universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Synthesis of indole derivatives as novel indoleamine 2,3-dioxygenase (IDO) inhibitors

angestrebter akademischer Grad

Magistra der Pharmazie (Mag. pharm.)

Eingereicht von:	Susanne Moroder
Matrikelnummer:	0302599
Studienrichtung:	A 449 Diplomstudium Pharmazie
Betreuer:	o. Univ.-Prof. Dipl.-Ing. Mag. Dr. Christian R. Noe

Wien, im Februar 2010

Acknowledgement

I wish to express my sincere gratitude to my supervisors, Prof. Noe and Prof. Pellicciari, who gave me the unique opportunity to do my diploma thesis abroad.

My special thanks to Dr. Kopelent and Prof. Marinozzi for the well-organized and unproblematic ERASMUS coordination, which made my stay in Perugia very enjoyable.

I am exceedingly grateful to all my former collegues from the CTF - Chimica Farmaceutica I - in Perugia, who encouraged me during my research work. I am especially obliged to Prof. Camaioni for the interesting synthetic work and the illuminating discussions about it, and Dr. Venturoni for gathering the NMR-Spectra and the pleasant laboratory climate.

I am thankful to Prof. Moroni and his research group from the University of Florence for the performed enzyme assays.

My sincere thanks to my critical proofreaders Dr. Kopelent, Dr. Lachmann, Prof. Camaioni, Andreas Slateff and Martin Piskernig for their sharp eyes, quick answers and important discussions.

I thank all my friends, who have been all the way through my time spent as a student – especially my boyfriend Martin for the several scientific discussions, his technical support as well as his immense patience.

Lastly, I express my most heartfelt gratitude to my family for all their unconditional support, patience and love throughout my student's life.

Abstract

The immunomodulatory indoleamine 2,3-dioxygenase, a key enzyme of the tryptophan catabolism, is strongly connected to tumoural immune tolerance due to overexpression in certain tumours. Local depletion of the essential amino acid and the accumulation of cytotoxic tryptophan-derived metabolites induce an arrest of T-cell proliferation and suppress the immune response. This immune escaping state prevents an efficient immunotherapy as well.
Natural just as synthetic compounds have been identified and developed for the novel drug target. Until now, only two of them have been assayed on tumour cells: 1-Methyl-tryptophan and Methyl-thiohydantoin-tryptophan. Both revealed, administered in combination with paclitaxel, a significant tumour regression. With the intent to develop novel small-molecule IDO inhibitors, we synthesized new derivatives based on Methyl-thiohydantoin-tryptophan scaffold. However, subsequently performed enzyme assay on human IDO displayed minor inhibitor activity as the parent substance.

Table of contents

Chapter 1

Introduction

1.1 What is IDO?

Indoleamine 2,3-dioxygenase (IDO) is one of the key enzymes of a branched enzymatic cascade, known as the kynurenine pathway (KP). The KP is the principal catabolic route for the essential amino acid *L*-tryptophan (*L*-trp) in mammals which is not required for protein biosynthesis. Although *L*-trp is the rarest of all amino acids, several biologically active compounds originate from it, among them the aminergic neurotransmitter serotonine (5-hydroxytryptamine, 5-HT) and the neurohormone melatonine.

The initial reaction of the KP involves the oxidative cleavage of *L*-tryptophan into N-formylkynurenine.[1] Two different but isofunctional, heme-containing enzymes catalyse this rate-determining degradation process: tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). N-formylkynurenine is then promptly hydrolysed to *L*-kynurenine and subsequently degraded to a series of partly neuroactive metabolites including 3-hydroxykynurenine, quinolinic acid, kynurenic acid and nicotineamide adeninedinucleotide (NAD).

Unlike TDO, which is found only in the liver, IDO is distributed in a huge number of human tissues, such as brain, lung, small intestine and placenta.[2] Whereas TDO converts only *L*-trp, IDO evinces a very widespread substrate specifity for various indoleamines. Ball and coworkers [3,4] have recently characterised a novel enzymatic isoform of IDO and termed it indoleamine 2,3-dioxygenase-2 (IDO-2).

1.2 Physiological functions

As an intracellular enzyme, IDO can be induced by several inflammatory stimuli, e.g. cytokines (IL-1, TNF). Its pivotal inducer *in vitro* and *in vivo* is interferon-γ (IFN-γ).[5] This is the reason why IDO has been assumed to be part of the innate immune system. However, its performance is not always in favour of the host.

1.2.1 Host defence

Increased IDO activity is reflected in local depletion of free serum *L*-trp and the accumulation of tryptophan-derived kynurenine pathway metabolites.
Some microorganisms (auxotrophs) cannot synthesize their own tryptophan. They depend on an external source and react very sensitively to the enhanced IDO activity. Furthermore, IDO is induced by bacterial lipopolysaccharide (LPS) and other endotoxines. IDO expression inhibits several pathogenic agents like bacteria (*Chlamydia pneumoniae*, *Toxoplasma gondii*) and viruses (*Cytomegalo virus*, *Herpes simplex virus*) from growing and replicating.[5]
Nevertheless, its infection-controlling role has become more and more nested to reconcile with its immunomodulating effect.

1.2.2 Immune supervisor

Immunomodulators affect the immune system for a balanced response: immunosuppression is justified and imperative at autoimmune diseases, organ transplantation, or immune-mediated abortion. Inappropriate suppression, however, causes uncontrolled infections and tumour growth.
Landmark work by Munn and colleagues [6] evinced that, during murine pregnacy, IDO expressing cells protects the allogenic fetus. IDO inhibition lets the maternal T-cells to instigate fetal rejection. Therefore, (interferon-γ-mediated) IDO, when expressed in antigen presenting cells (APC) like macrophages and dendritic cells (DC), plays a crucial role in adaptive T-cell immune responses.[7] In a similar manner to its antimicrobial effect, IDO is starving T-cells of tryptophan. These are extremely sensitive to the lack of the essential amino acid, leading to an arrest of their cell cycle and subsequently to apoptosis.[8] Moreover, certain downstream tryptophan catabolites suppress T-cell proliferation in order to facilitate immune tolerance.[9]

1.3 Pathophysiological part

Enhanced IDO activity and the production of high levels of kynurenines correlate with several immunological diseases, which affect the central nervous system, as well as inflammatory diseases. However, for the last decades, research has been focused on the connection between IDO and tumours, especially due to the fact that IDO is involved in tumour-induced tolerance. Tumours are capable to escape from immune surveillance evolving a pathological condition of tolerance against their own antigens. [10,11]
The role of IDO is not clear yet: First, elevated IDO activity in cancer patients was inferred with IFN-γ exposure to have an antitumoural effect by depletion of tryptophan and production of toxic kynurenines. However, tumour cells are highly pliable: They are able to reverse the IDO immune suppressive activity in favour of their own profit and to establish a rampart towards the immune response. Not only does this pathological state render possible that tumour grow undisturbed, but it also makes difficult for clinical immunotherapy difficult to succeed.
Muller and coworkers [12] explained one way of IDO becoming deregulated in cancer cells, which involves the tumour suppression gene *Bin1*. *Bin1*, which is decreased in several cancer types, controls the expression of the *Indo* gene. The *Indo* gene encodes the IDO enzyme. Mouse knockout studies demonstrate that a lack of *Bin1* augments the expression of IDO.

Therefore, IDO illustrates an interesting and important novel target for cancer drug development.

1.4 Medicinal Chemistry of IDO inhibitors

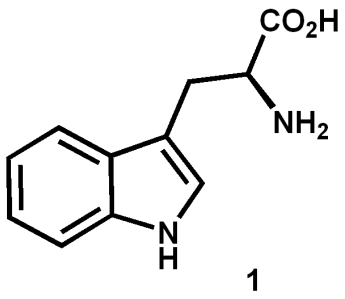
Introduction

The development of IDO-inhibitors has been focused, so far, on the natural substrate *L*-Tryptophan. Competitive as well as non-competitive inhibitors have been identified endowed with potency in the high micromolar range. Recent reviews summarize the variety of compounds that have been discovered to possess IDO inhibitor activities. [13,14,15]
However, reported pharmacological data must be interpreted cautiously: Two enzymatic isoforms of IDO – IDO-1 and IDO-2 [3,4] – exist and different IDO sources (human, murine or recombinant) are in use. Austin and coworkers [16] recently reported that the competitive inhibitor 1-Methyl-*L*-tryptophan is a better inhibitor for recombinant mouse IDO (K_i = 105 µM) than for recombinant human IDO (K_i = 62 µM).

1.4.1 Competitive inhibitors

Tryptophan Analogues

The amino acid substrate *L*-trp (**1**, figure 1.1) has earlier been found to be endowed with inhibitory activity at high concentrations (> 0.2 mM). [17,18] Therefore, most known competitive IDO inhibitors are tryptophan analogues.



1

Figure 1.1

The following modifications have been performed on the tryptophan scaffold

1. Indole modifications

   Tryptophan analogues with substitution of the indolic nitrogen with a methyl group (1-Methyl-tryptophan, 1-MT, **2**, figure 1.2) or replacing it with a sulphur atom (benzothiazole derivative, **3**) or an oxygen atom (benzofuran derivative, **4**) led to inhibitors with K_i ranging from low to high micromolar concentration. [19]
   Thus, 1-MT, a commercially available compound, is the prototype of this class of inhibitors and possesses a K_i about 30 µM. [20] Several studies reported the docking process to the active site of IDO to be stereoselective. Thus, (*S*)-1-MT was more potent than its (*R*)-antipode. (63% and 12% of inhibition at 100 µM, respectively). [20] All of them, lacking the acidic proton at the N-1 position of Trp, may prevent the first step of the enzymatic oxidation.

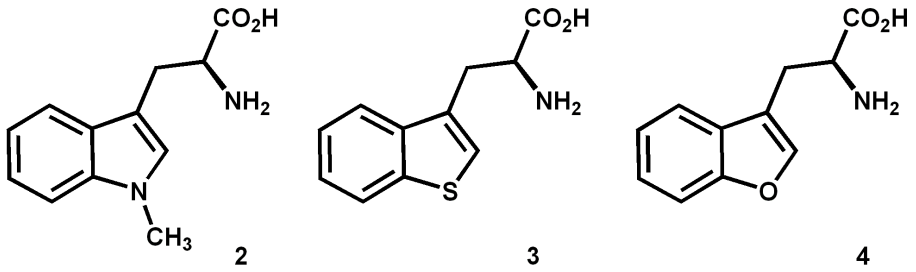


Figure 1.2

2. Substitutions at the phenyl ring

   Substitutions with electron-withdrawing groups on the phenyl ring of Trp at C-5, C-6, and C-7 positions lead to active compounds whereas the presence of electron-releasing group speeds up the IDO oxidation. Indeed, 5-Bromo (**5**, figure 1.3, 56% inhibition at 1mM), 6-Fluoro (**6**, 54% inhibition at 1mM), 6-Nitro (**7**, 52% inhibition at 1mM, K_i = 180 µM for (*S*)-enantiomer), 7-Fluoro (**8**, K_i = 37 µM) as well as 4,7-Difluoro (**9**, K_i = 40 µM) and 5,7-Difluoro (**10**, K_i = 24 µM) resulted in the most active inhibitors. [21,22]
   However, many members of this class of compounds act as substrates as well and thus they undergo IDO oxidation, at least in part.

