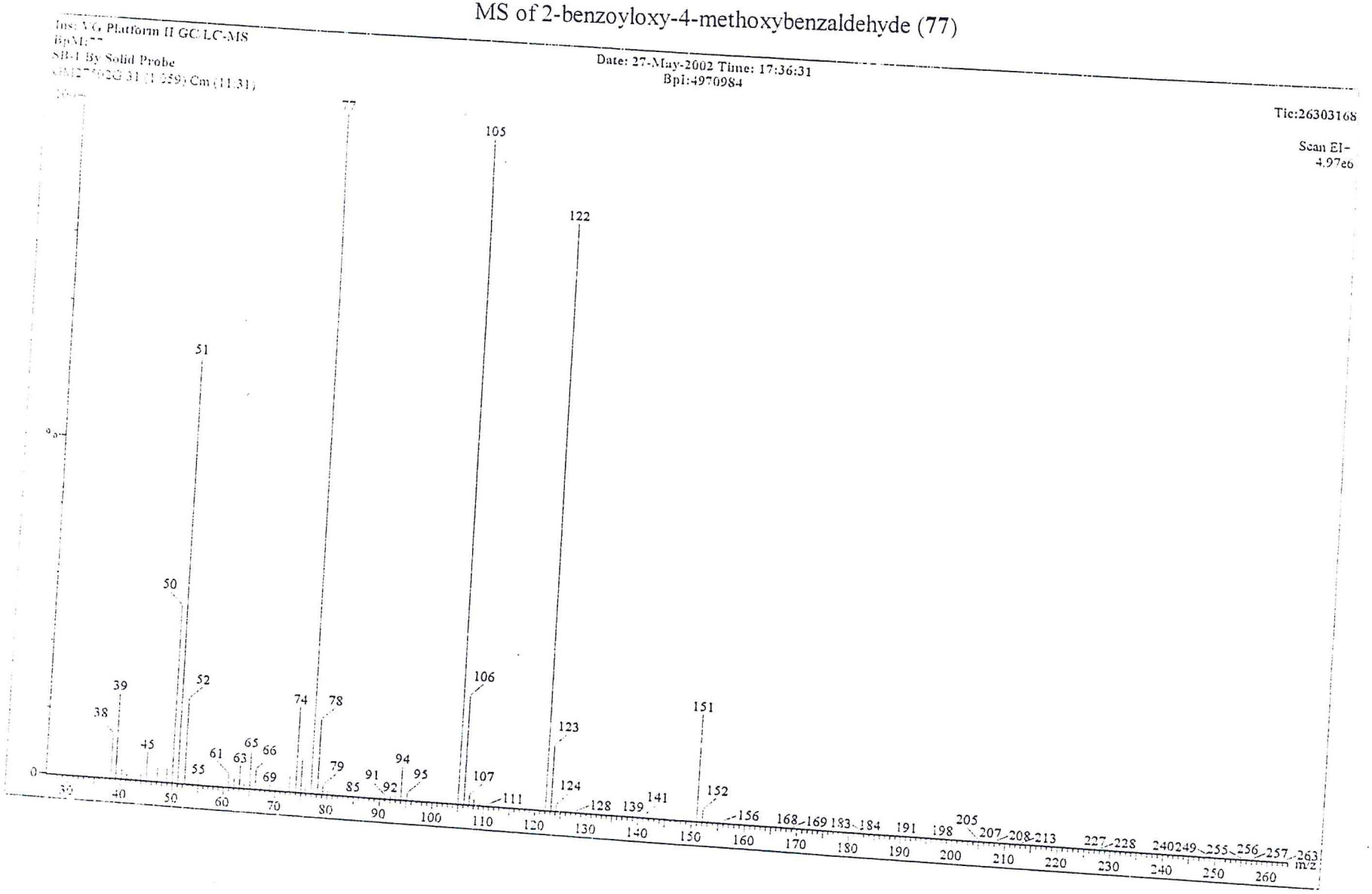


^{1}H NMR Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (**78**)

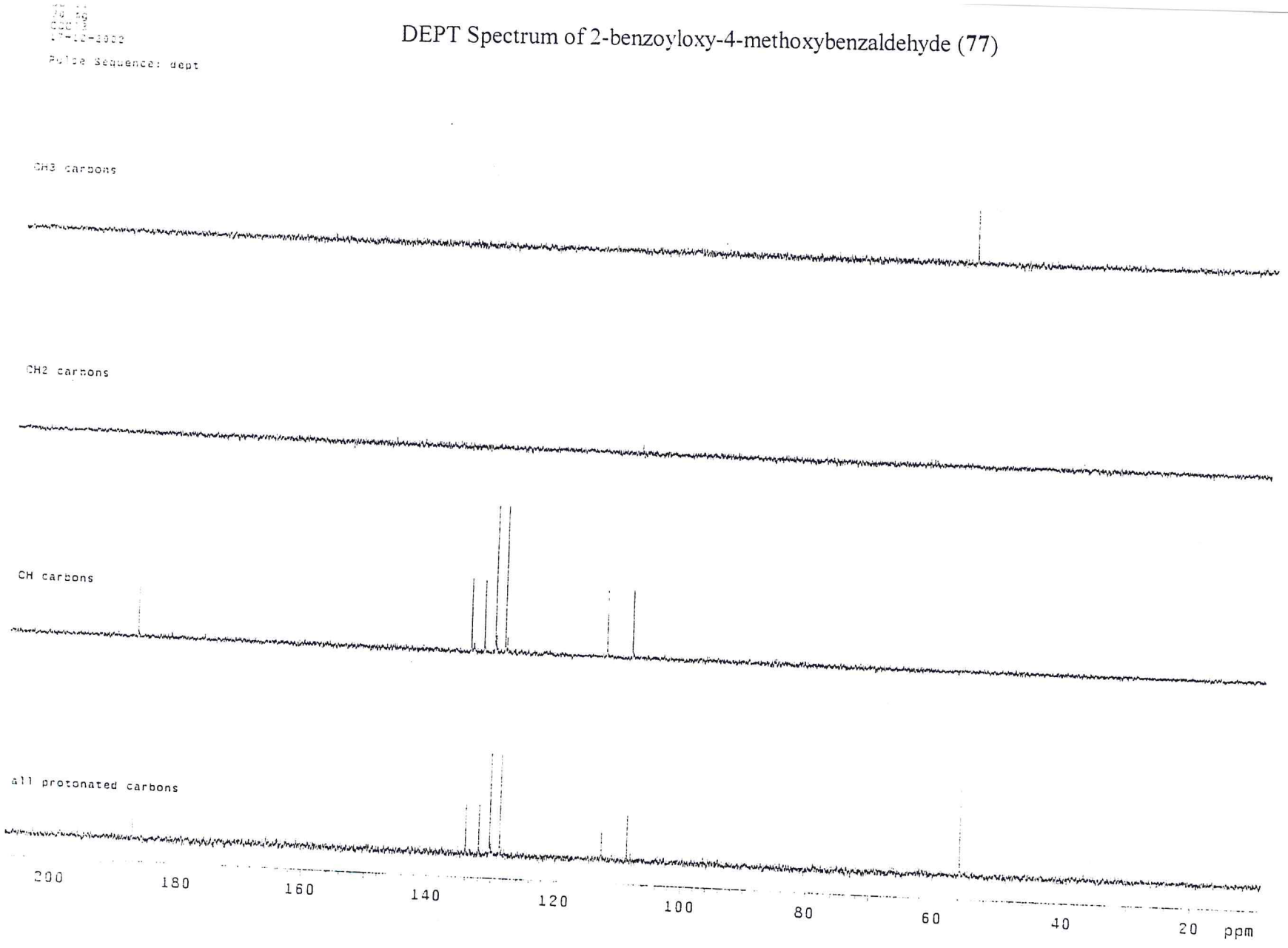



IR Spectrum of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (78)
%T
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
-20.0
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
3284.5
2933.5
2831.3
1882.4
1612.4
1587.3
1504.4
1423.4
1386.7
1361.7
1292.2
1255.6
1195.4
1124.4
1082.0
1014.5
896.8
829.3
798.5
771.5
734.8
570.9
DEFAULT.IRS: GP
Date: 31/07/2002
Time: 13:06:15
NScans: 20
Type: HYPER IR
User: KRA, C _E Dept.
Detector: standard
Abscissa: 1/cm
Ordinate: %T
Apodization: Happ
Min: 401.17
Max: 3998.16
Range: 1/cm
Ndp: 1866
Data Interval: 1.92868
Resolution: 4.0
Gain: auto
Aperture: auto
Mirror Speed: 2.8(low)

MS of 2-benzoyloxy-4-methoxybenzaldehyde (77)



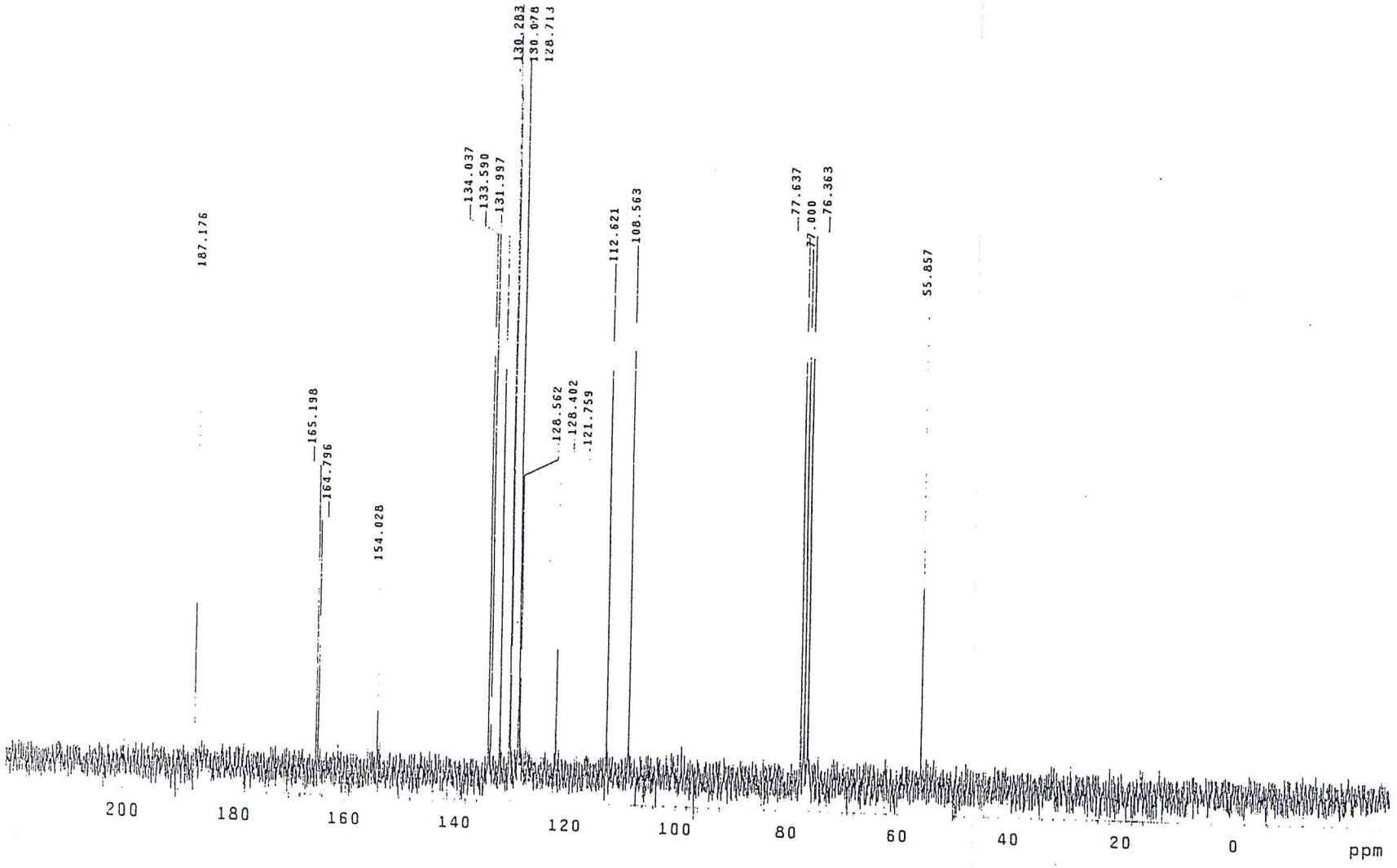
DEPT Spectrum of 2-benzoyloxy-4-methoxybenzaldehyde (77)