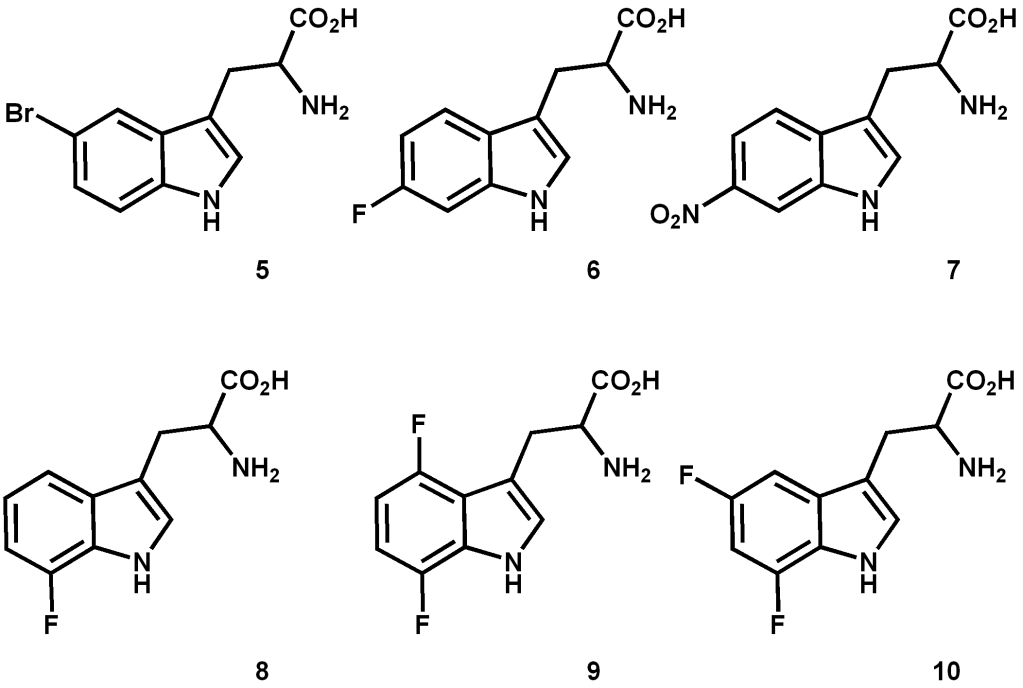


Figure 1.3

3. Side chain modifications

   Only few modifications are tolerated on the distal aminoacidic moiety: it can be methylated or esterified but the amino group could not be removed. Recently potent inhibitors were discovered in which the aminoacidic moiety has been cyclized to form a thiohydantoine ring. Compound Methyl-thiohydantoin-tryptophan (MTH, **11**, figure 1.4), reported as a necroptosis inhibitor as well ("Necrostatin-1" [23]), displayed a K_i of 11.4 µM, being a potent IDO inhibitor. [12]

Sulfur containing inhibitors

Various dithiocarbamate derivatives have been reported to present IDO inhibitory activity. By screening of commercially available indole derivatives, Gaspari and coworkers [24] discovered the natural product brassinin (**12**, figure 1.4) to display a moderate competitive IDO inhibitory activity with a K_i about 98 µM. Further structure activity relationship (SAR) studies at synthetic brassinin derivatives showed that the dithiocarbamate side chain is crucial for the inhibitory activity. Most likely this moiety binds to the heme iron acting as chelating agent.

Moreover, the indole ring is not necessary in this class of compounds and the distal thiomethyl group could be substituted with more hindered aromatic groups.

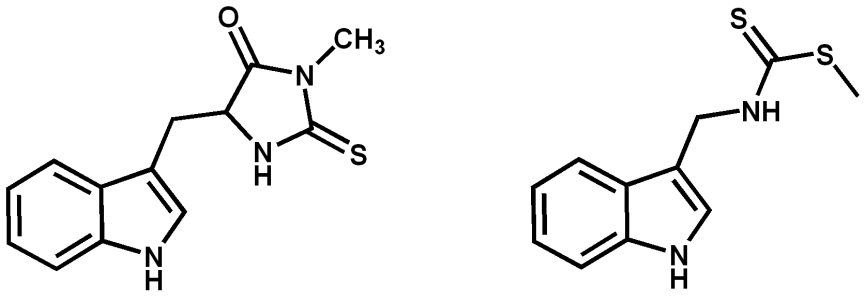
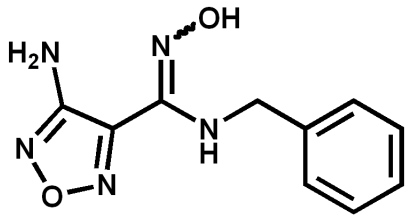


Figure 1.4

A new lead compound

Yue and coworkers [25] have recently identified a novel non-indolic-based lead structure for competitive IDO inhibitors: 4-amino-1,2,5-oxadiazole-3-carboximidamide (**13**, figure 1.5). Further SAR studies showed the phenyl derivatives to be the most potent ones.



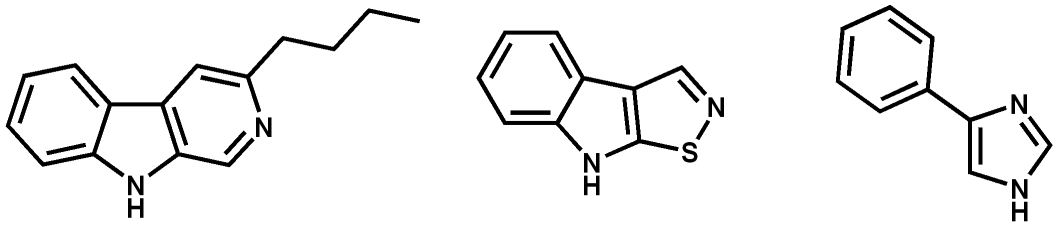
13

Figure 1.5

1.4.2 Non-competitive inhibitors

1. β-carboline derivatives

   About two decades ago, a series of β-carboline derivatives was reported to act as IDO inhibitors, among them, norharman as the most active one.[26] They show non-competitive inhibition behavior.[27] Modifications at the β-carboline structure have been performed on the pyridine and phenyl rings evidencing that large alkyl chain in the C-3 position of the β-carboline scaffold lead to potent inhibitors; i.e.: 3-butyl-β-carboline (**14**, figure 1.6) possesses an IDO inhibition K_i about 3 µM. [28]
   The natural product brassilexin(**15**, K_i about 5 µM) and 4-phenylimidazole (**16**) are other compounds of this class, reported as potent non-competitive IDO inhibitors. [27,28]



14 15 16

Figure 1.6

2. Various structures

The screening of a library of marine invertebrates extracts unveiled the new polyketide annulin C (**17**, figure 1.7) and the known compounds annulin A and annulin B, which are natural products isolated from the marine hydroid *Garveia annulata*, to act as submicromolar inhibitors of IDO.[29] Another compound, found by the same screening program, was exiguamine A (**18**), a novel alkaloid of the marine sponge *Neopetrosia exigua*. It displayed a K_i of 120 nM.[30]

Kumar and coworkers [31] supposed the naphthoquinone core of the annulins as the key pharmacophore. Thus, led them to identify a series of naphthoquinone derivatives, which showed potent inhibitory activity. Among them, three pyrano-naphthoquinone-based compounds were reported to be the most potent IDO inhibitors so far (**19**, K_i = 61–70 nM).

Yeast target-based screening disclosed another potent non-indolic IDO inhibitor. Thus, the N-methyl-N'-9-phenanthrenyl-imidodicarbonimic diamide (**20**) was reported to possess a K_i of 1.5 µM against recombinant human IDO. [32]

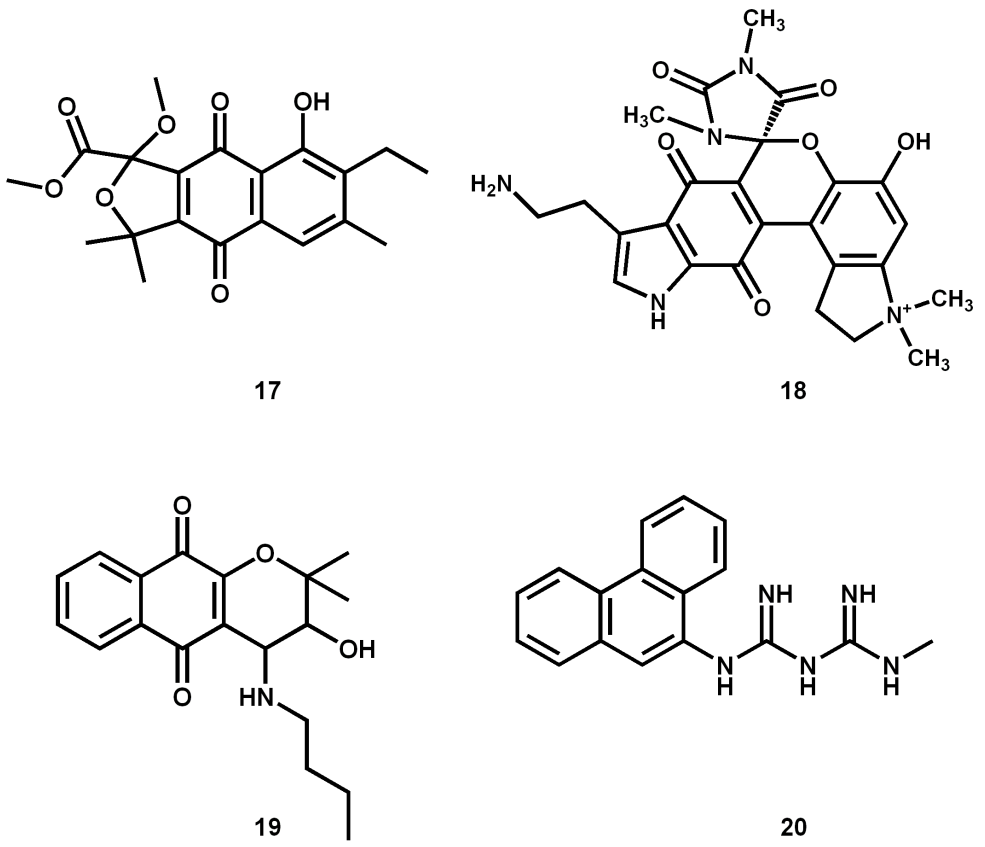


Figure 1.7

1.4.3 Therapeutical use of IDO inhibitors

Since elevated IDO activity is linked to tumoural immune tolerance and immunosuppression, it has become a crucial new drug target. Muller and colleagues [12] demonstrate the antitumoural effect of IDO inhibitors (1-MT, MTH) on a mouse model of breast cancer. 1-MT and MTH, by themselves, only slowed but were not able to arrest tumour growing. Combining 1-MT with common chemotherapeutical drugs, which are used for breast cancer treatment, lead to significant results. 1-MT with paclitaxel, a taxane, caused an average of 30% in tumour volume regression as well as combining MTH with paclitaxel; whereas paclitaxel treatment by itself only inhibited tumour growth. Significant tumour decrease was achieved even combining 1-MT with cisplatin, cyclophosphamide and doxorubicin.

1-MT is a rather weak IDO inhibitor with poor enzyme selectivity: However, it is one of few inhibitors that has been assayed *in vivo*, so far.

Chapter 2

Research tasks

2.1 Scope

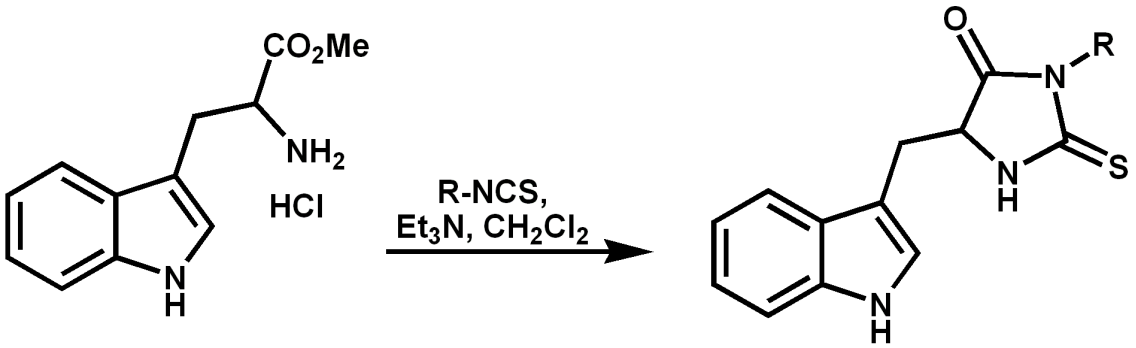
Muller and coworkers [12] discovered, by screening commercially available indoleamines, a competitive IDO inhibitor that displayed a threefold inhibitor activity and much better enzyme selectivity related to 1-MT in cell-based assays. Pharmacological experiments with Methyl-thiohydantoin-tryptophan revealed similar oral bioavailability and in combination with paclitaxel similar tumour regression as 1-MT *in vivo*.
Natural as well as synthetic compounds were characterised as more potent IDO inhibitors than 1-MT. However, 1-MT and MTH are, so far, the only two competitive inhibitors assayed on tumour tissue *in vivo*.