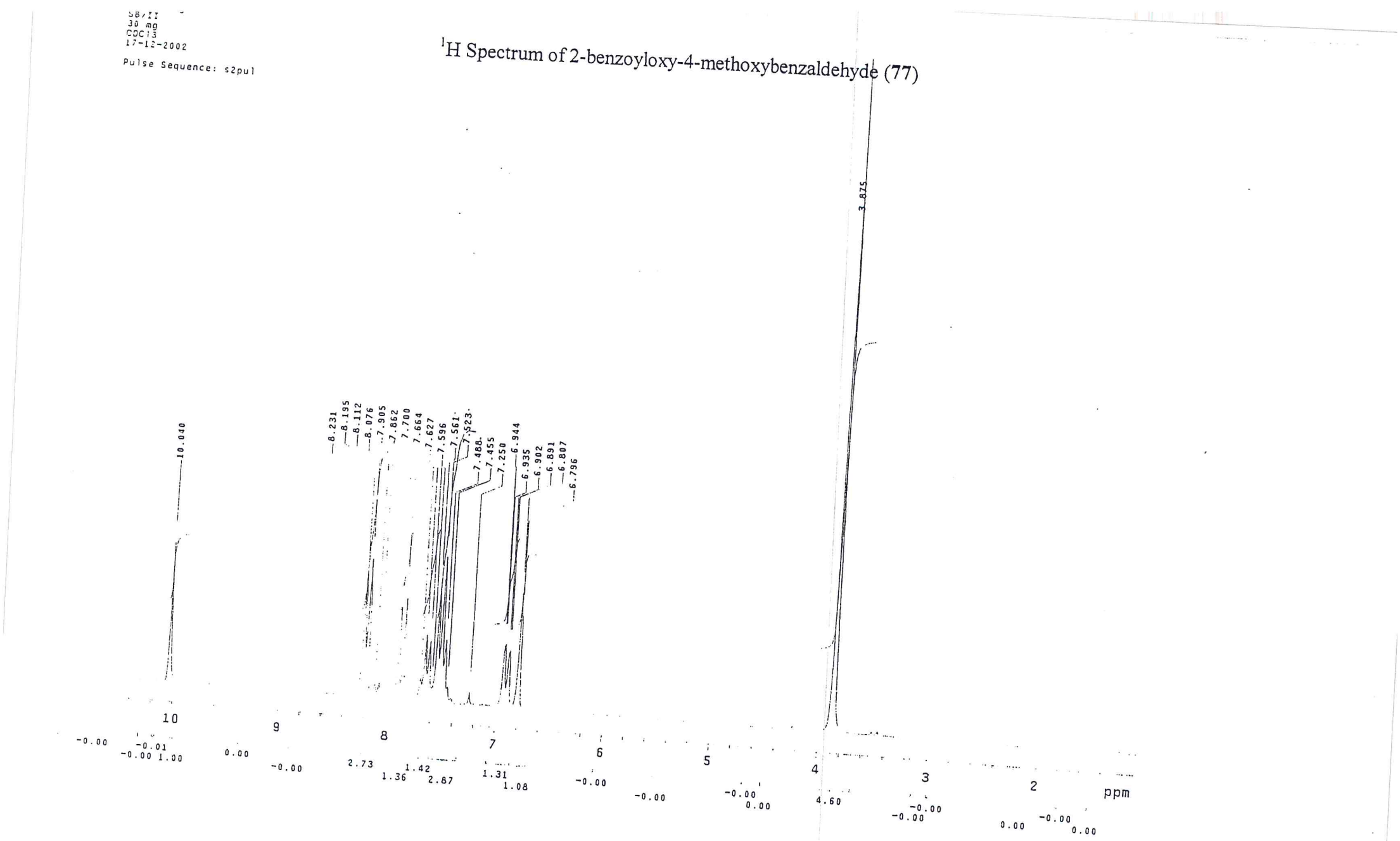
G. Mahamga
SB/II
30 mg
CDCl3
17-12-2002

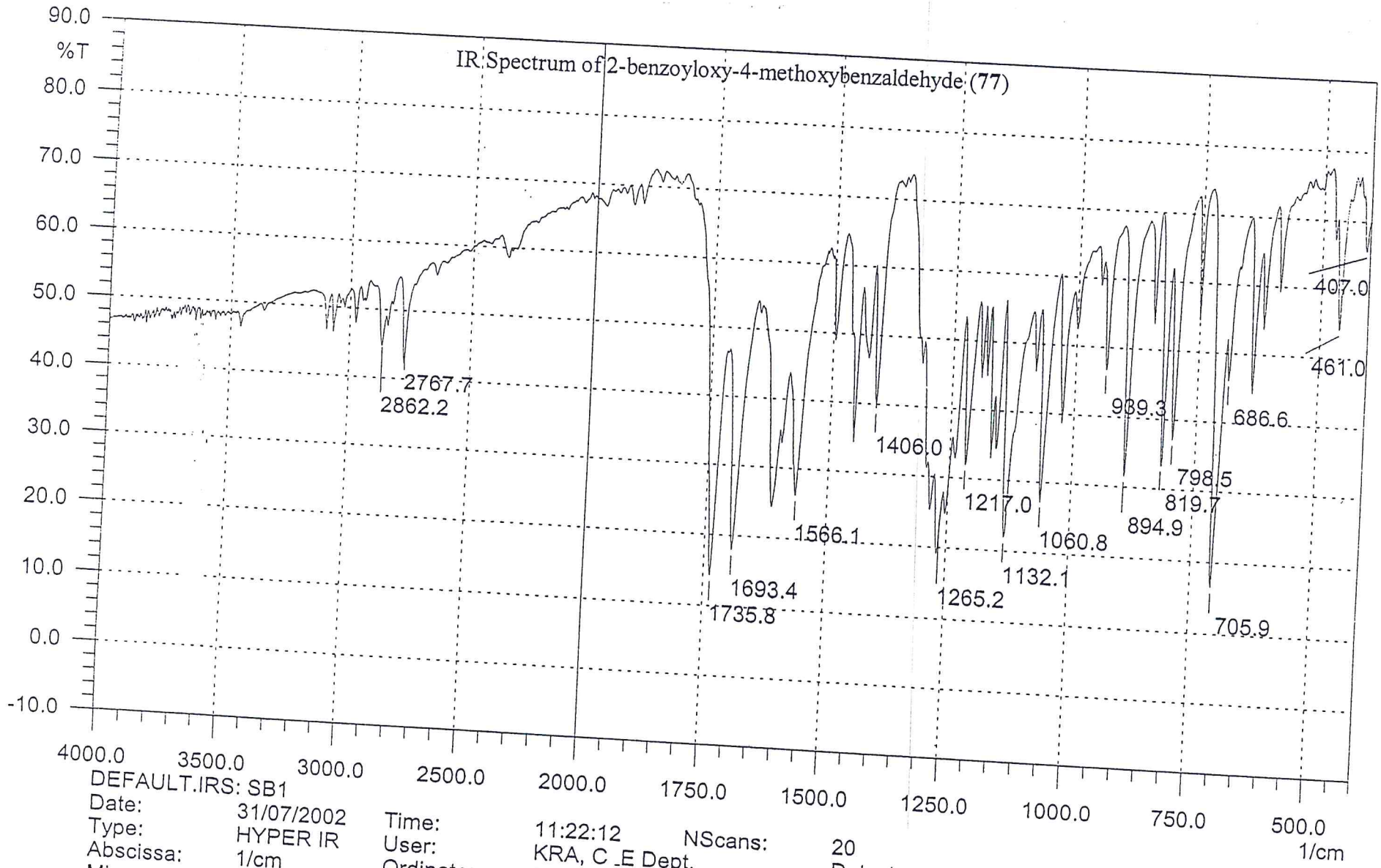
Pulse Sequence: s2pul

^{13}C NMR Spectrum of 2-benzoyloxy-4-methoxybenzaldehyde (77)



^{1}H Spectrum of 2-benzoyloxy-4-methoxybenzaldehyde (77)





DEFAULT.IRS: SB1

Date:	31/07/2002	Time:	11:22:12	NScans:	20
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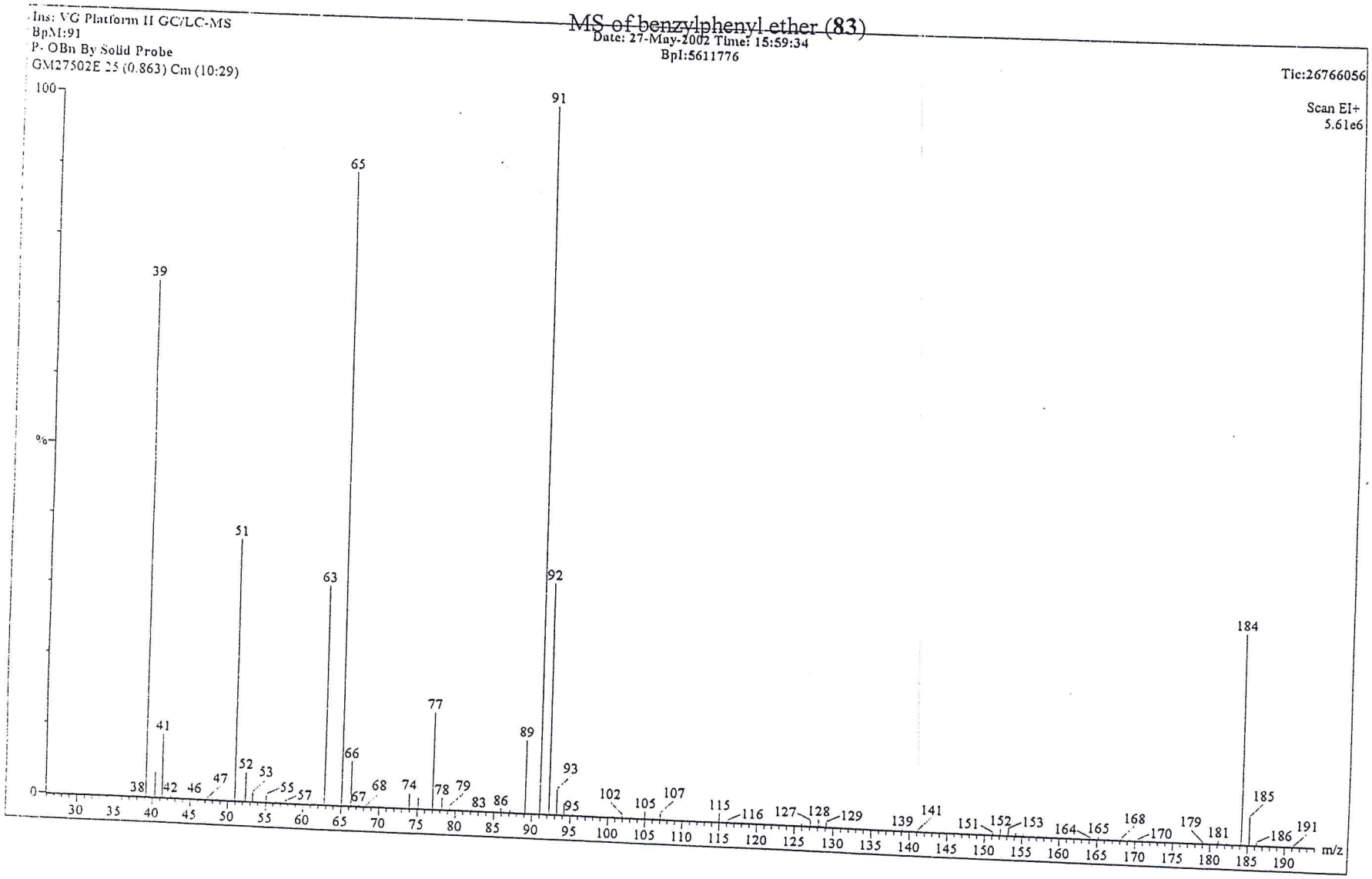
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ
Min:	401.17	Max:	3998.16	Range:	1/cm
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

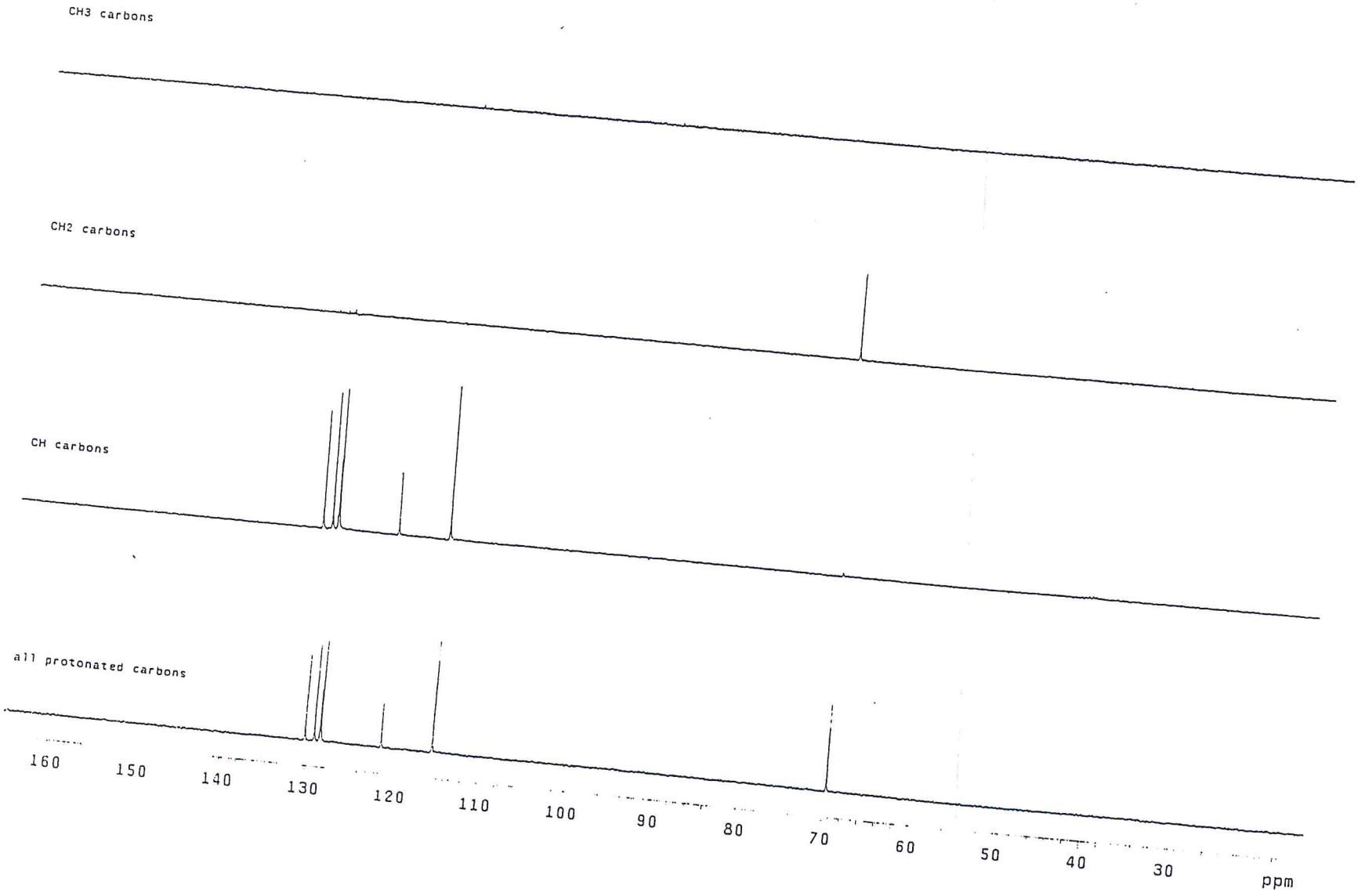
MS of benzylphenyl ether (83)



DR AKENGA
P-OBn
DMSO
DMSO
50 MHZ
30-11-02

Pulse Sequence: dept

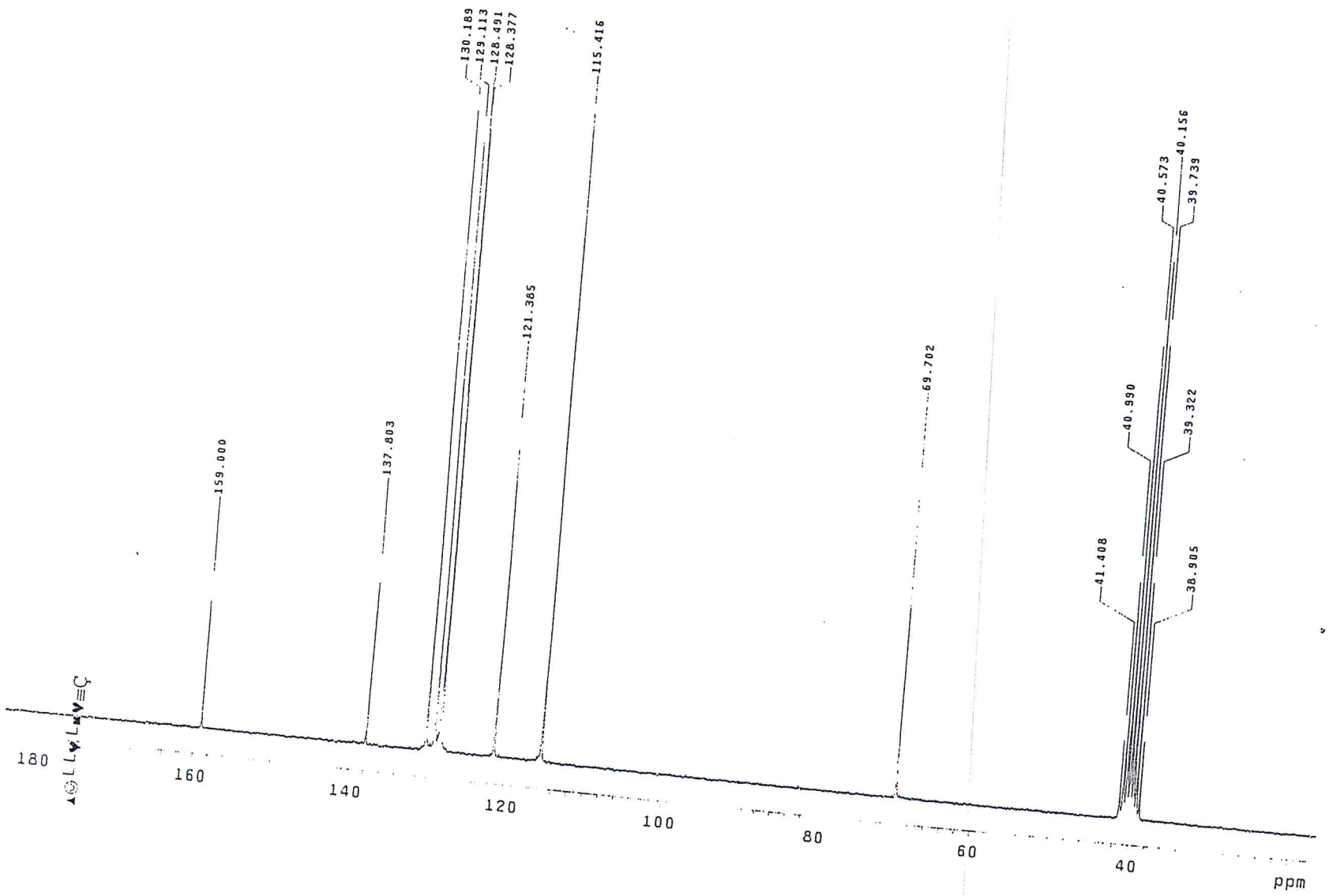
DEPT Spectrum of benzylphenyl ether (**83**)



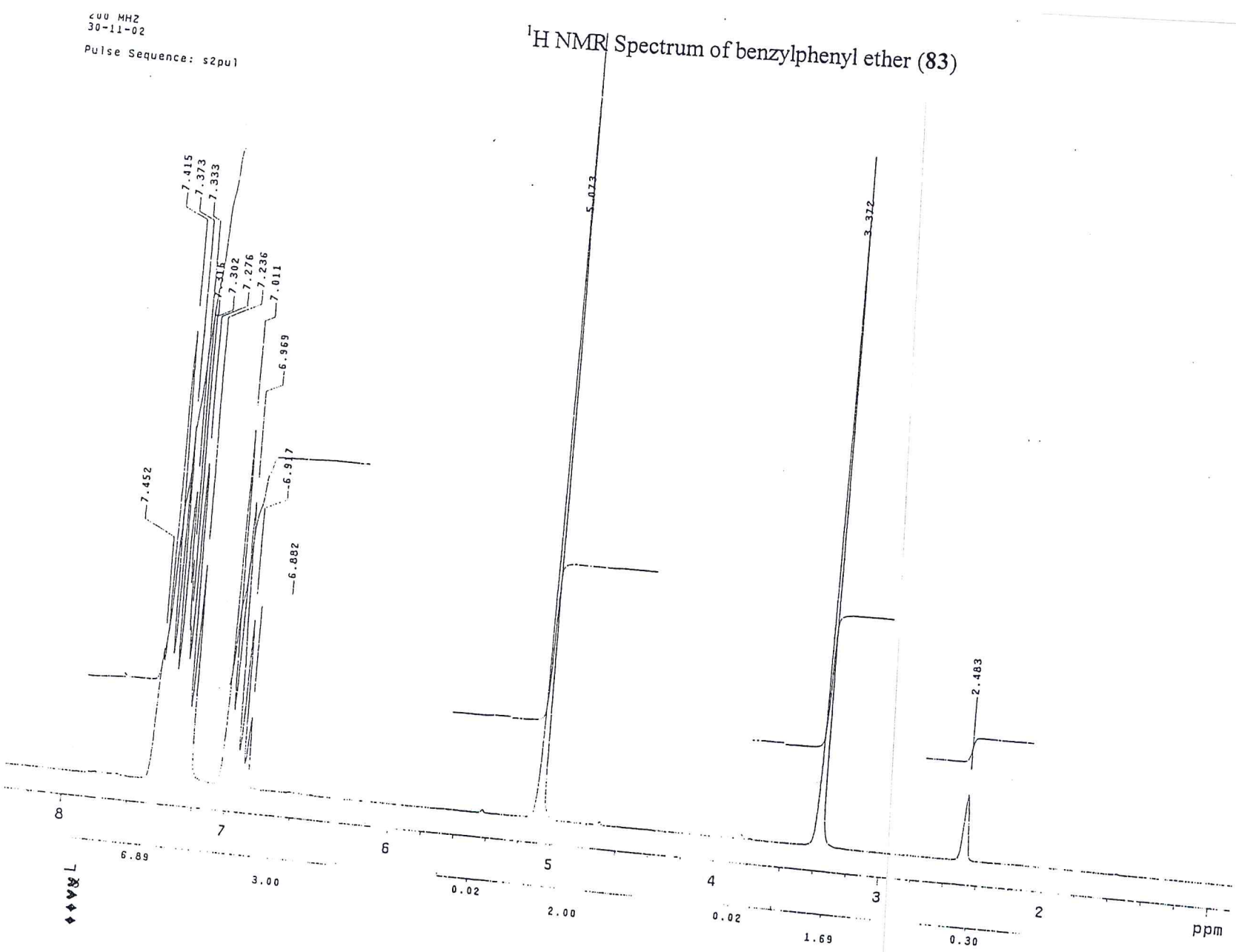
DR AKENGA
P-OBn
DMSO
13C NMR
50 MHZ
30-11-02

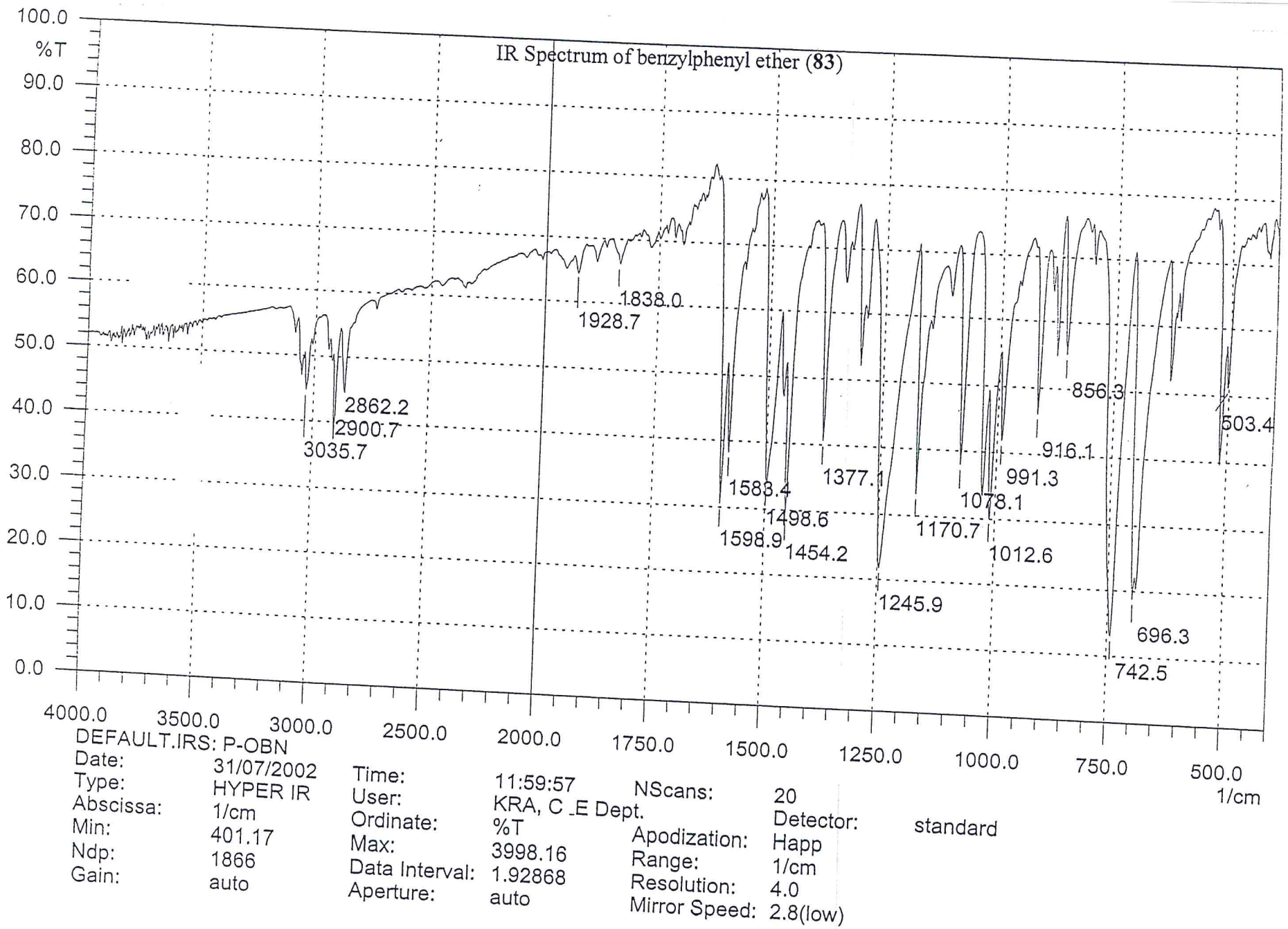
Pulse Sequence: s2pul

^{13}C NMR Spectrum of benzylphenyl ether (**83**)



^{1}H NMR Spectrum of benzylphenyl ether (**83**)




IR Spectrum of benzylphenyl ether (83)
100.0
%T
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
3035.7
2862.2
2900.7
1928.7
1838.0
1598.9
1583.4
1498.6
1454.2
1377.1
1245.9
1170.7
1078.1
1012.6
991.3
916.1
856.3
742.5
696.3
503.4
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Date: 31/07/2002
Type: HYPER IR
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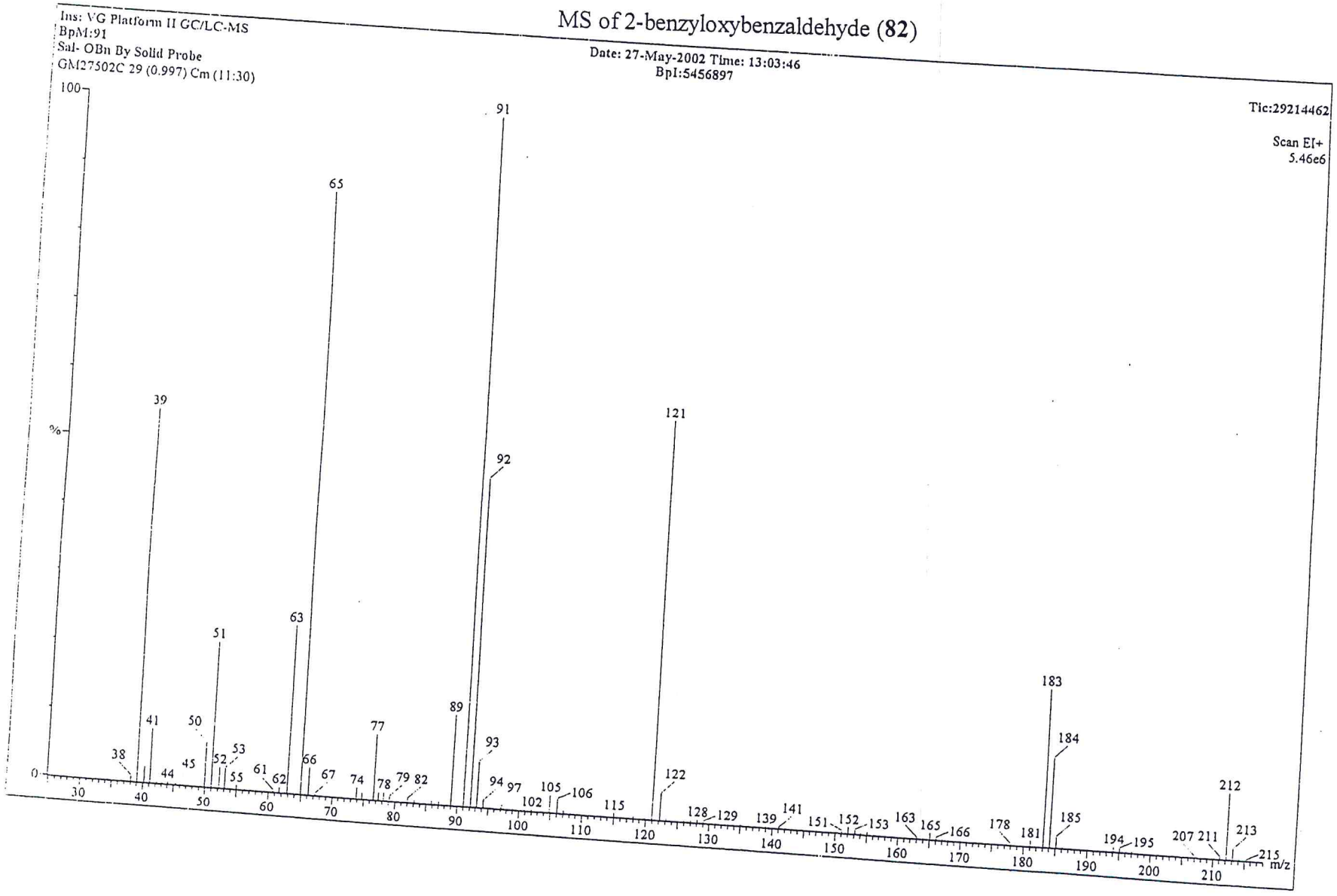
Min: 401.17
Ndp: 1866
Gain: auto
Time: 11:59:57
User: KRA, C _E Dept.
Ordinate: %T
Max: 3998.16
Data Interval: 1.92868
Aperture: auto
NScans: 20
Detector: standard
Apodization: Happ
Range: 1/cm
Resolution: 4.0
Mirror Speed: 2.8(low)