Cancer immunotherapeutic strategies have focused mainly on immune system's stimulation. Biological agents, such as cytokines, which are currently the main immunotherapeutics, acitivate and amplify immune response towards tumour antigens. However, in the last decade, targeted therapies with small molecules become rampant - even due to the fact that small molecules have further advantages compared to biological agents, including production, application and immunogenicity. [33]

Based on these facts and, additionally, that no derivatives of MTH ever appeared in context with IDO inhibition or cancer treatment, we targeted to develop novel small-molecule IDO inhibitors: We designed and synthesized new derivatives starting from Methyl-thiohydantoin-tryptophan scaffold, suitable for pharmacological studies.

2.2 Synthesis of 5-(1*H*-indol-3ylmethyl)-2-thiohydantoin derivatives

Scheme 1



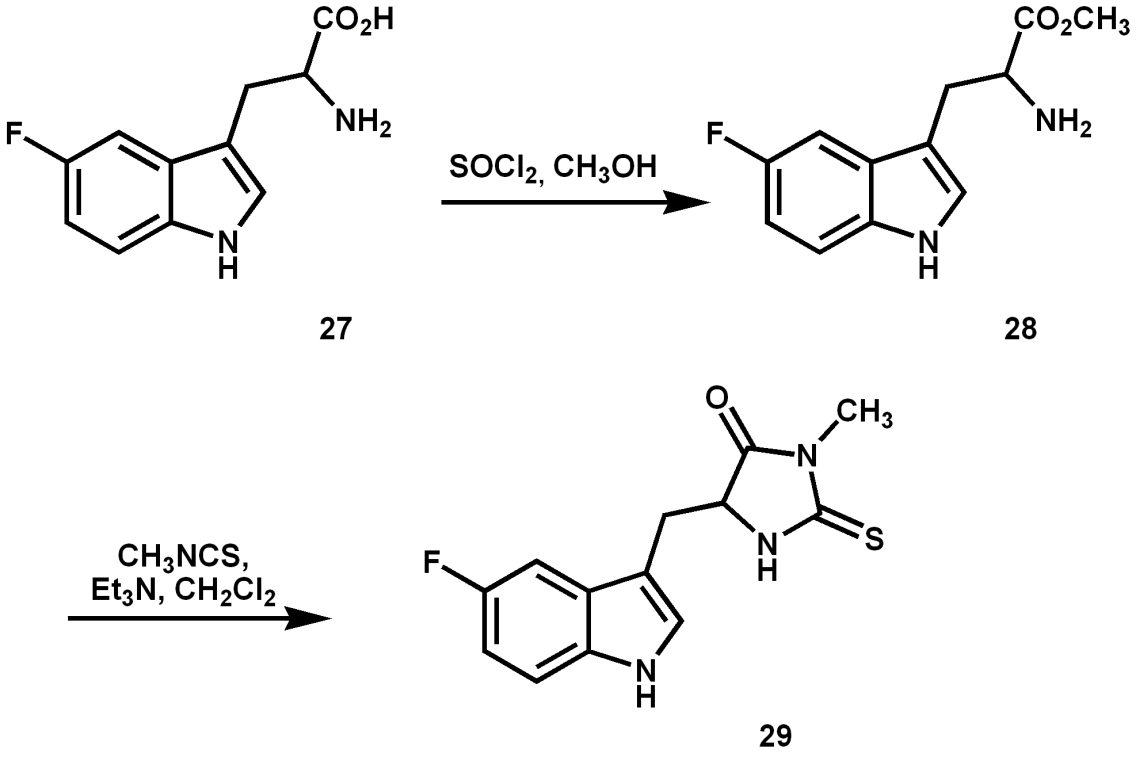
21

R = *n*Pr, 22
R = Bn, 23
R = Ph, 24
R = Me, 25 (*S*-enantiomer)
R = Me, 26 (*R*-enantiomer)

2-Thiohydantoin derivatives **22** – **26** have been synthesized starting from the appropriate Tryptophan methyl ester hydrochloride (**21**) according to the known procedure.[23] Thus, as displayed in Scheme 1, by treating **21** with the appropriate alkyl isothiocyanate in the presence of triethylamine in dichloromethane (DCM), the corresponding 5-(1*H*-indol-3ylmethyl)-2-thiohydantoin derivatives **22** – **26** were obtained in high yields.

2.3 Synthesis of 5-(5-Fluoro-1*H*-indol-3ylmethyl)-3-methyl-2-thiohydantoin

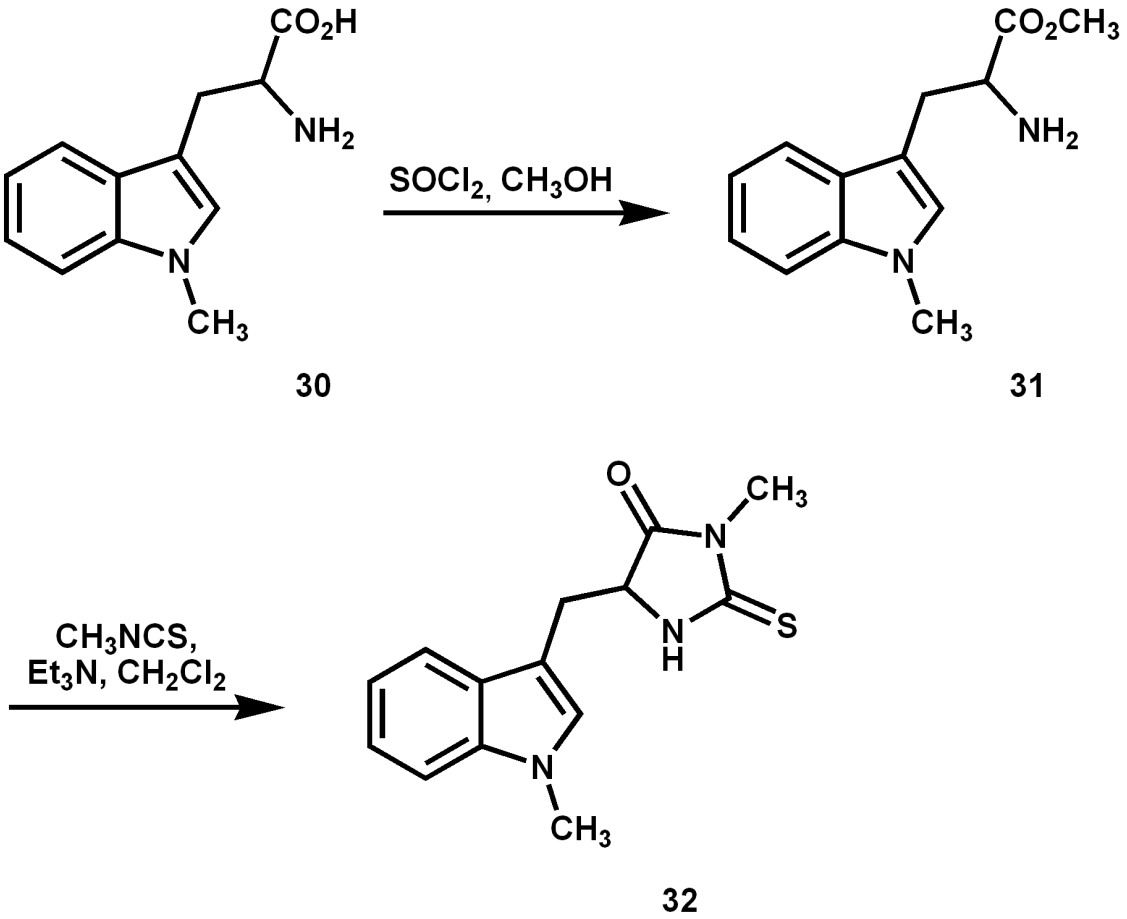
Scheme 2



5-Fluoro-Necrostatin (**29**) has been obtained starting from 5-Fluoro-tryptophan (**27**), according to Scheme 2. Thus, derivative **27** has been transformed in the corresponding methyl ester **28** through the reaction with thionyl chloride in dry methanol solution. Compound **28** was treated without further purification with methylisothiocyanate as reported above to afford the corresponding 5-(5-Fluoro-1*H*-indol-3ylmethyl)-3-methyl-2-thiohydantoin (**29**), in 37% yield.

2.4 Synthesis of 5-(1-*N*-Methyl-1*H*-indol-3ylmethyl)-3-methyl-2-thiohydantoin

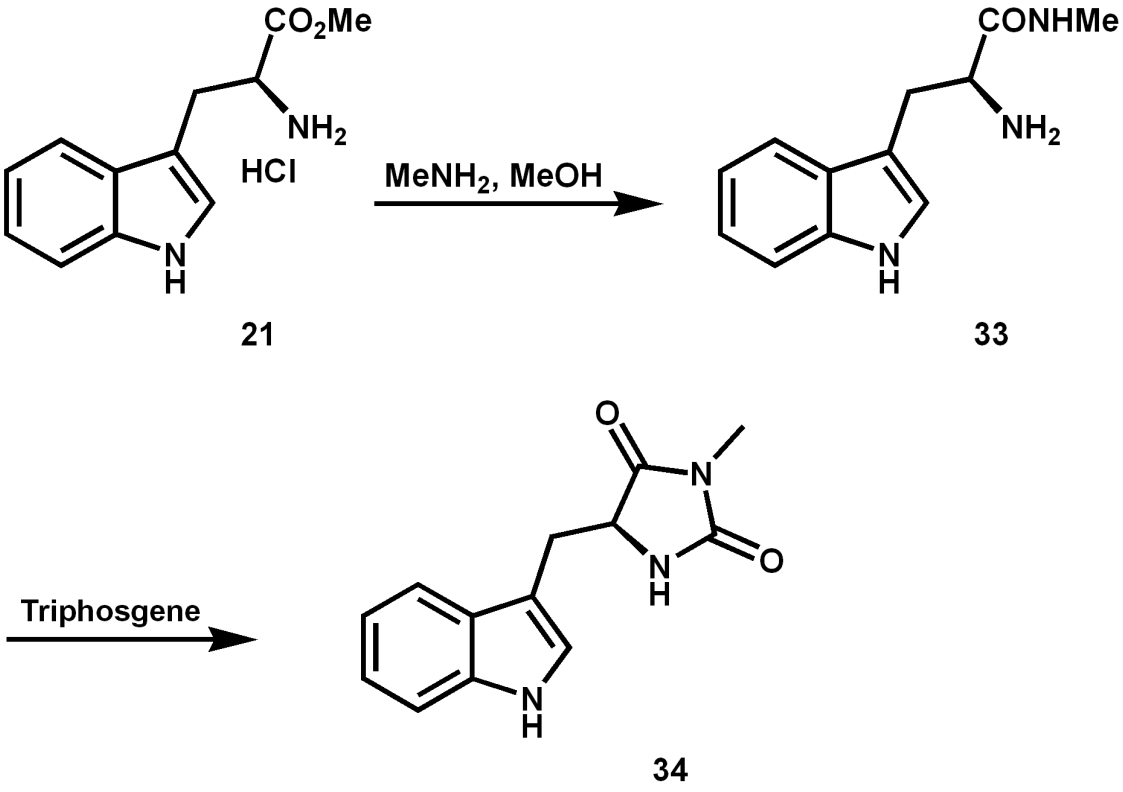
Scheme 3



1-Methyl-Necrostatin (**32**) has been prepared in two steps as figured in Scheme 3. Briefly, 1-*N*-Methyl-tryptophan methyl ester (**31**) has been obtained in 75% yield, by reacting at room temperature commercially available 1-Methyl-tryptophan (**30**) with thionyl chloride in dry methanol solution. The latter intermediate **31** was treated without further purification with methylisothiocyanate as reported above to afford the corresponding 5-(1-*N*-Methyl-1*H*-indol-3ylmethyl)-3-methyl-2-thiohydantoin (**32**), in 73% yield.

2.5 Synthesis of 5-(1*H*-Indol-3-ylmethyl)-3-methyl-hydantoin (O-Necrostatin-1)

Scheme 4



O-Necrostatin-1 (**34**) has been synthesized as reported in scheme 4. **21** was treated with methylamine in methanol (4 d) to provide Tryptophan methyl amide (**33**) in 98% yield. It worked treating **21** with methylamine in ethanol (16 h) as well, obtaining Tryptophan methyl amid in 94% yield. The intermediate **33** was arranged with triphosgene in the presence of triethylamine in dry dichloromethane to give 5-(1*H*-Indol-3-ylmethyl)-3-methyl-hydantoin (**34**). The reaction failed when arranging **33** with triphosgene in the presence of pyridine in dry dichloromethane.

2.6 Chiral HPLC

IDO inhibitor activity is related to stereospecifity. Peterson and colleagues [20] showed that (*S*)-MT is a more potent inhibitor than (*R*)-MT. Starting from these premises, we wanted to determine if IDO inhibitor activity differentiates between the two stereoisomers of MTH as well. The compounds **25** and **26** have been submitted to a chiral HPLC analysis proving their enantiomeric purity. Unfortunately, as shown in the chromatogram in 2.1, the two reaction products are racemic. Racemisation might occur through the basic reaction conditions.

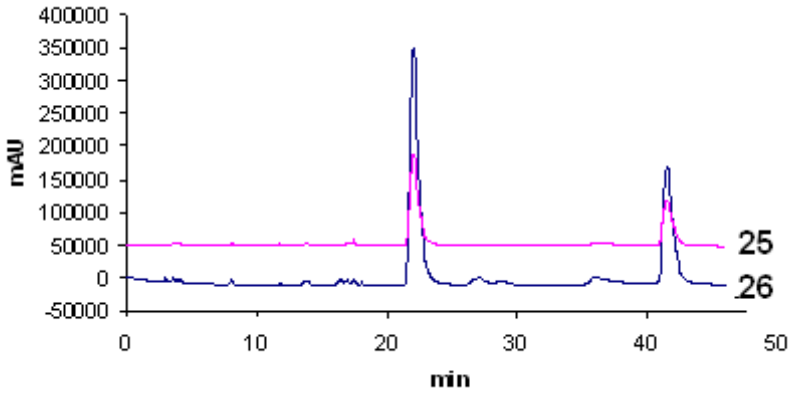
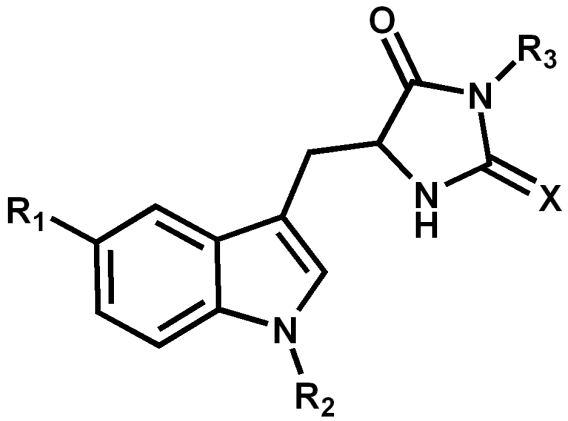


Figure 2.1

2.7 Enzyme activity

All synthesis products were evaluated by the pharmacological research group of Prof. Moroni at the University of Florence, as novel competitive inhibitors of human indoleamine 2,3-dioxygenase, extracted from placenta. The inhibition activity was determined by measuring the amount of kynurine product (HPLC), after incubating the purified enzyme with a constant quantity of *L*-tryptophan (30 µM) in presence of the IDO inhibitor (100 µM). As reported in table 2.1, the percentage of inhibition for 1-MT and MTH (reference compounds) was 45% and 35% respectively.



compound	R_1	R_2	R_3	X	inhibition % at 100 µM[a]
1-MT **2**					45
MTH **11**	H	H	Me	S	35
22	H	H	*n*-Pr	S	30
23	H	H	Bn	S	16
24	H	H	Ph	S	12
29	F	H	Me	S	32
32	H	Me	Me	S	21
34	H	H	Me	O	not active

[a] percentage of inhibition obtained by comparison of the amount of kynurenine produced in the presence of *L*-tryptophan (30 µM) and IDO inhibitor (100 µM)

Table 2.1: Inhibition assay for the synthesized hydantoin derivatives

Unfortunatly, our synthetic compounds displayed lower inhibition potency and solubility as their parent substance. Difficulty in reproducibility of the inhibition assay have emerged as well.

However, indoleamine 2,3-dioxygenase is still an interesting drug target, precisely because its crystal structure has been recently reported.[34] Computer-guided design will might lead to efficient inhibitors alternatives and they might support a successful cancer treatment.

Chapter 3

Experimental section

3.1 General procedures

3.1.1 Chemistry

All the necessary reagents were purchased from Sigma Aldrich and used as received. All syntheses were prepared under an inert argon or nitrogen atmosphere. Precoated TLC plates with silica gel 60 F-254 (Merck) were used for thin layer chromatography. Spots were visualized by UV lamp (254 nm) or by staining and warming with phosphomolybdate (5% in EtOH) or permanganate reagent. Flash column chromatography was performed using Merck silica gel 60 (0.040 – 0.063 mm) and on Biotage SP1 apparatus. Melting points were determined with a Büchi 535 electrothermal apparatus (capillary method) and are reported uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a Bruker AC 200 and 400 MHz spectrometer, and the chemical shifts are reported in parts per million (ppm). The abbreviations used are as follows: s, singlet; d, doublet; dd, double of a doublet; t, triplet; dt, double of a triplet; m, multiplet; b, broad; p, pseudo.

The chiral analysis of compounds **25** and **26** were performed on chiral HPLC (CHIRALPAK® IB a stationary phase consisted of tris(3,5-dimethylphenylcarbamate) derivatized cellulose and immobilized on silica 5 µm). The chromatogram was recorded with a PDA detector (set on from 190 to 350 nm), visualizing the absorption in mAU on the optimized wavelength of absorbance (220 nm, 254 nm). The most effective mobile phase for resolution and enantioselectivity was a mixture of n-hexane / i-propanol (85:15).

3.1.2 Pharmacology

Tissue preparation

Placental human IDO was purified as reported in literature [2] with some modification. Briefly, the placenta were cut in pieces and washed with physiological solution of 0.9% NaCl. The tissues were homogenized with 3 vol. of potassium phosphate buffer 50 mM (pH 6.5), 1M EDTA and 1M DTT. After centrifugation at 14000 rpm for 30 min, the clear solution was poured into an ion exchange column (Sephaldex S-100).

Enzymatic inhibition assay

The evaluation of IDO activity was conducted as reported in literature.[35] Briefly, the reaction mixture consisted of 20 µL of cytosol preparation and 80 µL of phosphate buffer (pH 6.5), containing 10 mM ascorbate, 50 µM methylene blue, 200 µg catalase, and 30 µM L-tryptophan (the inhibitor is included within the 80 µL). The resulted mixture was incubated for 2h at 37°C. By the addition of 100 µL of 20% (w/v) trichloroacetic acid the reaction was terminated. The mixture was centrifuged at 14000 rpm for 5 min. An aliquot of the supernatant was assayed by HPLC to evaluate the amount of kynurenine product. Kynurenine was determined by HPLC, using a reverse-phase column (Sperisorb S5 ODS2, 10 cm) and a mobile phase of 1 M ammonium acetate, 0.1 M acetic acid and 2% acetonitrile, and an ultraviolet detector (Perkin Elmer model LC 90, 365 nm).

3.2 Preparations

3.2.1 5-(1*H*-Indol-3-ylmethyl)-3-propyl-2-thiohydantoin (22)

Propylisothiocyanate (0.07 mL, 0.68 mmol) and triethylamine (0.18 mL, 1.30 mmol) were added to a solution of *L*-Tryptophan methyl ester hydrochloride (0.15 g, 0.59 mmol) in dry DCM (15 mL) under argon. The resulting mixture was stirred at room temperature for 30 h. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, eluting with DCM / EtOAC (from 3 up to 30% of EtOAc) to give derivative **22**.

Yield: 0.13 g (77%) of a yellow solid, mp 123-124°C

^{1}**H-NMR** (DMSO, 400 MHz, Appendix: figure 5.1 – 5.3) δ 0.44 (t, J = 7.5 Hz, 3H), 1.09 (dq, J = 7.3 and 2.1 Hz), 3.16 (dq, J = 14.98 and 4.6 Hz, 2H), 3.35 (m, 2H under H2O), 4.53 (pt, 1H), 6.95 (dt, J = 7.4 and 0.9 Hz, 1H), 7.03 (dt, J = 7.5 and 1.1 Hz, 1H), 7.06 (d, J = 2.4 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 10,32 (s, 1H), 10.88 (s, 1H).

^{13}**C-NMR** (DMSO, 100.6 MHz, figure 5.4 – 5.5) δ 13.00, 22.55, 28.33, 43.73, 61.79, 109.38, 113.56, 120.75, 120.95, 123.30, 126.63, 129.69, 138.26, 176.95, 185.14.

3.2.2 5-(1*H*-Indol-3-ylmethyl)-3-benzyl-2-thiohydantoin (23)

Benzylisothiocyanate (0.86 mL, 0.65 mmol) and triethylamine (0.181 mL, 1.30 mmol) were added to a solution of *L*-Tryptophan methyl ester hydrochloride (0.15 g, 0.59 mmol) in dry DCM (15 mL) under argon. The resulting mixture was stirred at room temperature for 24 h. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, eluting with DCM / EtOAc (from 3 up to 30% of EtOAc) to give derivative **23**.

Yield: 0.08 g (41%) of a yellow solid, mp 182-183°C.

^{1}**H-NMR** (DMSO, 400 MHz, figure 5.6 – 5.7) δ 3.21 (dq, J = 23.6 and 4.4 Hz, 2H), 4.68 (m, 3H), 6.56 (d, J = 7.4 Hz, 2H), 6.95 (dt, J = 7.2 and 0.9 Hz, 1H), 7.00 – 7.09

(m, 5H), 7.36 (d, J = 8.1 Hz, 1H), 7.57 (d, J = 7.9 Hz, 1H), 10,54 (s, 1H), 10.93 (s, 1H).

^{13}**C-NMR** (DMSO, 100.6 MHz, figure 5.8 – 5.9) δ 28.01, 45.41, 62.27, 109.40, 113.67, 120.94, 121.06, 123.38, 126.78, 128.61, 129.03, 129.77, 130.44, 138.29, 176.77, 184.86.

3.2.3 5-(1*H*-Indol-3-ylmethyl)-3-phenyl-2-thiohydantoin (24)

Phenylisothiocyanate (0.12 mL, 0.65 mmol) and triethylamine (1.81 ml, 1.30 mmol) were added to a solution of *L*-Tryptophan methyl ester hydrochloride (0.15 g, 0.59 mmol) in dry DCM (15 mL) under argon. The resulting mixture was stirred at room temperature for 4 h. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, eluting with DCM / EtOAc (from 0 up to 40% of EtOAc) to give derivative **24**.

Yield: 0.12 g (63%) of a yellow solid, mp 180°C.

^{1}**H-NMR** (DMSO, 400 MHz, figure 5.10 – 5.11) δ 3.33 (m, 2H under H2O), 4.71 (t, J = 4.4 Hz, 1H), 6.63 – 6.65 (m, 2H), 6.96 (dt, J = 7.3 and 0.9 Hz, 1H), 7.07 (dt, J = 7.3 and 0.5 Hz, 1H), 7.14 (d, J = 2.3 Hz, 1H), 7.27 – 7.31 (m, 3H), 7.35 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 7.9, 1H), 10,54 (s, 1H), 10.96 (s, 1H).

^{13}**C-NMR** (DMSO, 100.6 MHz, figure 5.12 – 5.13) δ 28.81, 62.47, 109.29, 113.69, 120.88, 121.07, 123.39, 126.94, 129.67, 130.84, 130.91, 135.67, 138.29, 176.56, 184.88.

3.2.4 *(S)*-5-(1*H*-Indol-3-ylmethyl)-3-methyl-2-thiohydantoin (25)

Methylisothiocyanate (0.047 g, 0.65 mmol) and triethylamine (0.18 mL, 1.3 mmol) were added to a solution of *L*-Tryptophan methyl ester hydrochloride (0.15 g, 0.59 mmol) in dry DCM (15 mL) under argon. The resulting mixture was stirred at room temperature for 24 h. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, eluting only with DCM to give derivative **25**.

Yield: 0.096 g (63%) of a yellow solid, mp 152-154°C.

^{1}**H-NMR** (DMSO, 400 MHz, figure 5.14 – 5.15) δ 2.85 (s, 3H), 3.14 (d, J = 5.0 Hz, 2H), 4.55 (t, J = 5.0 Hz, 1H), 6.95 (t, J = 7.4 Hz, 1H), 7.01 – 7.07 (m, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H), 10,33 (s,1H), 10.88 (s, 1H).

^{13}**C-NMR** (DMSO, 100.6 MHz, figure 5.16 – 5.17) δ 28.43, 29.08, 62.07, 109.80, 113.72, 120.83, 123.36, 126.46, 129.67, 138.26, 177.01, 185.27.