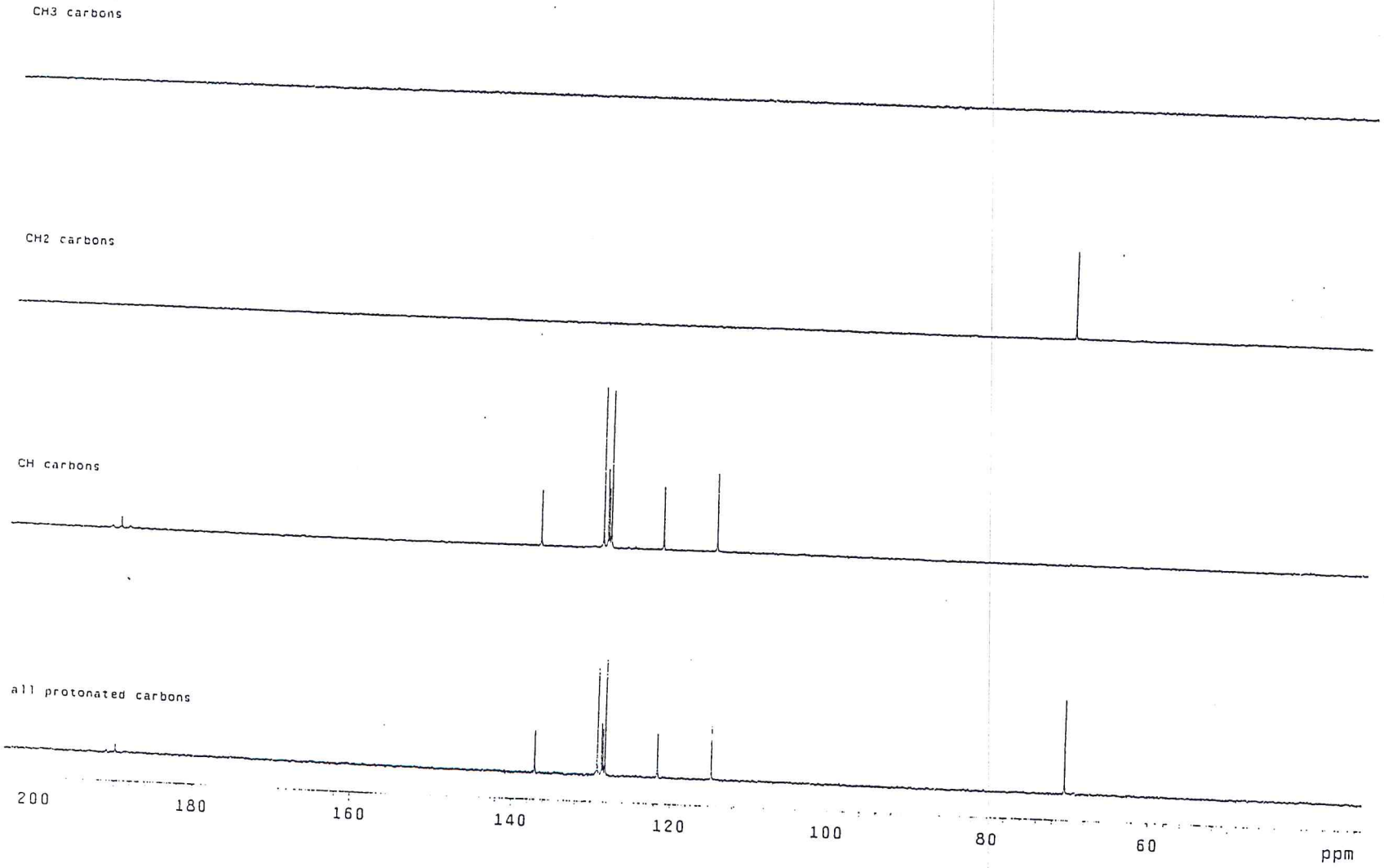
MS of 2-benzyloxybenzaldehyde (**82**)



DR AKENGA
SOL-OBn
DMSO
DEPT
50 MHZ
02-12-02

Pulse Sequence: dept

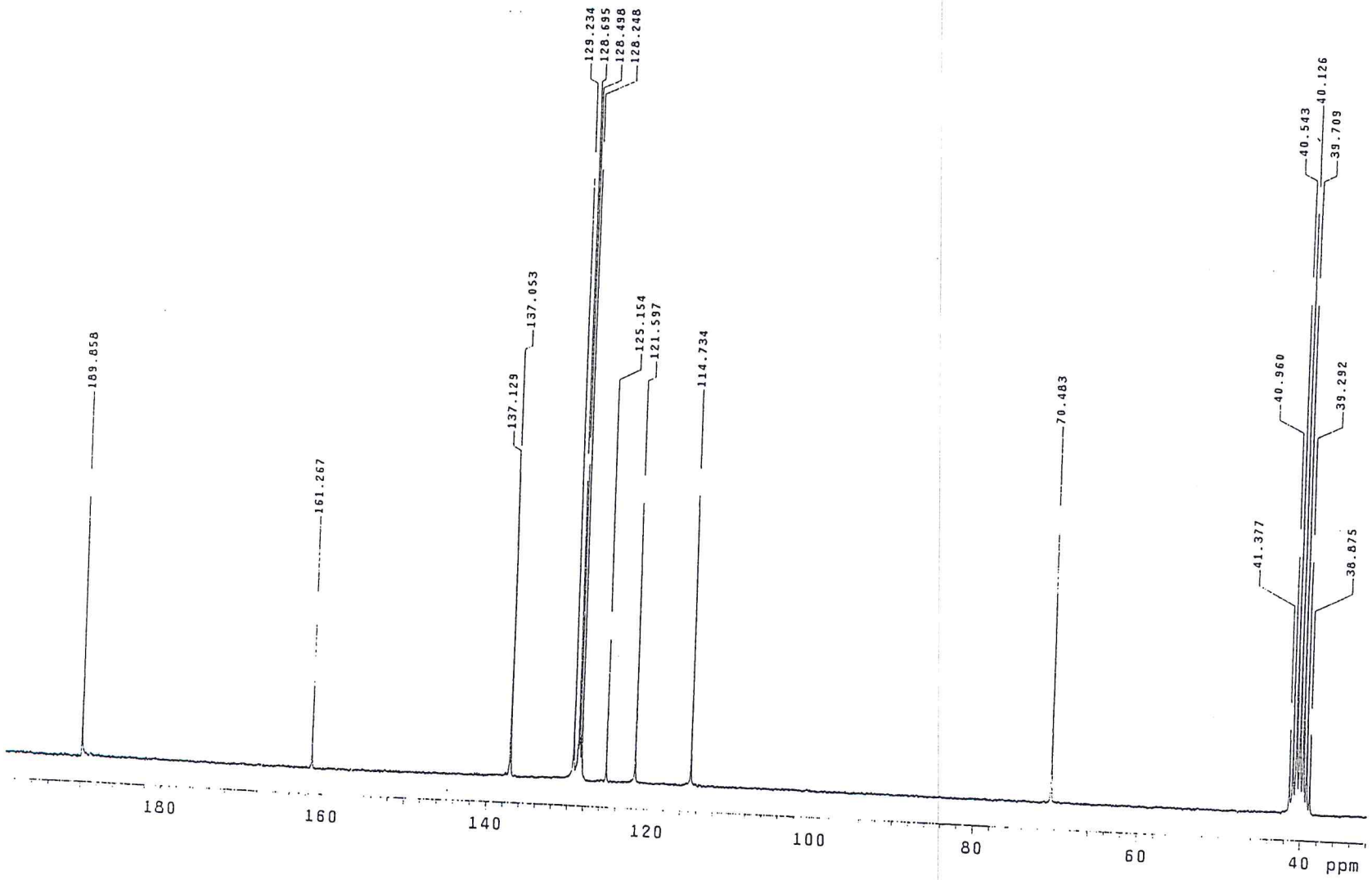
DEPT Spectrum of 2-benzyloxybenzaldehyde (**82**)



DR AKENGA
SOL-08a
DMSO
13C NMR
50 MHZ
02-12-02

Pulse Sequence: s2pul

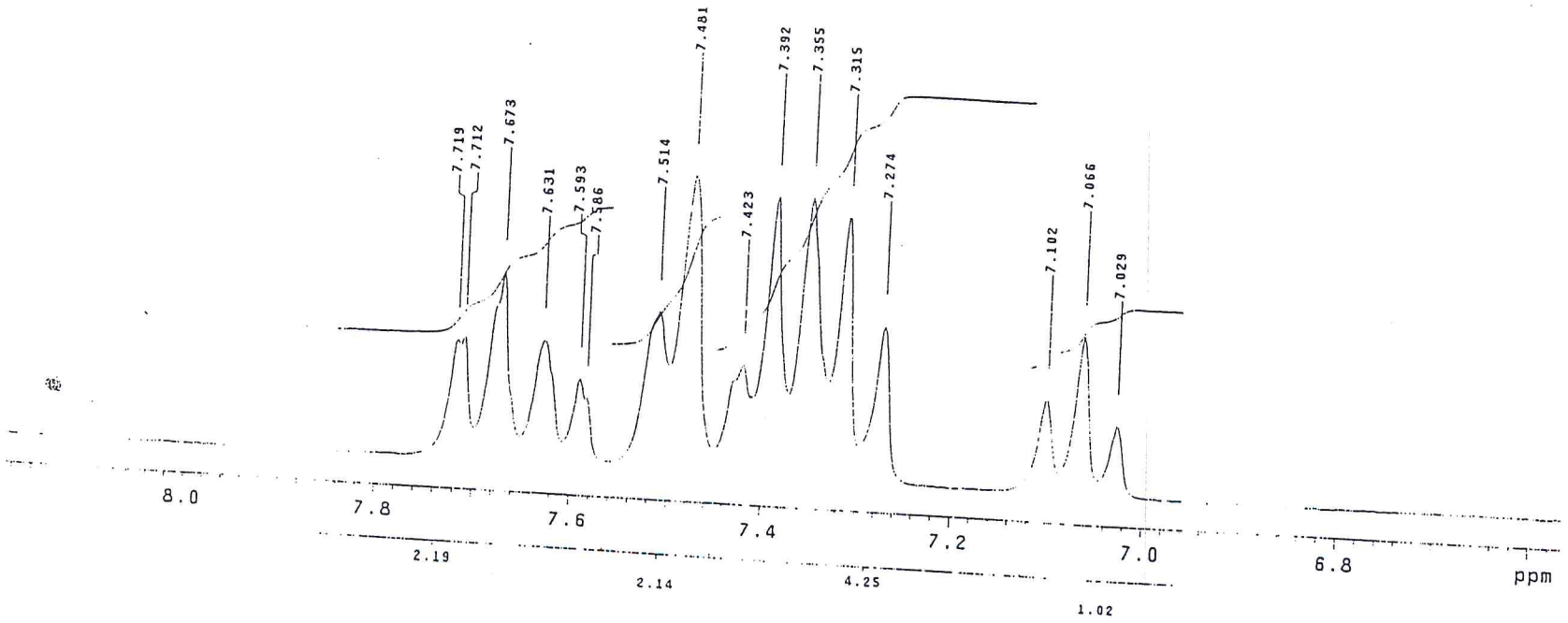
^{13}C NMR Spectrum of 2-benzyloxybenzaldehyde (**82**)



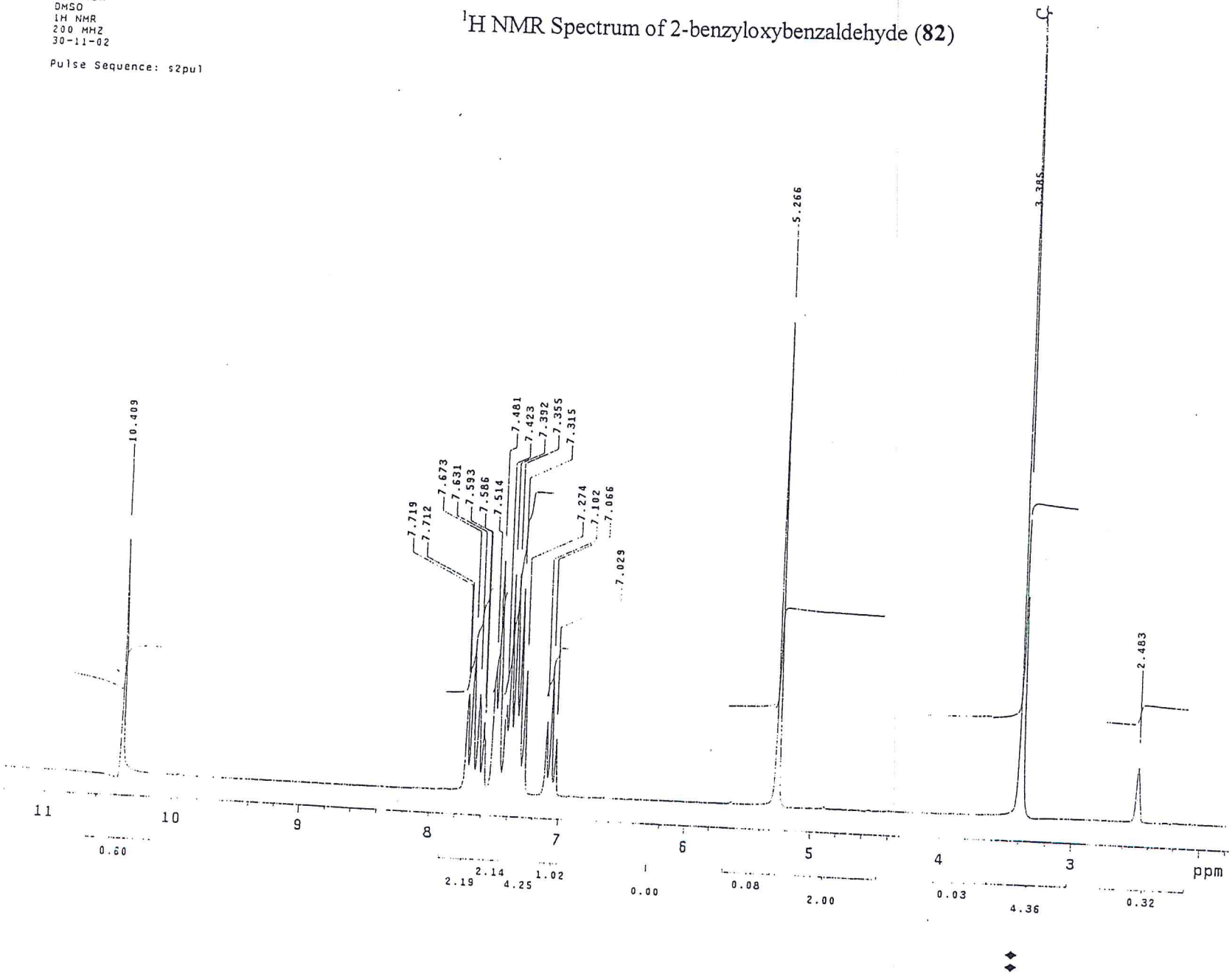
DR AKENGA
SOL-OBn
DMSO
1H NMR
200 MHZ
30-11-02