3.2.5 *(R)*-5-(1*H*-Indol-3-ylmethyl)-3-methyl-2-thiohydantoin (26)

Methylisothiocyanate (0.047 g, 0.65 mmol) and triethylamine (0.18 mL, 1.3 mmol) were added to a solution of *D*-Tryptophan methyl ester hydrochloride (0.15 g, 0.59 mmol) in dry DCM (15 mL) under argon. The resulting mixture was stirred at room temperature for 24 h under argon. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, eluting only with DCM to give derivative **26**.

Yield: 0.06 g (39%) of a yellow solid, mp 152-154°C.

^{1}**H-NMR** (DMSO, 400 MHz, figure 5.18 – 5.19) δ 2.84 (s, 3H), 3.14 (d, J = 5.0 Hz, 2H), 4.55 (pt, 1H), 6.96 (dt, J = 7.2 and 0.8 Hz, 1H), 7.01 – 7.06 (m, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H), 10,33 (s, 1H), 10.88 (s, 1H).

3.2.6 5-(5-Fluoro-1*H*-indol-3-ylmethyl)-3-methyl-2-thiohydantoin (29)

Methylisothiocyanate (0.041 g, 0.56 mmol) and triethylamine (0.15 mL, 1.1 mmol) were added to a solution of 5-Fluoro-tryptophan methyl ester **28** (0.12 g, 0.51 mmol) in dry DCM (15 mL) under argon. The resulting mixture was stirred at room temperature for 3 h under argon. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, by eluting only with DCM to give derivative **29**.

Yield: 0.052 g (37%) of a yellow solid, mp 140-141°C.

^{1}**H-NMR** (DMSO, 400 MHz, figure 5.20 – 5.21) δ 2.82 (s, 3H), 3.11 (d, J = 3.8 Hz, 2H), 4.54 (t, J = 4.8 Hz, 1H), 6.87 (dt, J = 9.15 and 2.4 Hz, 1H), 7.12 (d, J = 2.3 Hz,

1H), 7.25 – 7.30 (m, 2H), 10,31 (s, 1H), 10.98 (s, 1H).

^{13}**C-NMR** (DMSO, 100.6 MHz, figure 5.22 – 5.23) δ 28.27, 29.04, 62.10, 105.52, 105.75, 110.01, 110.06, 111.39, 111.65, 114.60, 114.69, 128.67, 129.94, 130.04, 134.92, 158.06, 160.35, 177.01, 185.24.

3.2.7 5-(1-*N*-Methyl-1*H*-Indol-3-ylmethyl)-3-methyl-2-thiohydantoin (32)

Methylisothiocyanate (0.02 g, 0.24 mmol) and triethylamine (0.04 mL, 1.2 mmol) were added to a solution of 1-*N*-Methyl-tryptophan methyl ester (**31**) (0.05 g, 0.22 mmol) in dry DCM (5 mL) under argon. The resulting mixture was stirred at room temperature for 2 h. It was taken up with DCM (about 10 mL) and evaporated under reduced pressure. The reaction mixture was purified by flash chromatography, by eluting with DCM / EtOAc (from 3 up to 30% of EtOAc) to give derivative **32**.

Yield: 0.044 g (73%) of a yellow solid, mp 160-162°C.

^{1}**H-NMR** (CD_3OD, 400 MHz, figure 5.24 – 5.25) δ 2.94 (pt, J = 12.47), 3.22 (pd, 3H), 3.50 (pt, J = 14.64 Hz), 3.77 (ps, 3H), 4.34 (pd, 1H), 6.95 (pd, 1H), 7.00 (s, 1H), 7.15 (pdt, 1H), 7.24 – 7.34 (m, 2H), 7.57 (pd).

^{13}**C-NMR** (CD_3OD, 100.6 MHz, figure 5.26 – 5.27) δ 27.46, 27.78, 32.77, 60.17, 107.68, 109.62, 118.40, 119.64, 122.27, 126.97, 127.70, 137.17, 173.54, 184.01.

3.2.8 Tryptophan methyl amide (33)

Tryptophan methyl ester hydrochloride (1 g, 3.93 mmol) was dissolved in methylamine (2M) in methanol (19.7 mL, 39.3 mmol) under argon. The resulting mixture was left stirring at room temperature for 48h. The remaining methylamine was removed with argon. The reaction mixture was taken up with methanol (about 10 mL) and evaporated under reduced pressure to give **33**.

Yield: 0.84 g (98%) of a bright brown sticky paste.

^{1}**H-NMR** (DMSO, 200 MHz, figure 5.28 - 5.30) δ 2.57 (d, J = 4.7, 3H), 2.75 (pd, 1H), 3.04 (pd, 1H), 3.17 (s, 1H), 3.40 (pd, 1H) 6.92 – 7.14 (m, 3H), 7.33 (pd, J = 6.9, 1H), 7.54 (pd, J = 8.0, 1H), 7.79 (d, J = 4.5, 1H), 10.83 (s, 1H).

3.2.9 5-(1*H*-Indol-3-ylmethyl)-3-methyl-hydantoin (34)

Anhydrous DCM (3 mL) and triethylamine (0.32 mL, 2.48 mmol) were added to a flask containing crude **33** (0.18 g, 0.83 mmol) under argon. The resulting mixture was stirred until the temperature internally reached -10°C (ice + salt). It was treated portionwise with triphosgene (0.074 g, 0.25 mmol) so that the temperature did not augmented more than -7°C. After stirring at -10°C for 30 min, the reaction mixture was allowed to warm to room temperature. A 0.5 M solution of sodium carbonate was added (= quenching).The aqueous layer was washed with DCM (10 mL x 3). The combined organic layers were dried over anhydrous sodium sulphate, filtrated and evaporated to dryness. The residue was purified by flash chromatography, by eluting with DCM/ MeOH (0 – 1%) to give **34**.

Yield: 0.021 g (10%) of a bright yellow solid, mp 182-183°C.

^{1}**H-NMR** (DMSO, 400 MHz, figure 5.31 – 5.32) δ 2.63 (s, 3H), 3.07 (pd, 2H), 4.33 (s, 1H), 6.95 – 7.09 (m, 3H), 7.30 (d, J = 8.0, 1H), 7.51 (d, J = 7.8, 1H), 8.14 (s, 1H), 10.86 (s, 1H).

^{13}**C-NMR** (DMSO, 100.6 MHz, figure 5.33 – 5.34) δ 26.27, 29.21, 59.61, 110.44, 113.72, 120.75, 120.88, 123.31, 126.48, 129.81, 138.36, 159.39, 176.63.

Chapter 4

References

1. Hayashi O. Properties and function of indoleamine 2,3-dioxygenase. *J Biochem* **1963**; 79(4):13-21

2. Yamazaki F, Kuroiwa T, Takikawa O, Kido R. Human indolylamine 2,3-dioxygenase. Its tissue distribution and characterization of the placental enzyme. *Biochem J* **1985**; 230:635-638

3. Ball HJ, Sachez-Perez A, Weiser S, Austin CJD, Astelbauer F, Miu J, McQuillan JA, Stocker R, Jermiin LS, Hunt NH. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene* **2007**; 396:203-213

4. Ball HJ, Yuasa HJ, Austin CJD, Weiser S, Hunt NH. Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int J Biochem Cell Biol* **2009**; 41:467-471

5. Taylor MW, Feng G. Relationship between interferon-γ, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB J* **1991**; 5(11):2516-2522

6. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* **1998**; 281:1191-1193

7. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev* **2004**; 4(10):762-774

8. Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? *Immunol Today* **1999**; 20(10):469-473

9. Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends in Immunol* **2003**; 24(5):242-248

10. Munn DH, Mellor AL. IDO and tolerance to tumors. *Trends in Molecular Medicine* **2004**; 10(1):15-18

11. Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and tumor-induced tolerance. *J Clin Invest* **2007**; 117(5):1147-1154

12. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene *Bin1*, potentiates cancer chemotherapy. *Nat Med* **2005**; 11(3):312-319

13. Malachowski WP, Metz R, Prendergast GC, Muller AJ. A new cancer immunosuppression target: indoleamine 2,3-dioxygenase (IDO). A review of the IDO mechanism, inhibition and therapeutic applications. *Drugs Future* **2005**; 30(9):897-909

14. Muller AJ, Malachowski WP, Prendergast GC. Indoleamine 2,3-dioxygenase in cancer: targeting pathological immune tolerance with small-molecule inhibitors. *Expert Opin Ther Targets* **2005**; 9(4):831-849

15. Macchiarulo A, Camaioni E, Nuti R, Pellicciari R. Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2,3-dioxygenase (IDO), a novel target in cancer disease. *Amino Acids*, **2009**; 37:219-229

16. Austin CJD, Astelbauer F, Kosim-Satyaputra P, Ball HJ, Willows RD, Jamie JF, Hunt NH. Mouse and human indoleamine 2,3-dioxygenase display some distinct biochemical and structural properties. *Amino Acids* **2009**; 36:99-106

17. Sono M, Taniguchi T, Watanabe Y, Hayaishi O. Indoleamine 2,3-dioxygenase. Equilibrium studies of the tryptophan binding to the ferric, ferrous, and CO-bound enzymes. *J Biol Chem* **1980**; 225(4):1339-1345

18. Sono M, Dawson JH. Extensive studies of the heme coordination structure of indoleamine 2,3-dioxygenase and tryptophan binding with magnetic and natural circular dichroism and electron paramagnetic resonance spectroscopy. *Biochim Biophys Acta* **1984**; 789(2):170-187

19. Cady SG, Sono M. 1-methyl-DL-tryptophan, 4β-(3-benzofuranyl)-DL-alanine (the oxygen analog of tryptophan), and β-[3-benzo(b)thienyl]-DL-alanine (the sulphur analog of tryptophan) are competitive inhibitors for indoleamine 2,3-dioxygenase. *Arch Biochem Biophys* **1991**; 291:326-333

20. Peterson AC, Migawa MT, Martin MJ, Hamaker LK, Czerwinski KM, Zhang W, Arend RA, Fisette PL, Ozaki Y, Will JA, Brown RR, Cook JM. Evaluation of functionalized tryptophan derivatives and related compounds as competitive inhibitors of indoleamine 2,3-dioxygenase. *Med Chem Res* **1994**; 3:531-544

21. Sono M, Roach MP, Coulter ED, Dawson JH. Heme-containing oxygenases. *Chem Rev* **1996**; 96:2841-2887

22. Southan MD, Truscott RJW, Jamie JF, Pelosi L, Walker MJ, Maeda H, Iwamoto Y, Toné S. Structural requirements of the competitive binding site of recombinant human indoleamine 2,3-dioxygenase. *Med Chem Res* **1996**; 6:343-352

23. Teng X, Degterev A, Jagtap P, Xing X, Choi S, Denu R, Yuan J, Cuny GD. Structure-activity relationship study of novel necroptosis inhibitors. *BMCL* **2005**; 15:5039-5044

24. Gaspari P, Banerjee T, Malachowski WP, Muller AJ, Prendergast GC, DuHadaway J, Bennett S, Donovan AM. Structure-activity study of brassinin derivatives as indoleamine 2,3-dioxygenase inhibitors. *J Med Chem* **2006**; 49:684-692

25. Yue EW, Douty B, Wayland B, Bower M, Liu X, Leffet L, Wang Q, Bowman KJ, Hansbury MJ, Liu C, Wei M, Li Y, Wynn R, Burn TC, Koblish HK, Fridman JS, Metcalf B, Scherle PA, Combs AP. Discovery of potent competitive inhibitors of indoleamine 2,3-dioxygenase with in vivo pharmacodynamic activity and efficacy in a mouse melanoma model. *J Med Chem* **2009**; 52: 7364-7367

26. Eguchi N, Watanabe Y, Kawanishi K, Hashimoto Y, Hayaishi O. Inhibition of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase by β-carboline and indole derivatives. *Arch Biochem Biophys* **1984**; 232:602-609

27. Sono M, Cady SG. Enzyme Kinetic and Spectroscopic Studies of Inhibitor and Effector Interactions with Indoleamine 2,3-Dioxygenase. 1. Norharman and 4-Phenylimidazole Binding to the Enzyme as Inhibitors and Heme Ligands. *Biochemistry* **1989**; 28:5392-5399