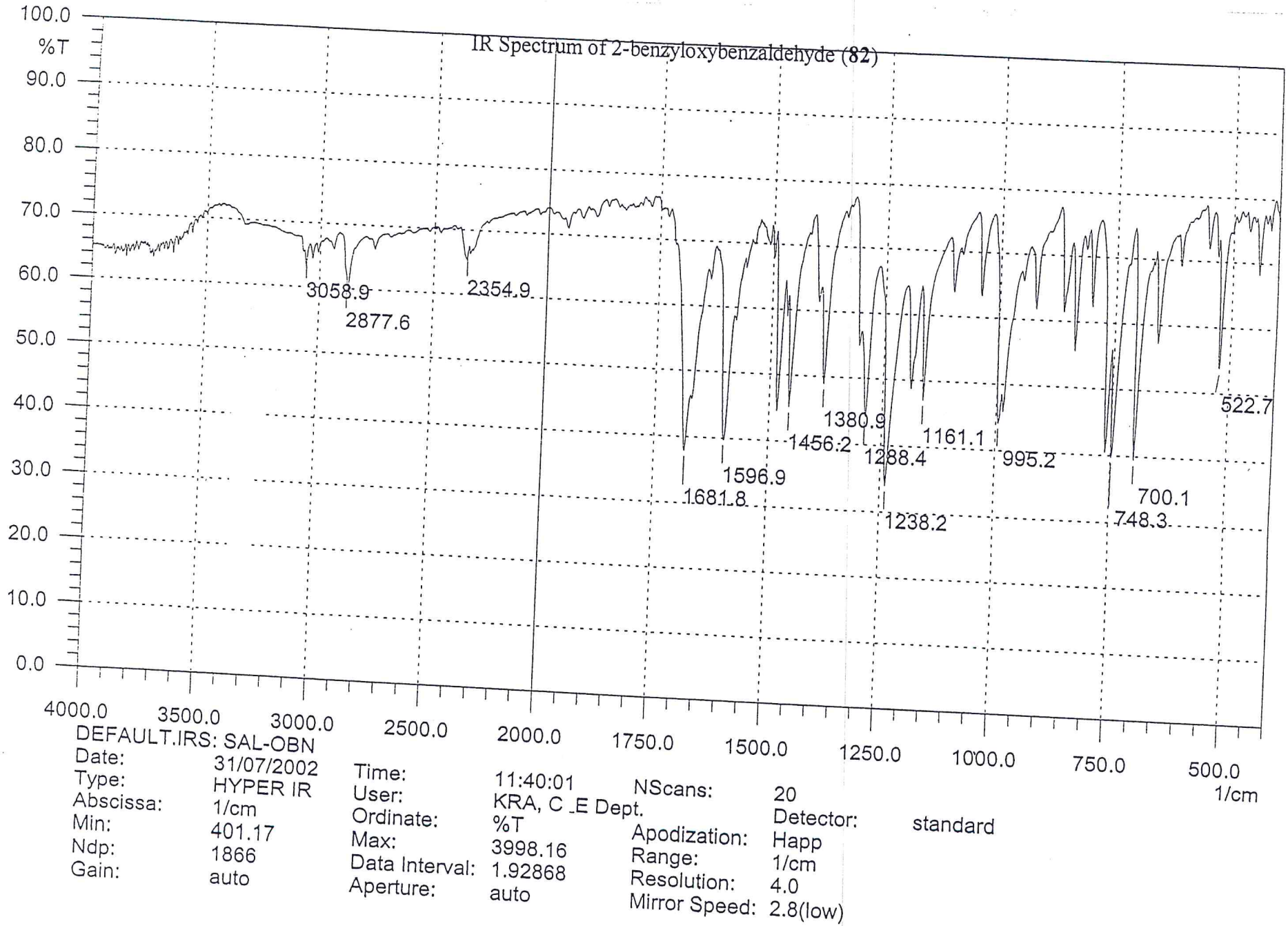
Pulse Sequence: s2pul

^{1}H NMR Spectrum of 2-benzyloxybenzaldehyde (**82**)



^{1}H NMR Spectrum of 2-benzyloxybenzaldehyde (**82**)




IR Spectrum of 2-benzyloxybenzaldehyde (82)
100.0
%T
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
3058.9
2877.6
2354.9
1681.8
1596.9
1456.2
1380.9
1288.4
1238.2
1161.1
995.2
748.3
700.1
522.7
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
DEFAULT.IRS: SAL-OBN
Date: 31/07/2002
Type: HYPER IR
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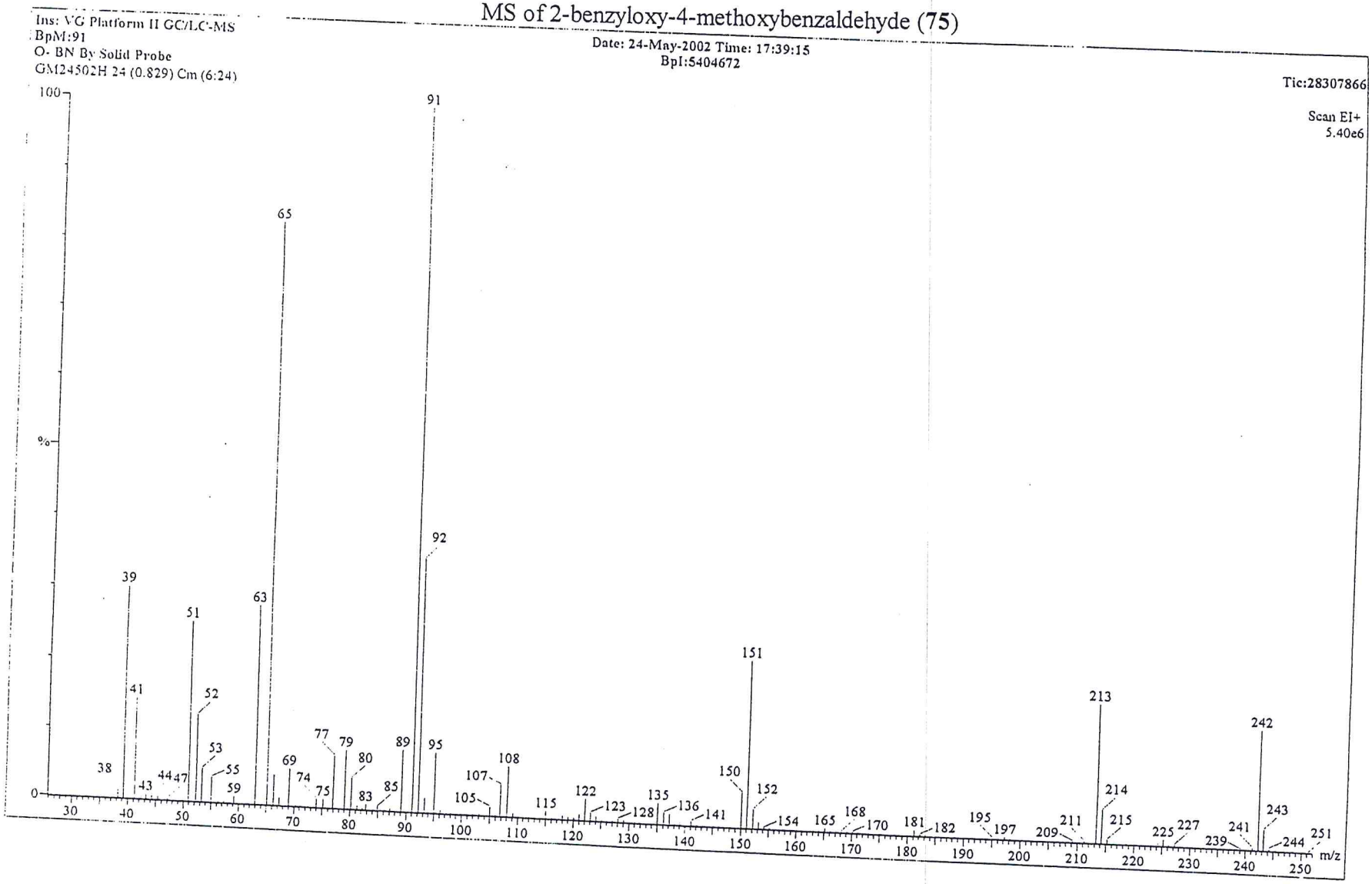
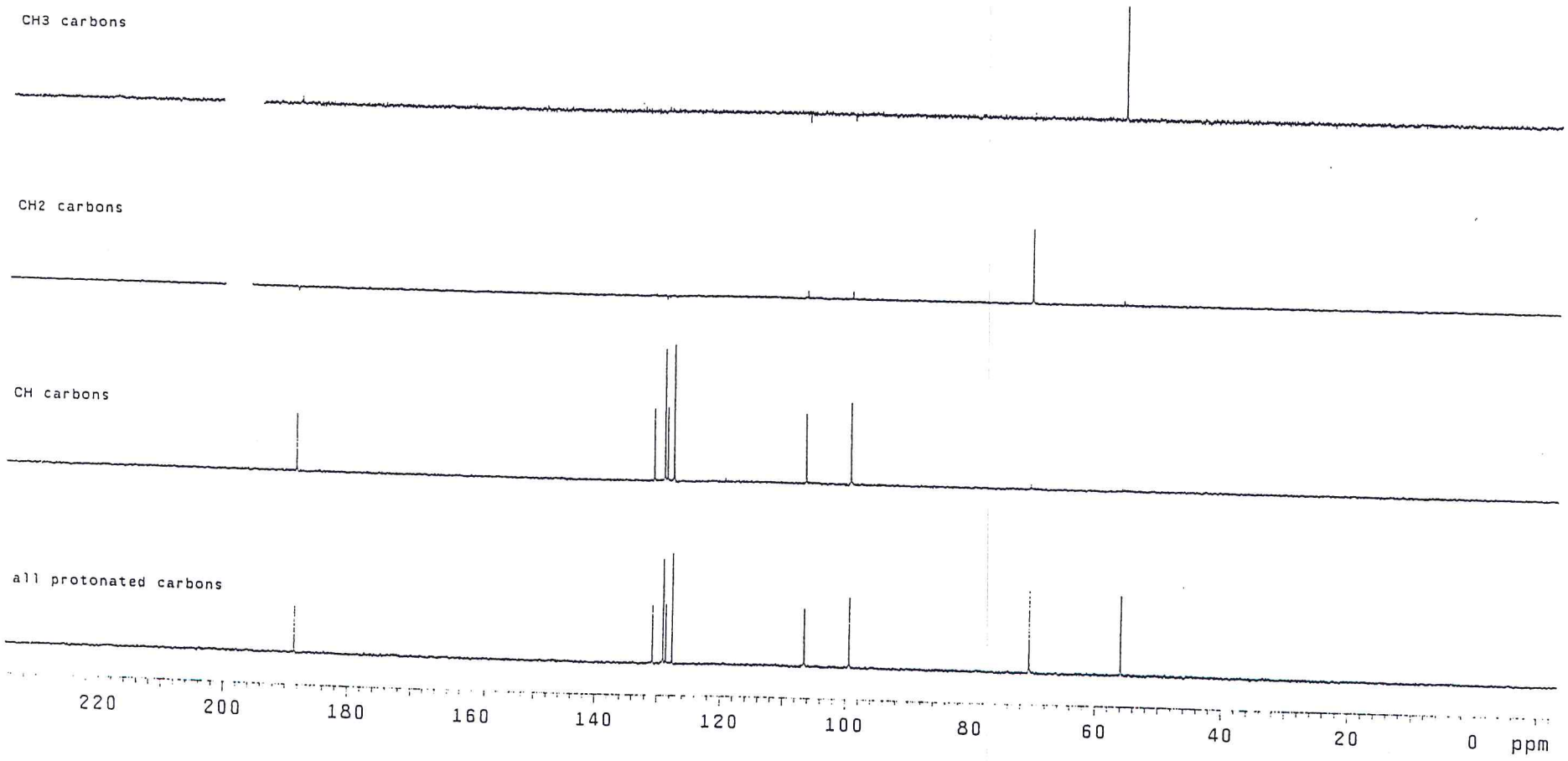
Min: 401.17
Ndp: 1866
Gain: auto
Time: 11:40:01
User: KRA, C .E Dept.
Ordinate: %T
Max: 3998.16
Data Interval: 1.92868
Aperture: auto
NScans: 20
Detector: standard
Apodization: Happ
Range: 1/cm
Resolution: 4.0
Mirror Speed: 2.8(low)

MS of 2-benzyloxy-4-methoxybenzaldehyde (**75**)

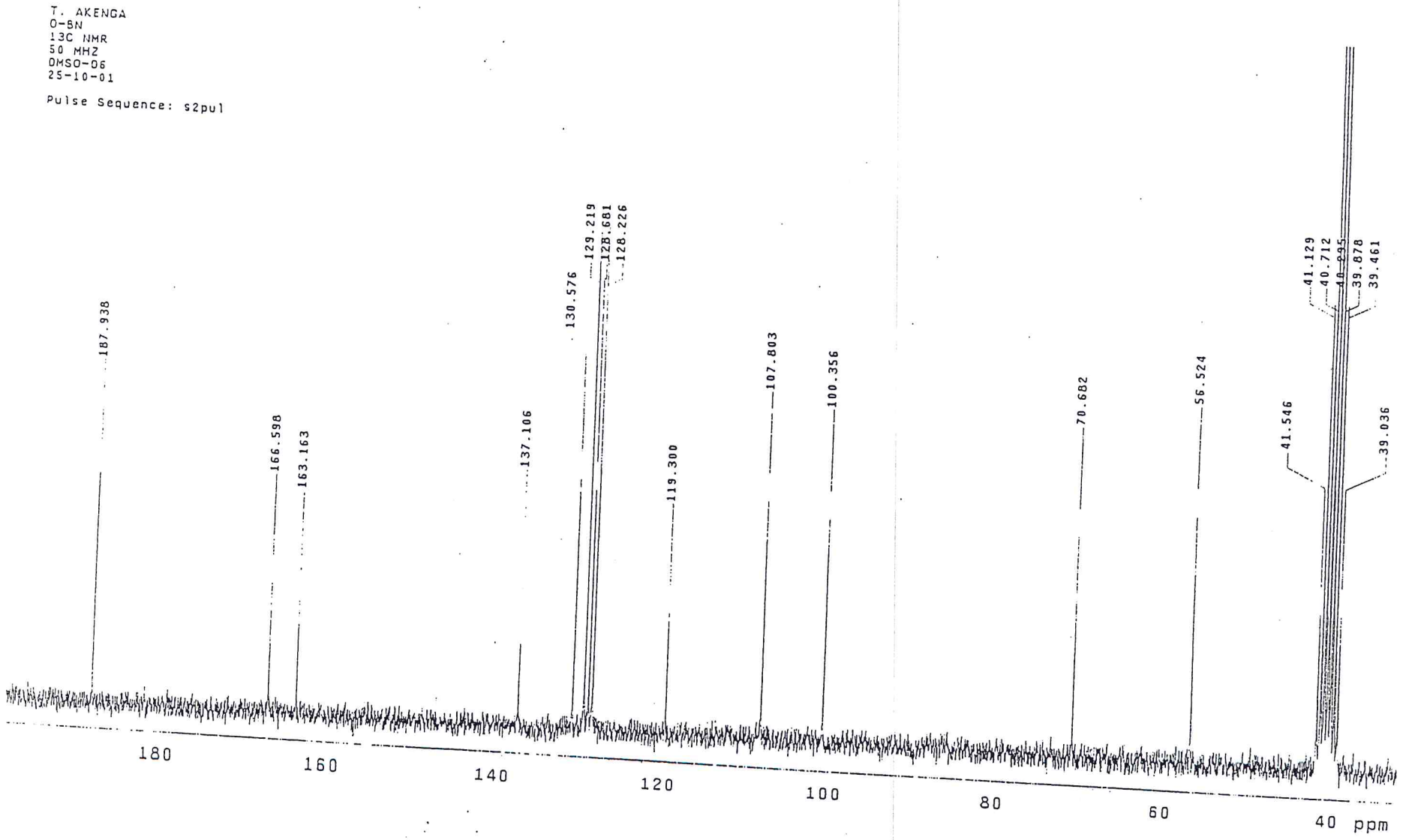


DEPT Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)