28. Peterson AC, La Loggia AJ, Hamaker LK, Arend RA, Fisette PL, Ozaki Y, Will JA, Brown RR, Cook JM. Evaluation of substituted β-carbolines as noncompetitive indoleamine 2,3-dioxygenase inhibitors. *Med Chem Res* **1993**; 3:473-482

29. Pereira A, Vottero E, Roberge M, Mauk AG, Andersen RJ. Indoleamine 2,3-dioxygenase inhibitors from the northeastern pacific marine hydroid *Garvenia annulata*. *J Nat Prod* **2006**; 69:1496-1499

30. Brastianos HC, Vottero E, Patrick BO, Van Soest R, Matainaho T, Mauk AG, Andersen RJ. Exiguamine A, an indoleamine 2,3-dioxygenase (IDO) inhibitor isolated from the marine sponge *Neopetrosia exigua*. *J Am Chem Soc* **2006**; 128: 16046-16047

31. Kumar S, Malachowski WP, DuHadaway JB, LaLonde JM, Carroll PJ, Jaller

D, Metz R, Prendergast GC, Muller AJ. Indoleamine 2,3-dioxygenase is the anticancer target for a novel series of potent naphthoquinone-based inhibitors. *J Med Chem* **2008** 51:1706-1718

32. Vottero E, Balgi A, Woods K, Tugendreich S, Melese T, Andersen RJ, Mauk AG, Roberge M. Inhibitors of human indoleamine 2,3-dioxygenase identified with a target-based screen in yeast. *Biotechnol J* **2006**; 1:282-288

33. Muller AJ, Scherle PA. Targeting the mechanisms of tumoral immune tolerance with small-molecule inhibitors. *Nat Rev Cancer* **2006**; 6:613-625

34. Sugimoto H, Oda S, Otsuki T, Hino T, Yoshida T, Shiro Y. Crystal structure of human indoleamine 2,3-dioxygenase: Catalytic mechanism of O_2 incorporation by a heme-containing dioxygenase. *PNAS* **2006**; 103(8):2611-1616

35. Chiarugi A, Dello Sbarba P, Paccagnini A, Donnini S, Filippi S, Moroni F. Combined inhibition of indoleamine 2,3-dioxygenase and nitric oxide synthase modulates neurotoxin release by interferon-γ-activated macrophages. *J Leukoc Biol* **2000**; 68:260-266

Chapter 5

Appendix

A NMR- Spectra

Figure 5.1: 5-(1*H*-Indol-3-ylmethyl)-3-propyl-2-thiohydantoin (**22**)

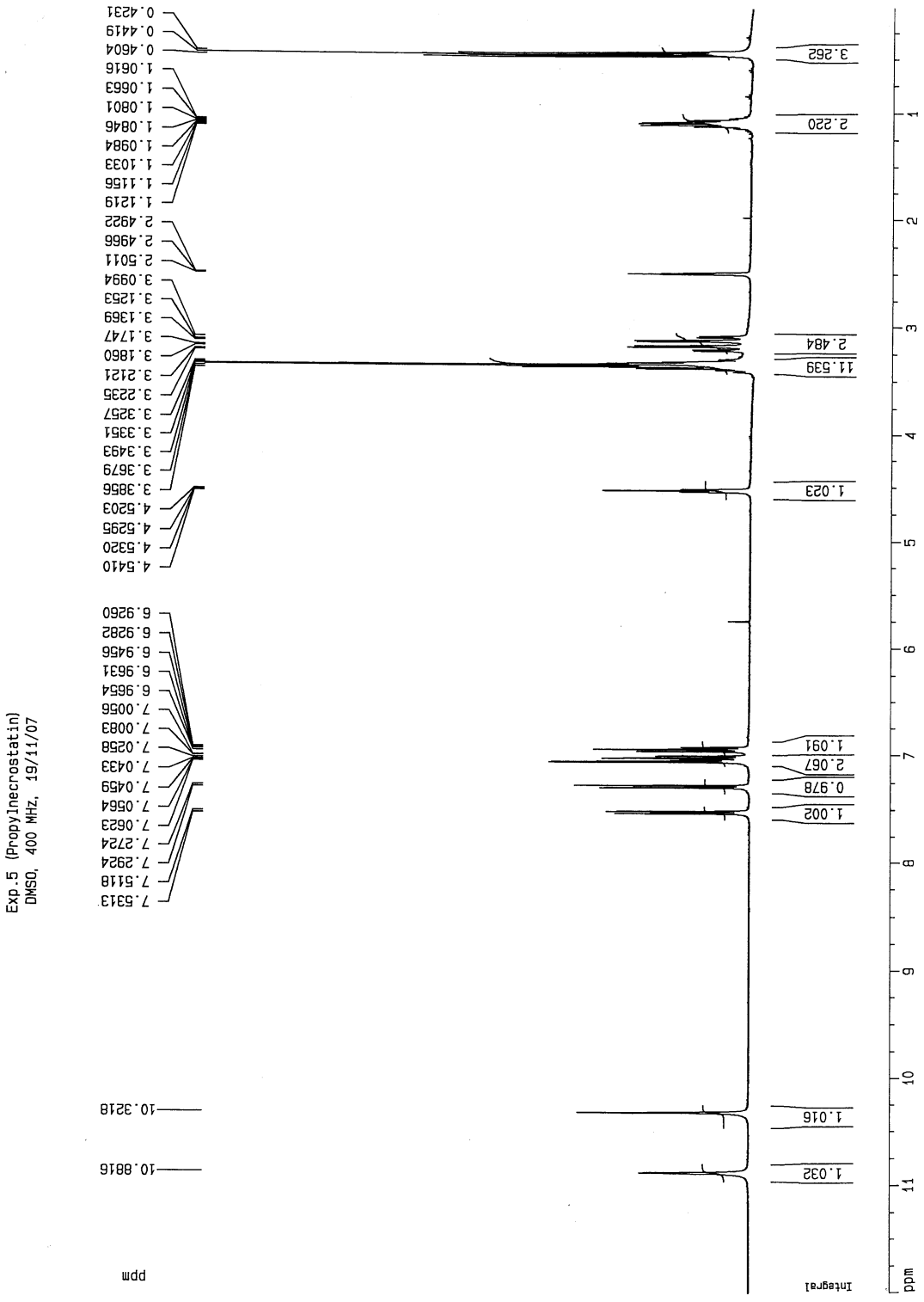


Figure 5.2: compound **22**

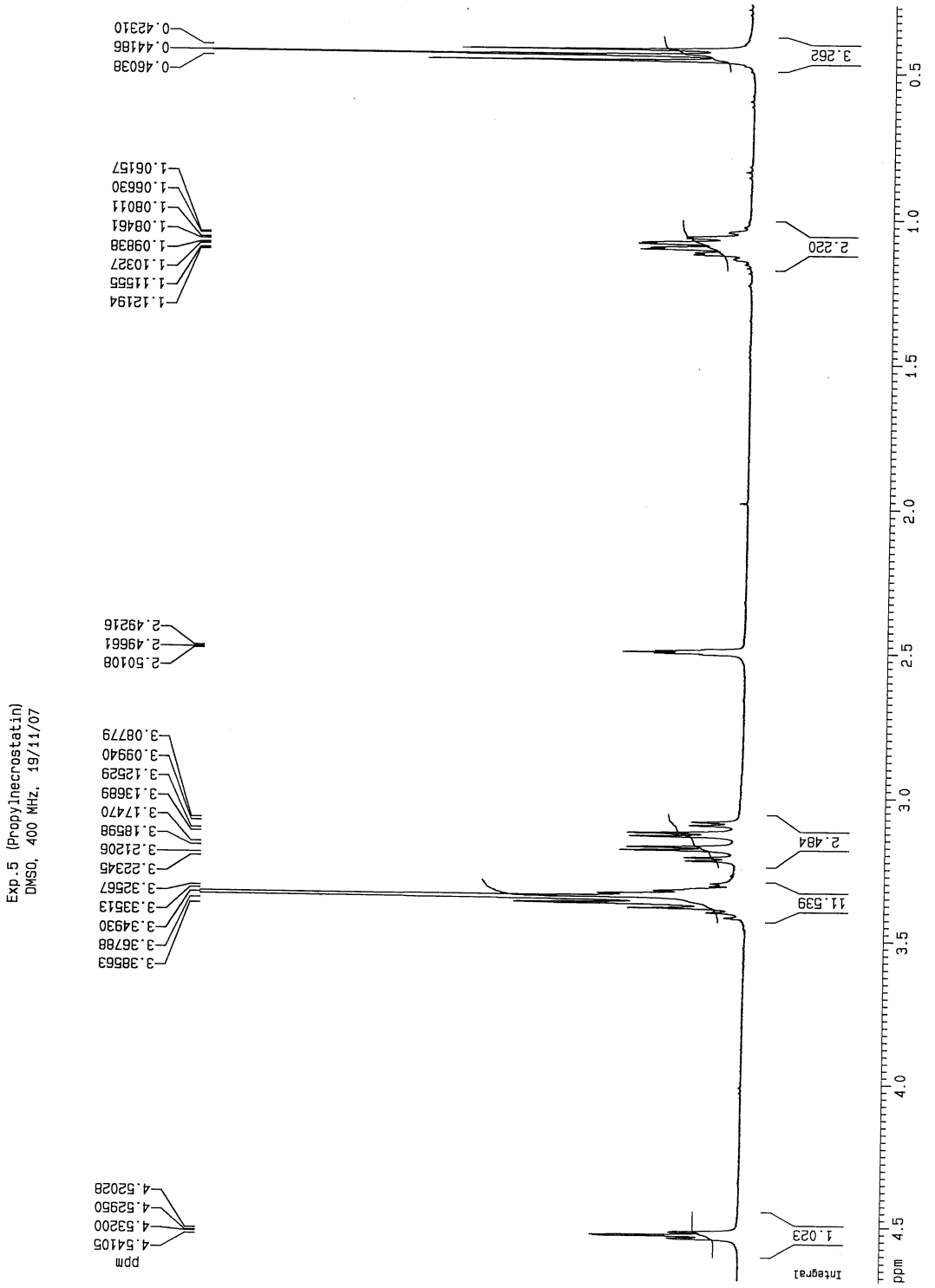


Figure 5.3: compound **22**

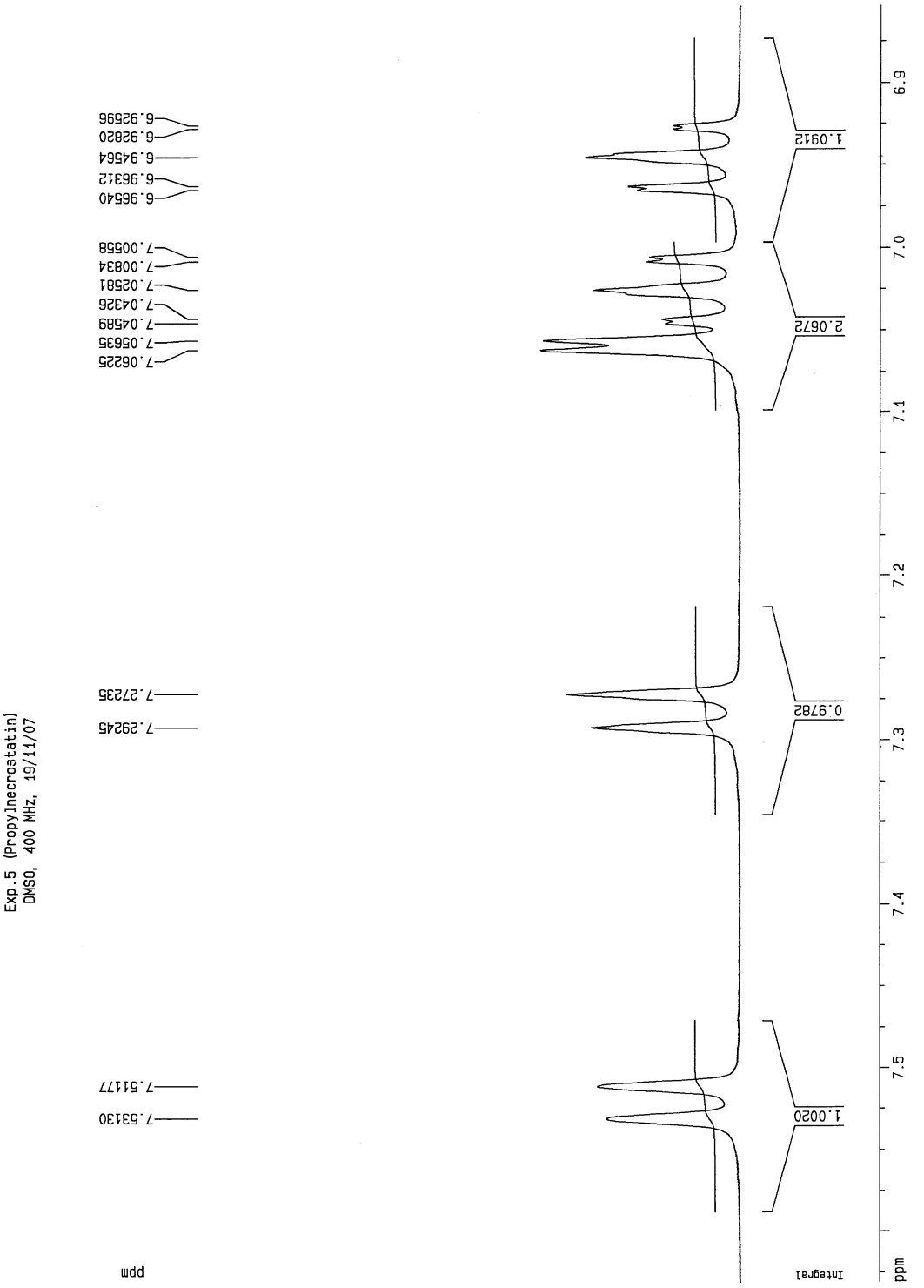


Figure 5.4: compound **22**

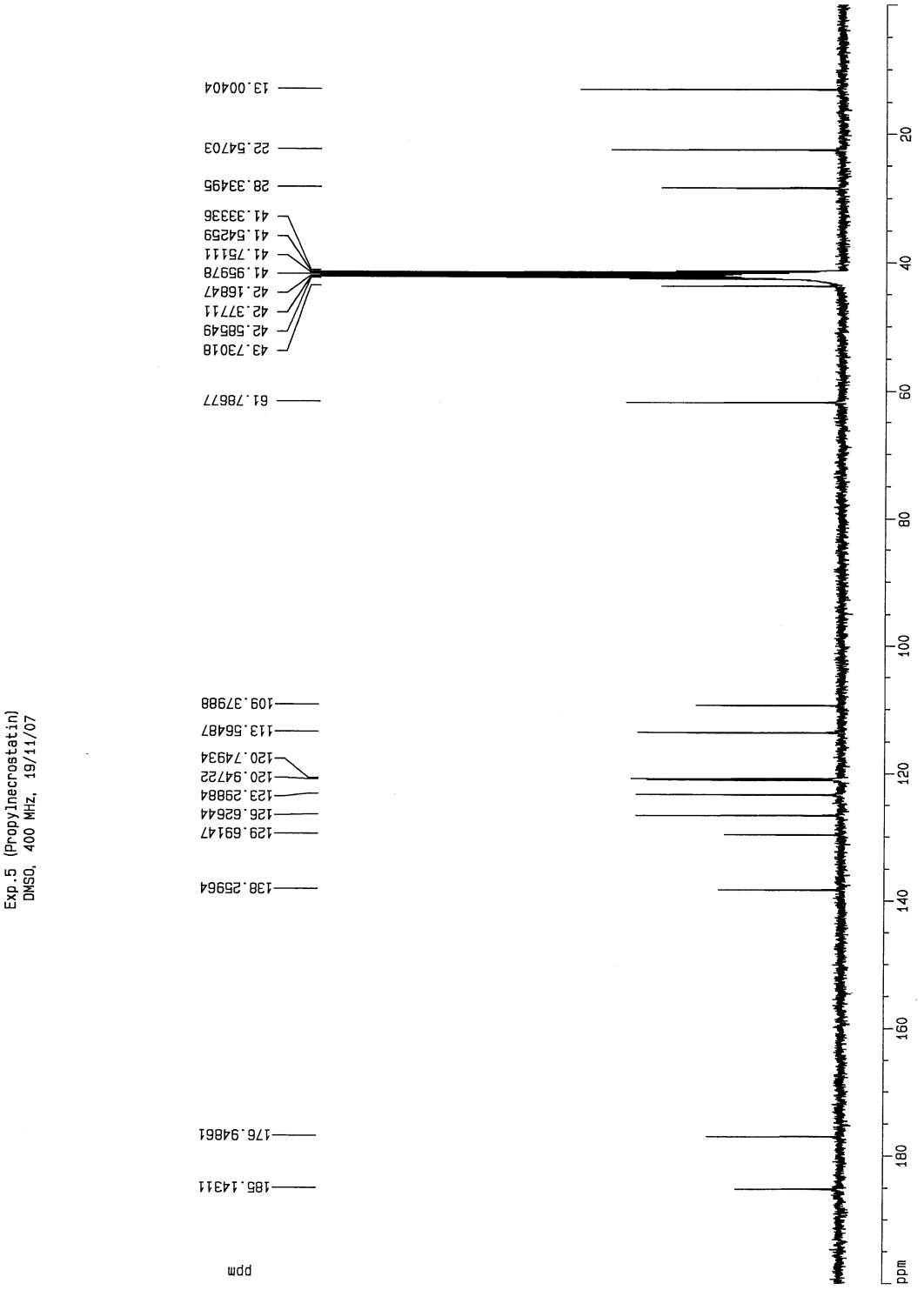


Figure 5.5: compound **22**

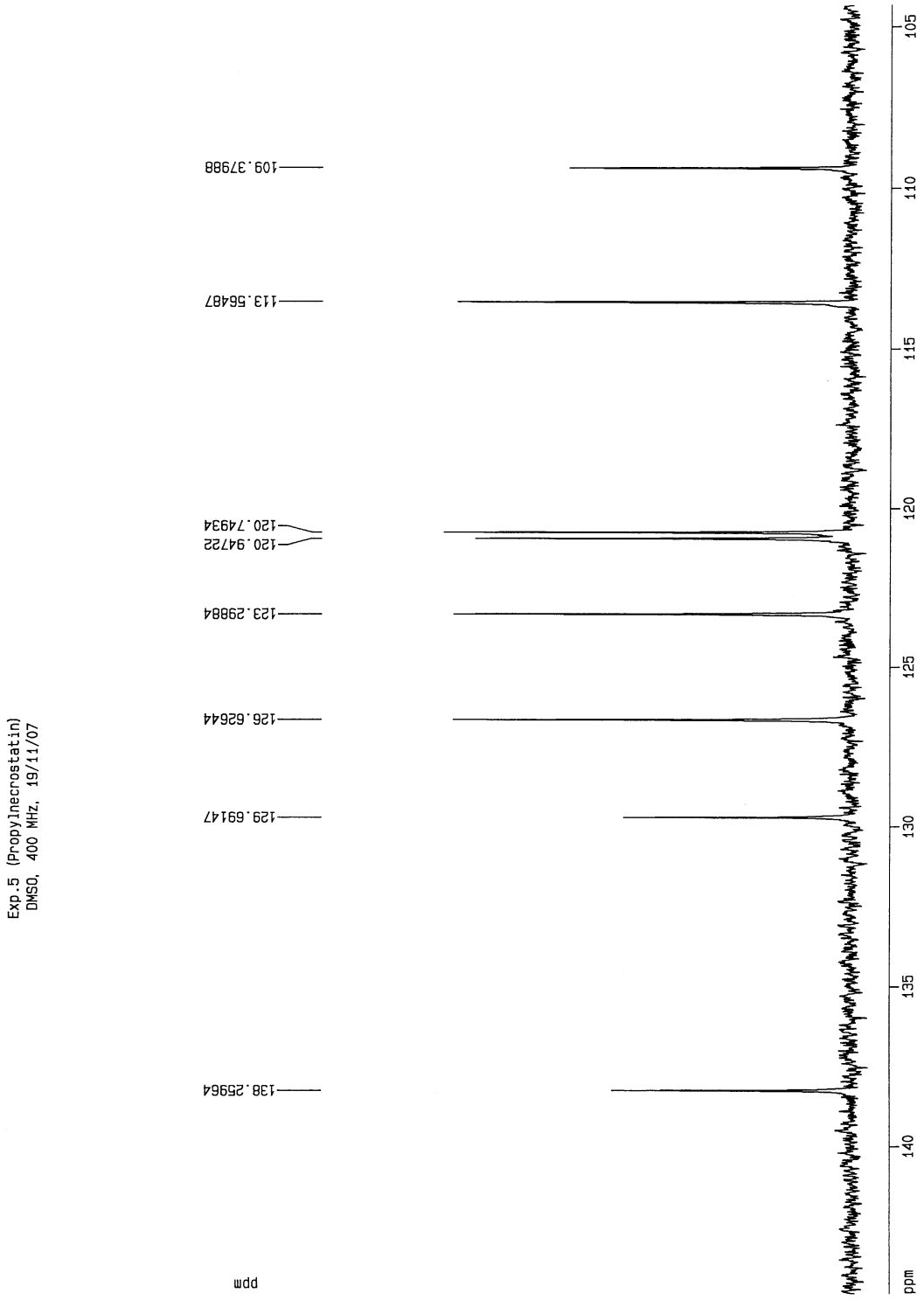


Figure 5.6: 5-(1*H*-Indol-3-ylmethyl)-3-benzyl-2-thiohydantoin (**23**)