^{13}C NMR Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)

^{13}C NMR Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)



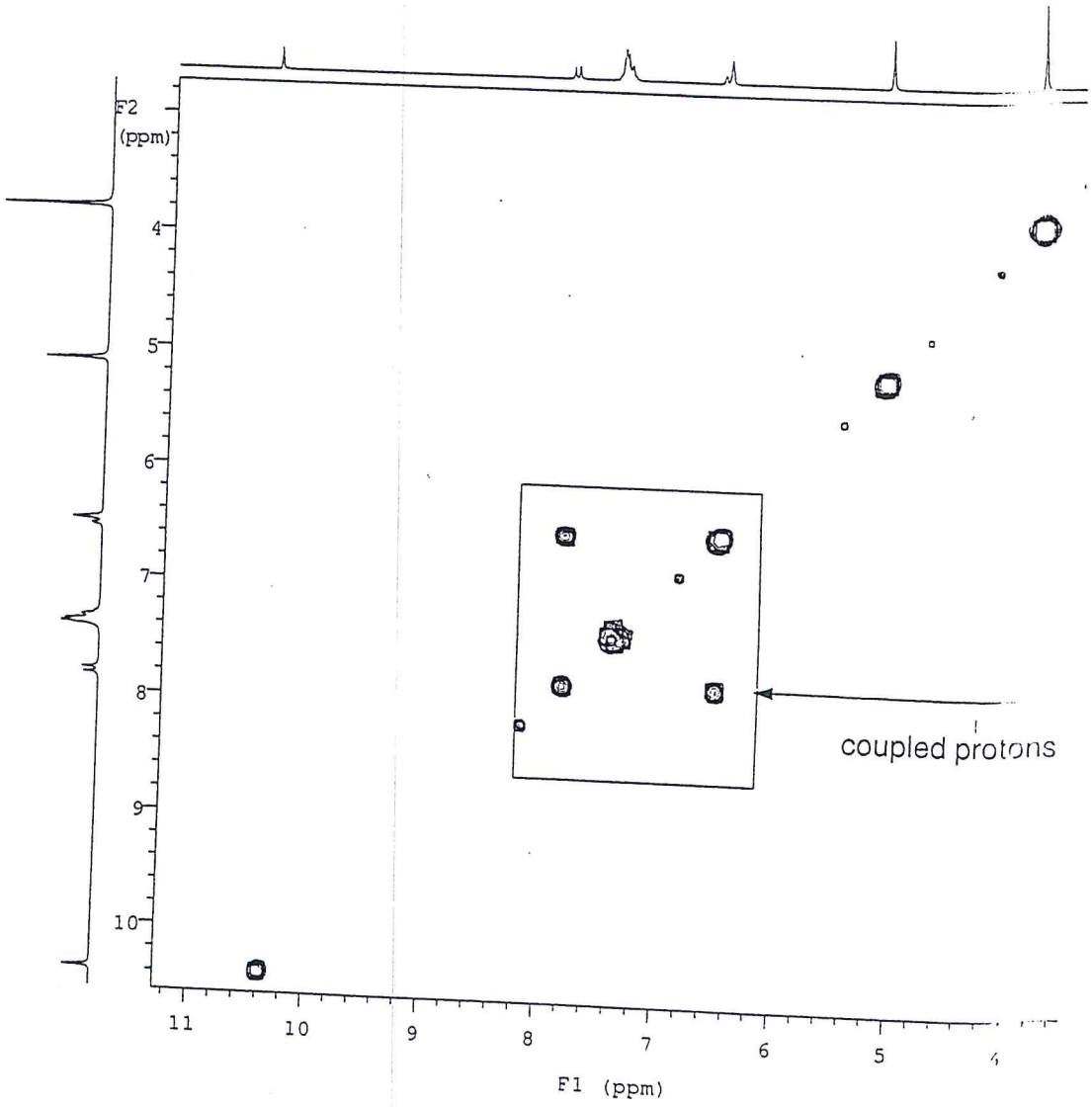
^{1}H-^{1}H Cosy Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)

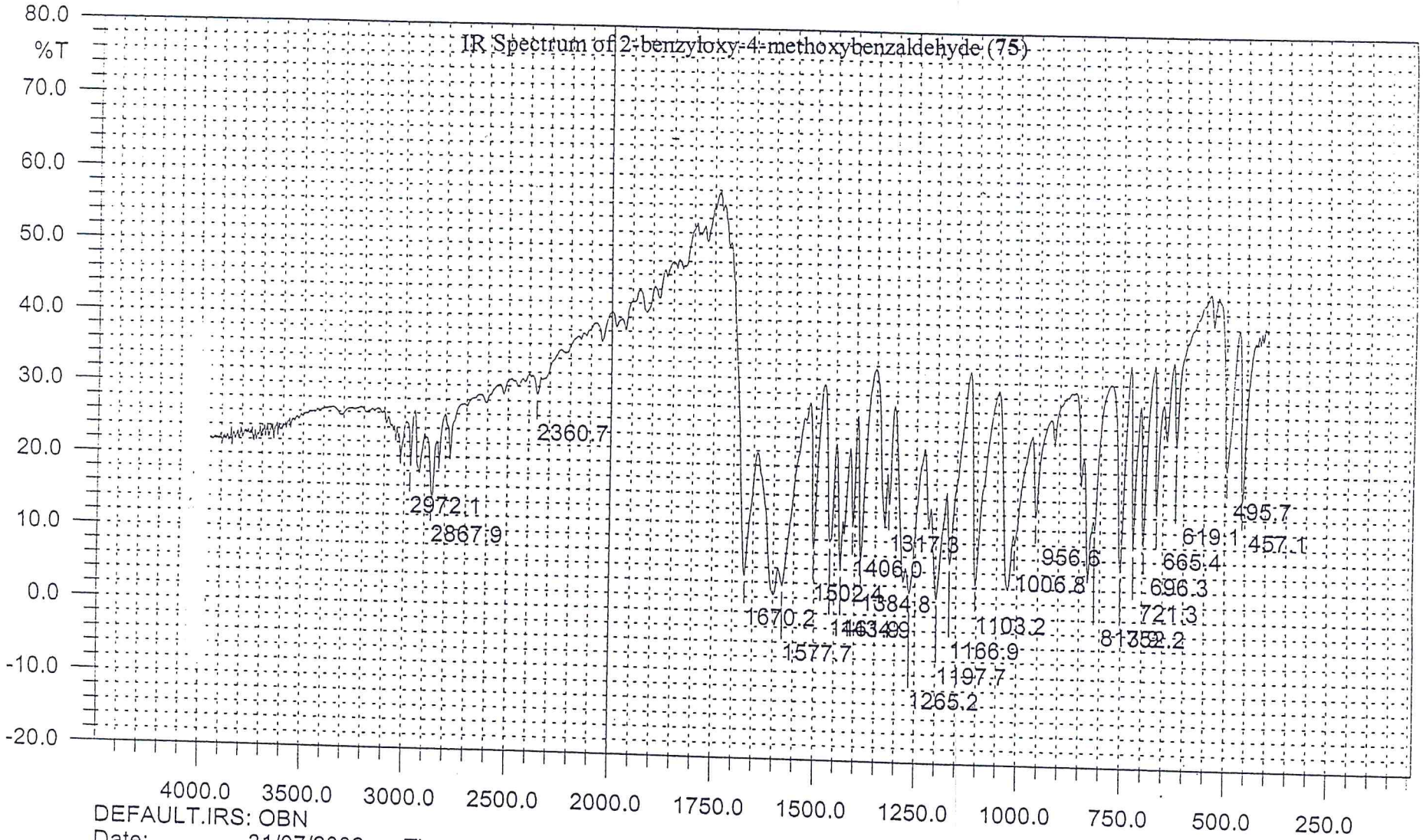
GMM3
CDCl3
50 mg

Pulse Sequence: COSY

Solvent: CDCl3
Ambient temperature
File: COSY
Mercury-200 "uonnmr200"

PULSE SEQUENCE: COSY
Relax. delay 1.000 sec
Acq. time 0.160 sec
Width 3200.9 Hz
2D Width 3200.9 Hz
2 repetitions
128 increments
OBSERVE H1, 200.0557687MHz
DATA PROCESSING
Sq. sine bell 0.080 sec
F1 DATA PROCESSING
Sq. sine bell 0.040 sec
FT size 1024 x 1024
Total time 6 min, 36 sec





DEFAULT.IRS: OBN

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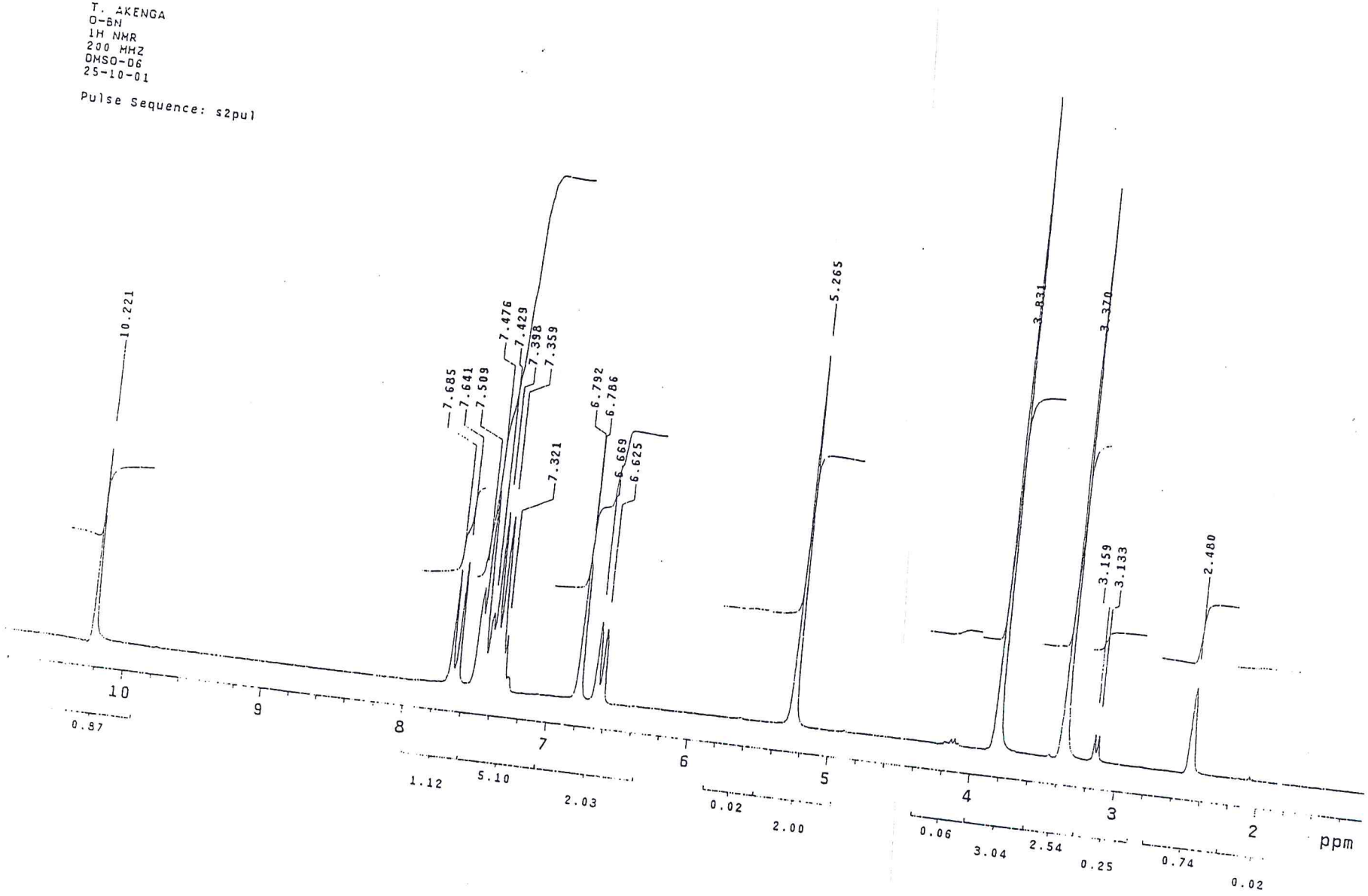
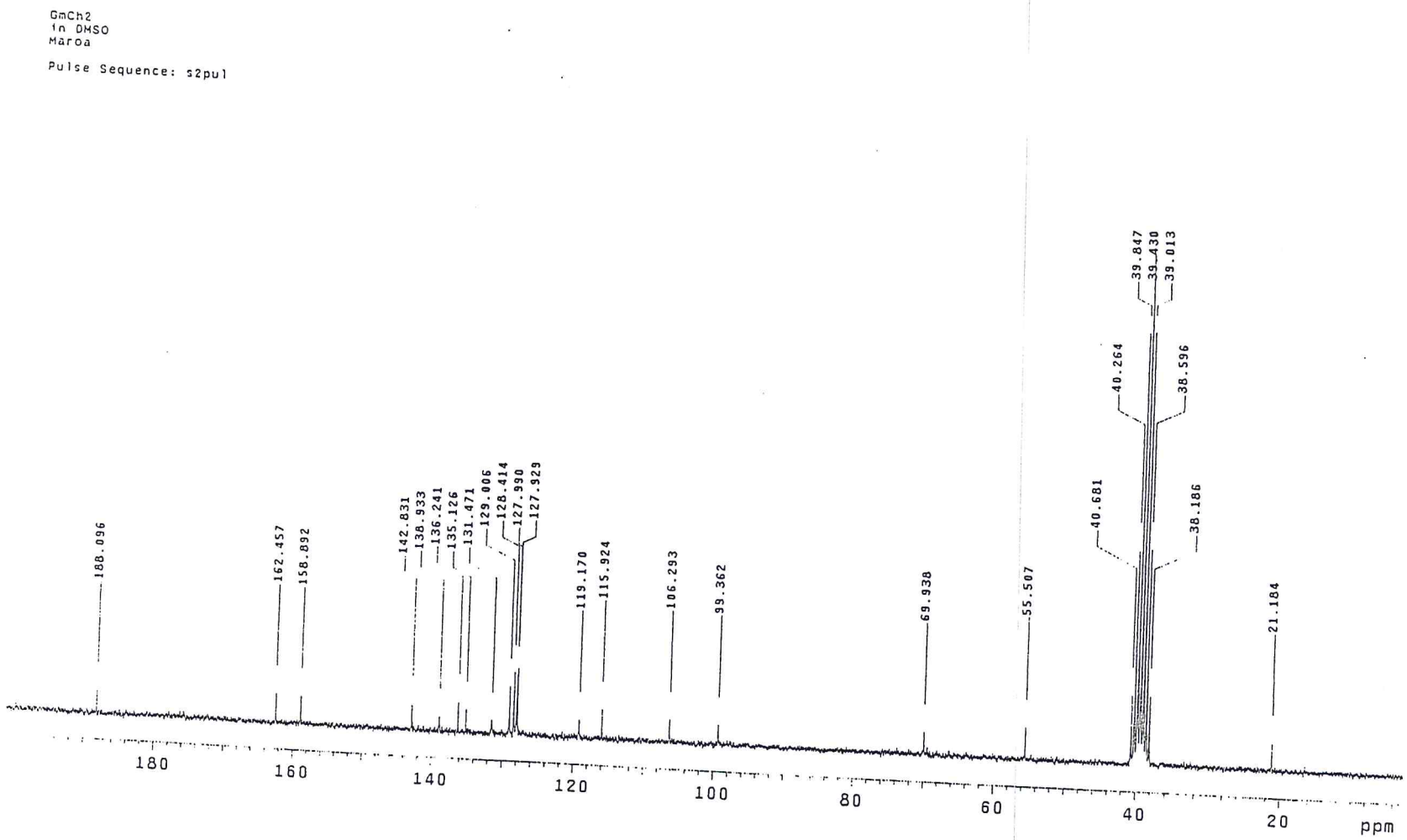
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ
Min:	401.17	Max:	3998.16	Range:	1/cm
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

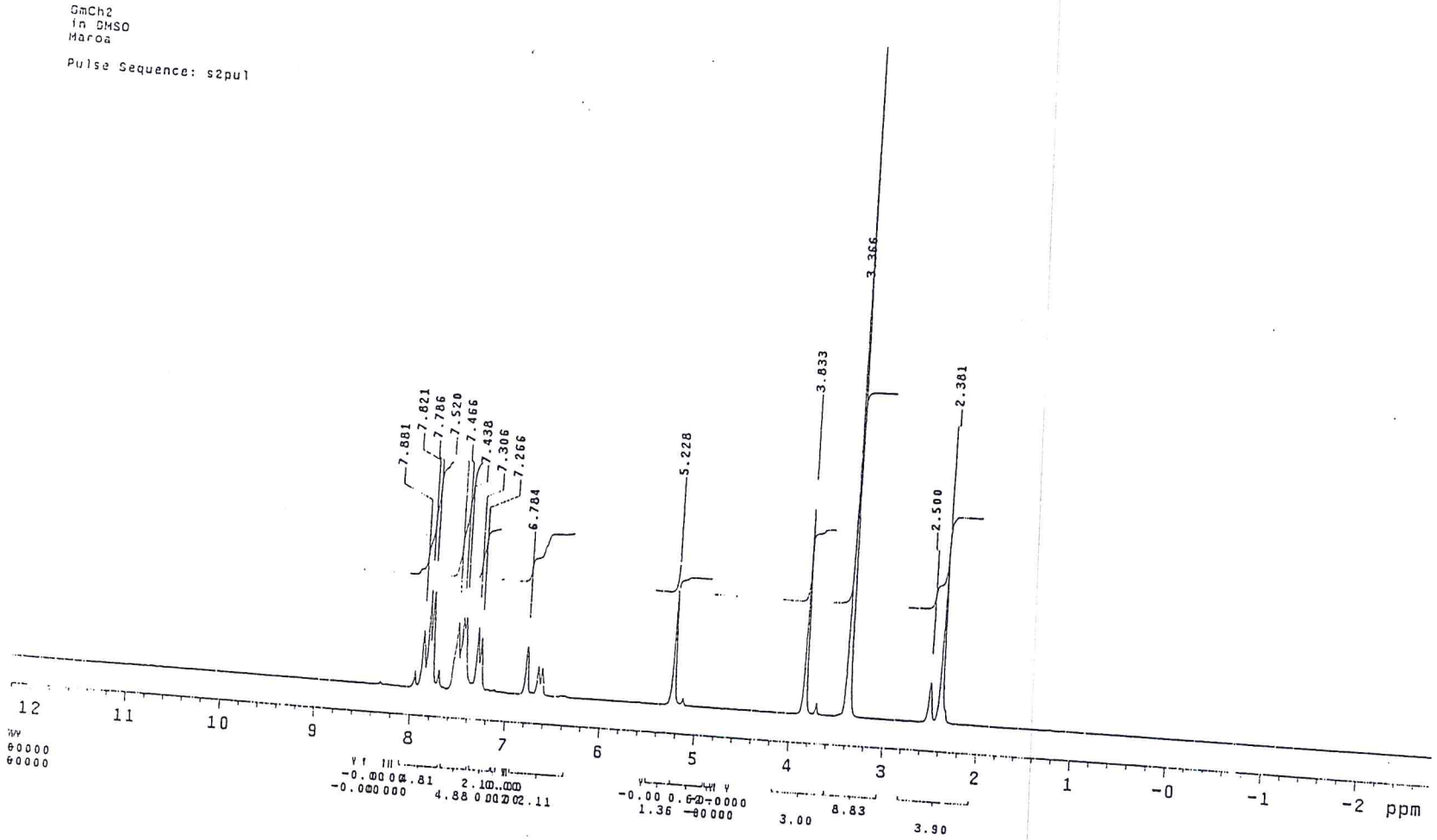
^{1}H NMR Spectrum of 2-benzyloxy-4-methoxybenzaldehyde (**75**)

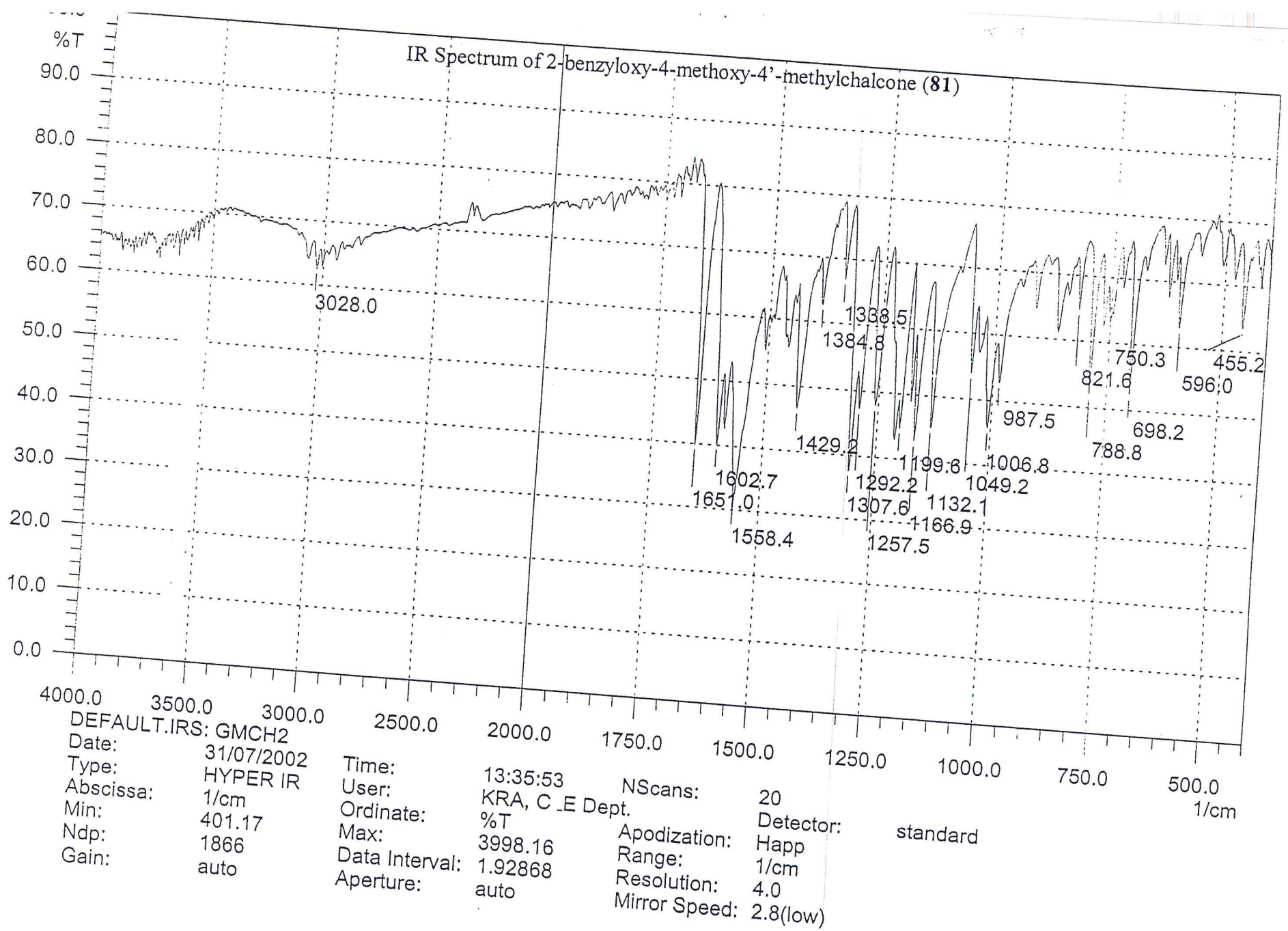


^{13}C NMR Spectrum of 2-benzyloxy-4-methoxy-4'-methylchalcone (**81**)



^{1}H NMR Spectrum of 2-benzyloxy-4-methoxy-4'-methylchalcone (**81**)




IR Spectrum of 2-benzyloxy-4-methoxy-4'-methylchalcone (81)
%T
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
3028.0
1602.7
1651.0
1558.4
1429.2
1388.5
1384.8
1292.2
1307.6
1257.5
1199.8
1166.9
1132.1
1049.2
1006.8
987.5
821.6
788.8
750.3
698.2
596.0
455.2
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Date: 31/07/2002
Time: 13:35:53
Type: HYPER IR
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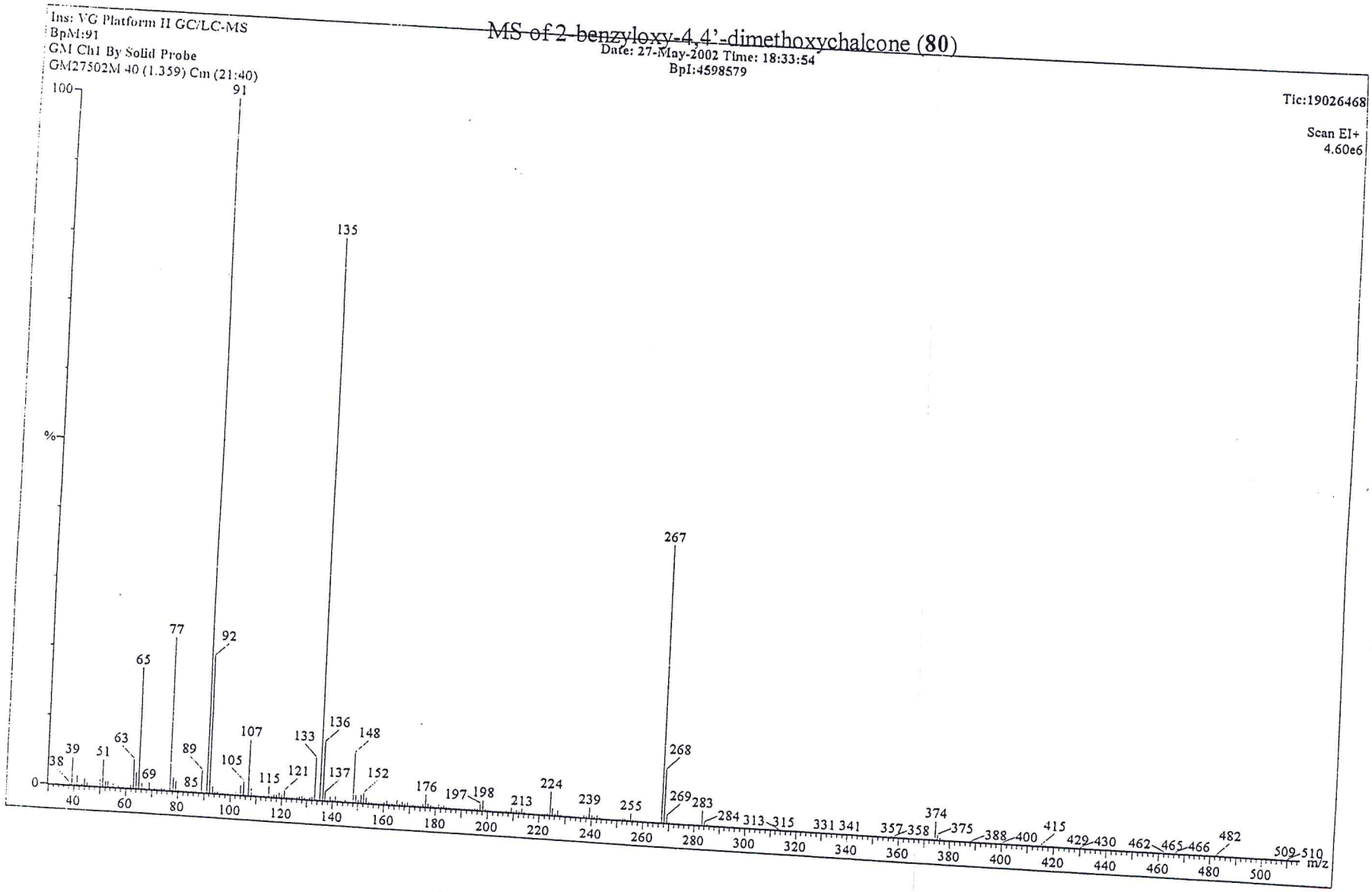
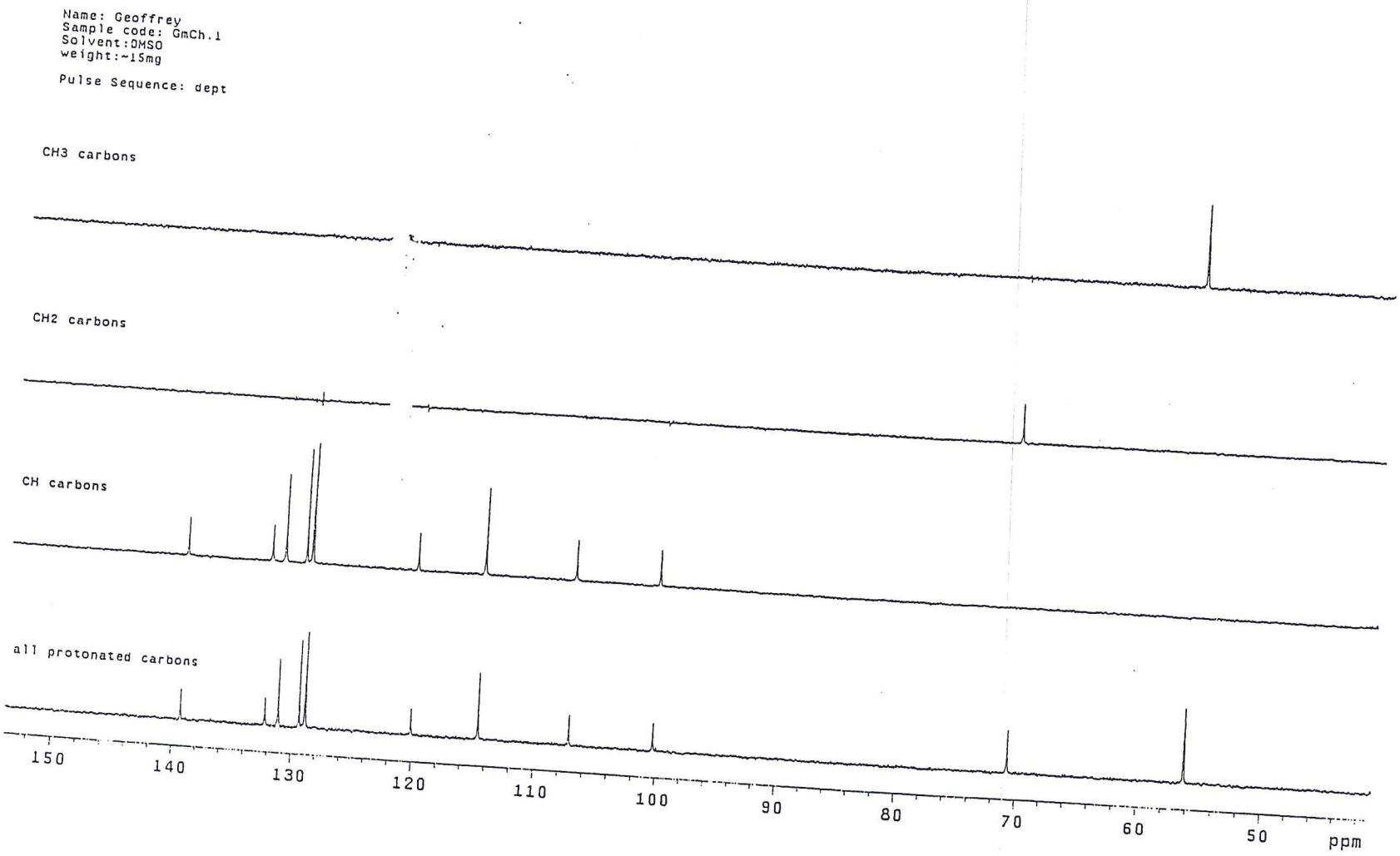
NScans: 20
Abscissa: 1/cm
Ordinate: %T
Detector: standard
Min: 401.17
Max: 3998.16
Apodization: Happ
Ndp: 1866
Data Interval: 1.92868
Range: 1/cm
Gain: auto
Aperture: auto
Resolution: 4.0
Mirror Speed: 2.8(low)

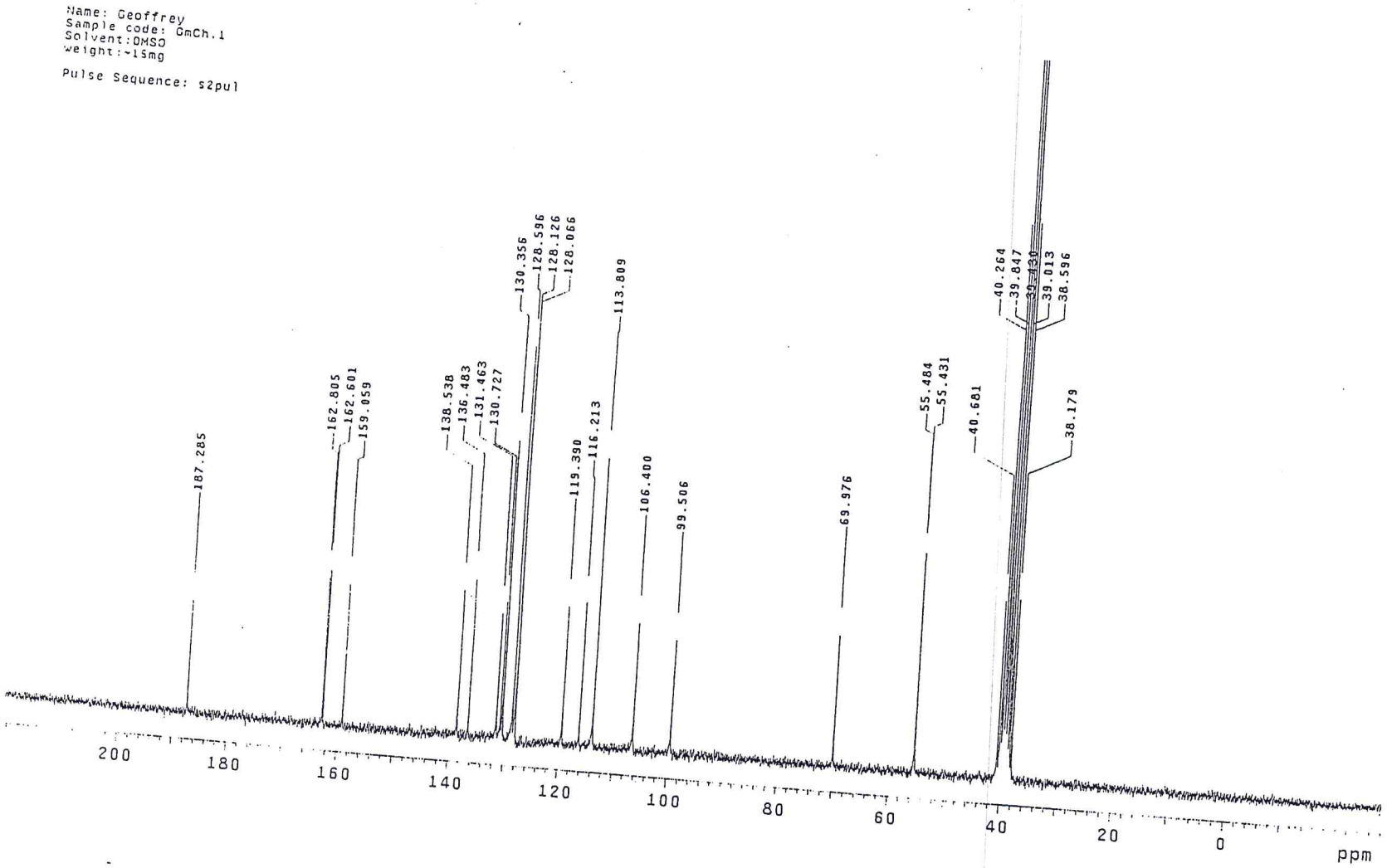
MS of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)



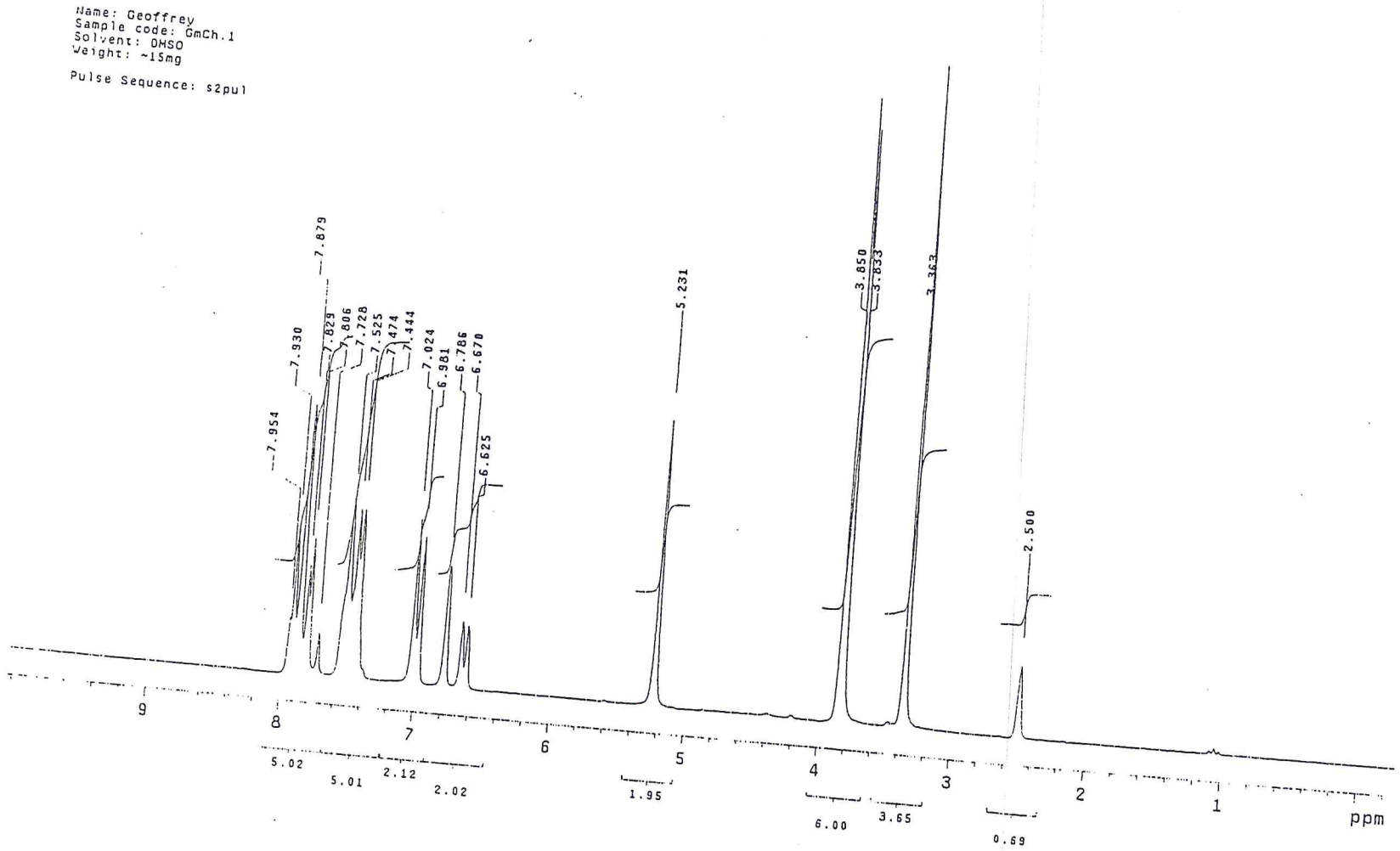
DEPT Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)

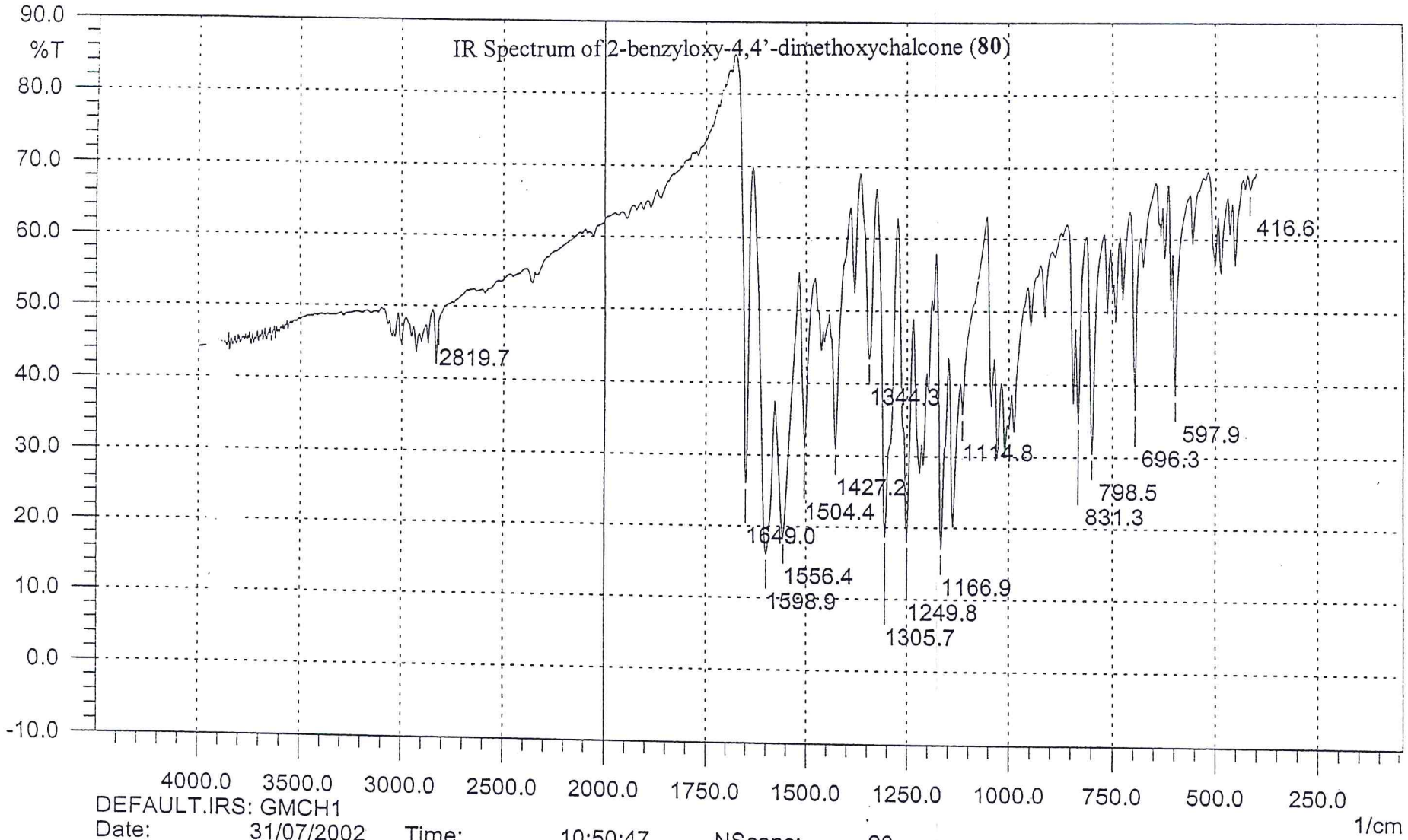


^{13}C NMR Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)



^{1}H NMR Spectrum of 2-benzyloxy-4,4'-dimethoxychalcone (**80**)





DEFAULT.IRS: GMCH1

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Type:	HYPER IR	User:	KRA, C _E Dept.	Detector:	standard
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ
Min:	401.17	Max:	3998.16	Range:	1/cm
Ndp:	1866	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

MS of benzyloxy-4-methoxy(I-methyl)benzyl alcohol (78)