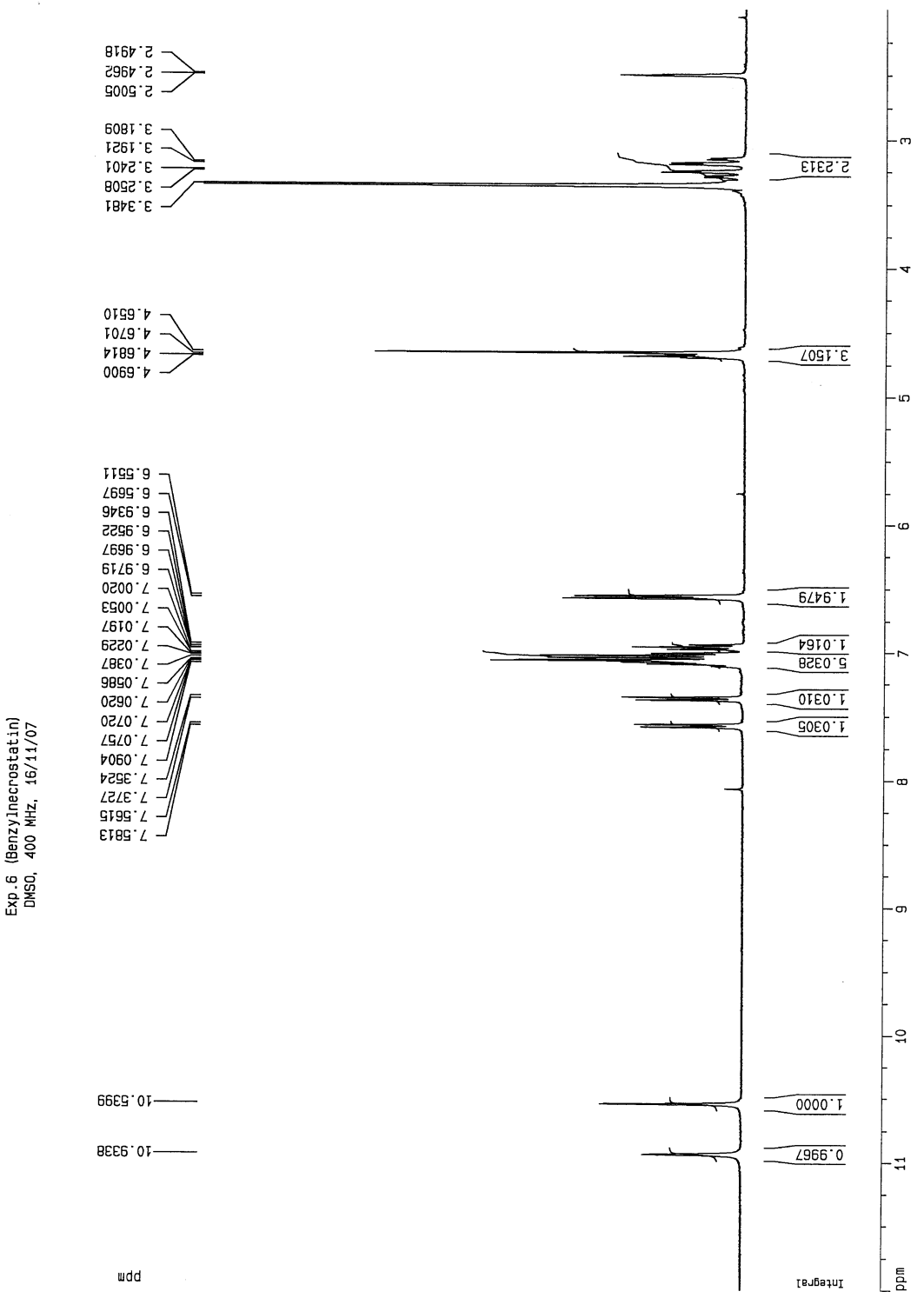


Figure 5.7: compound **23**

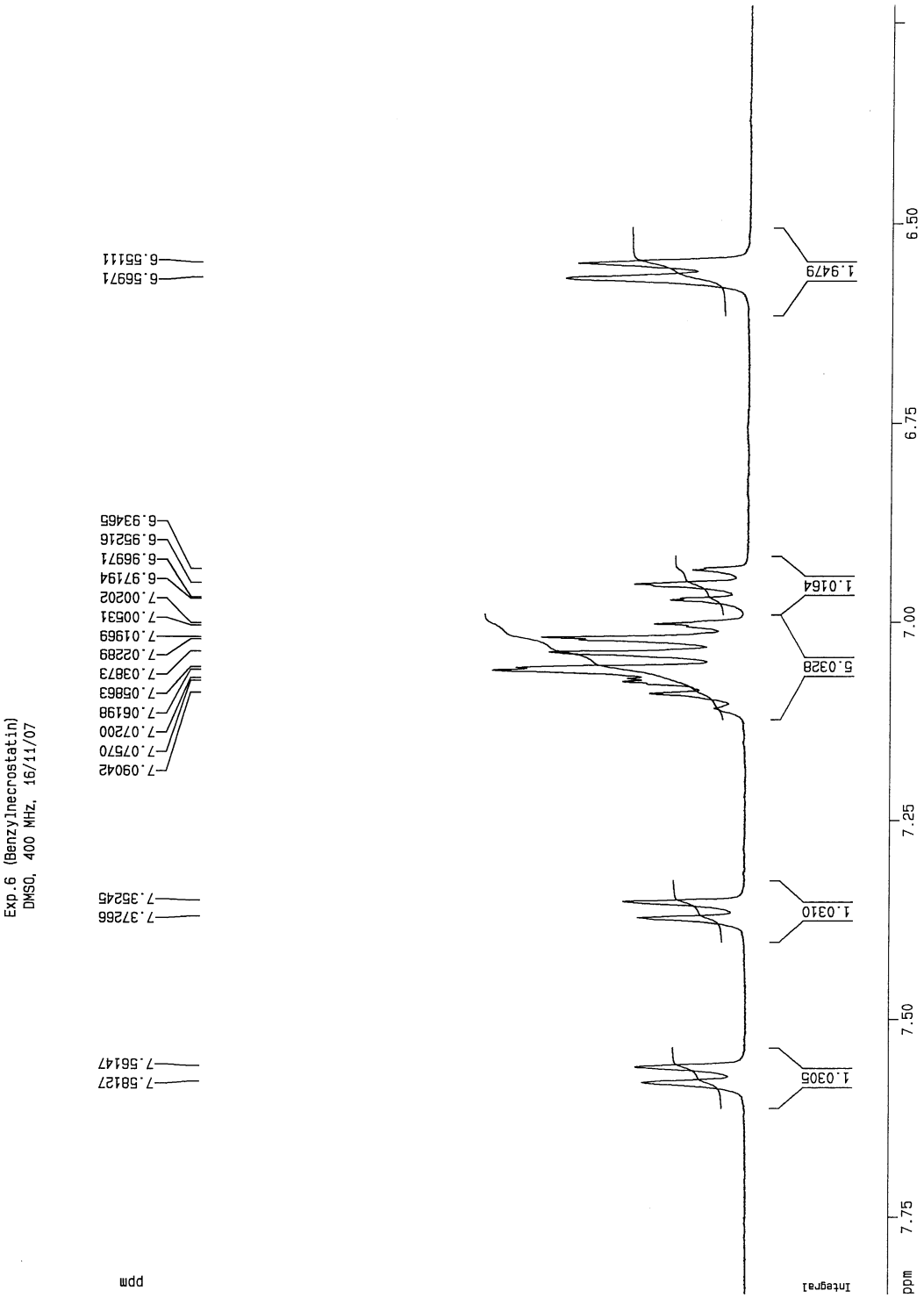


Figure 5.8: compound **23**

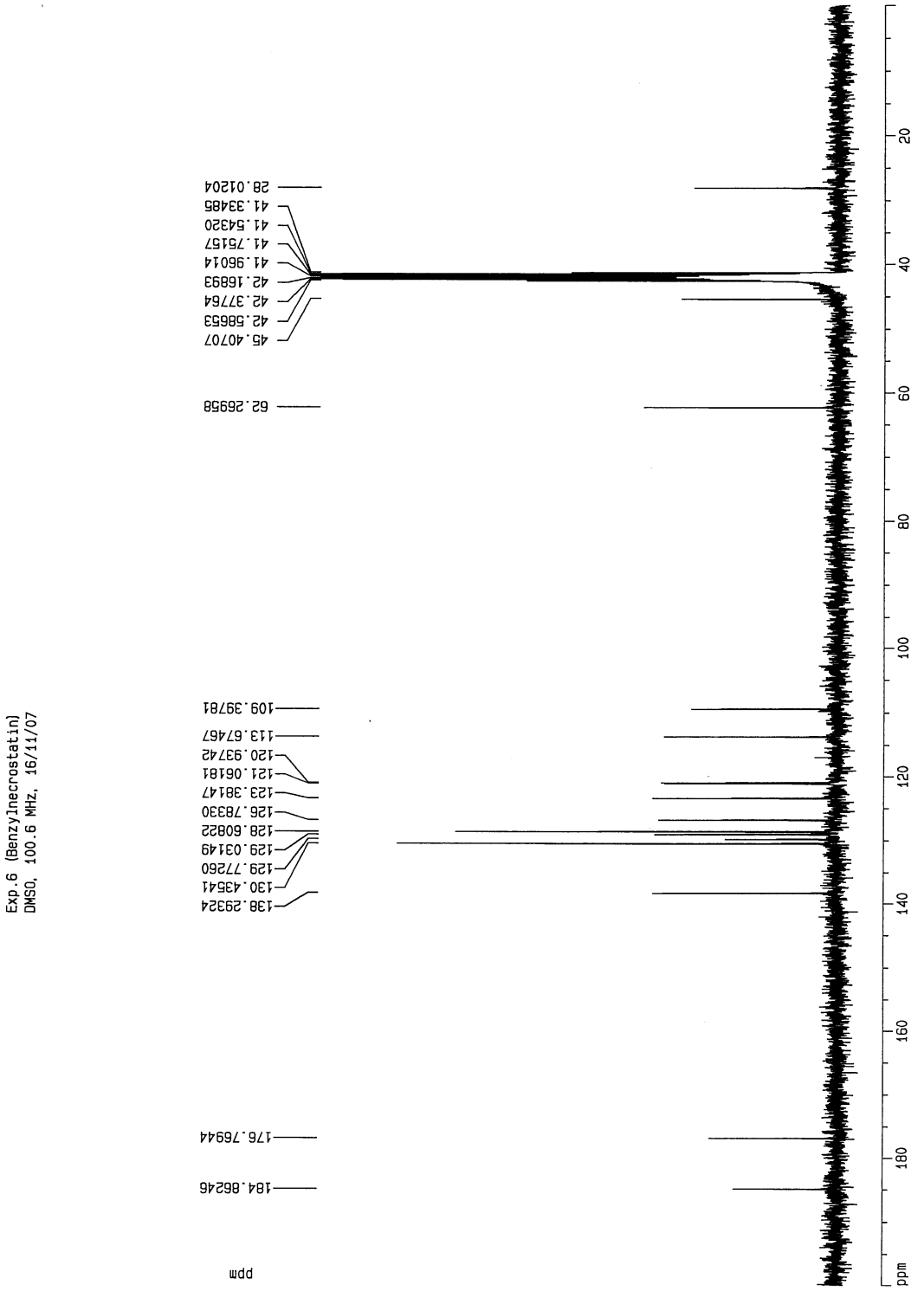


Figure 5.9: compound **23**

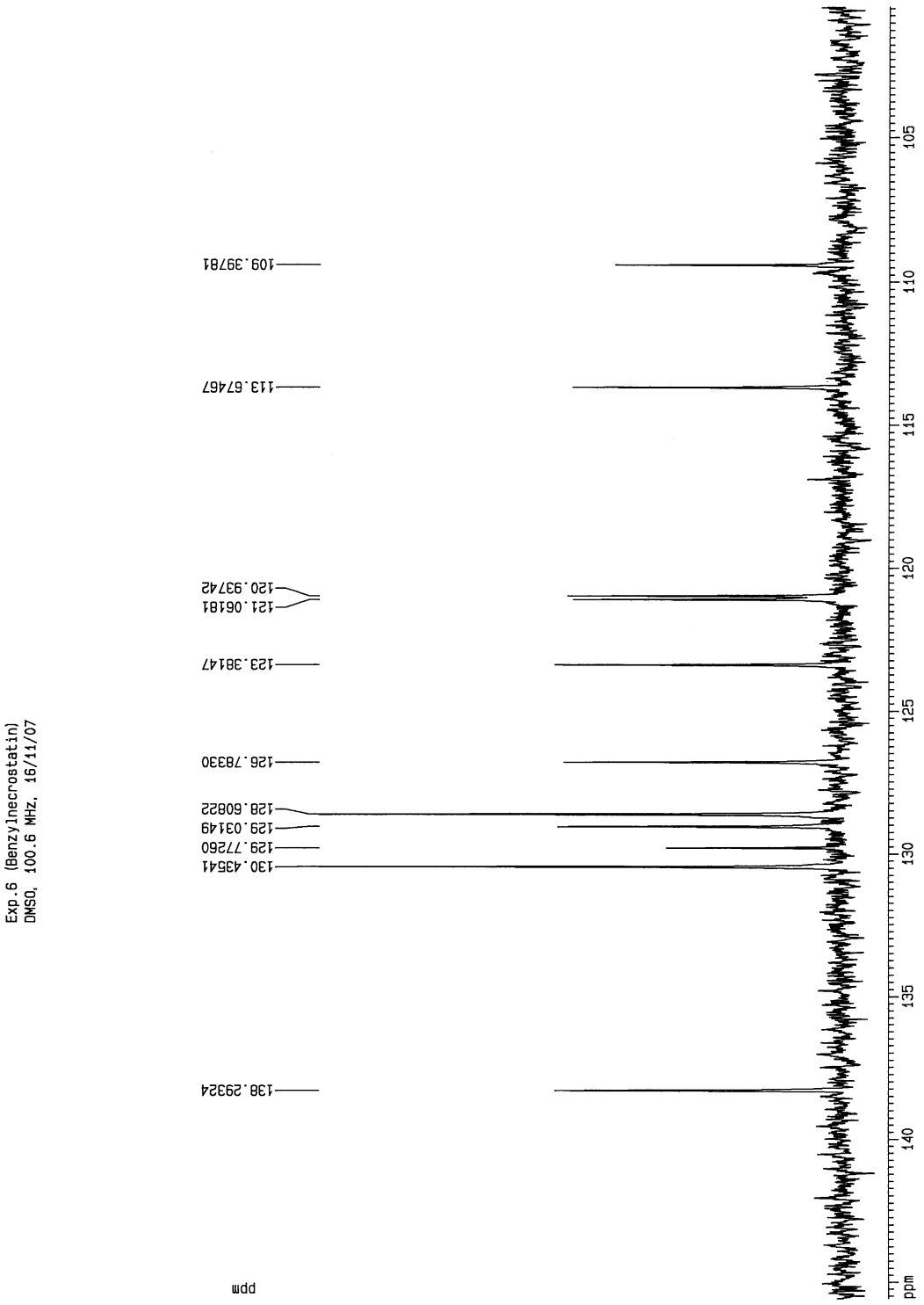


Figure 5.10: 5-(1*H*-Indol-3-ylmethyl)-3-phenyl-2-thiohydantoin (**24**)

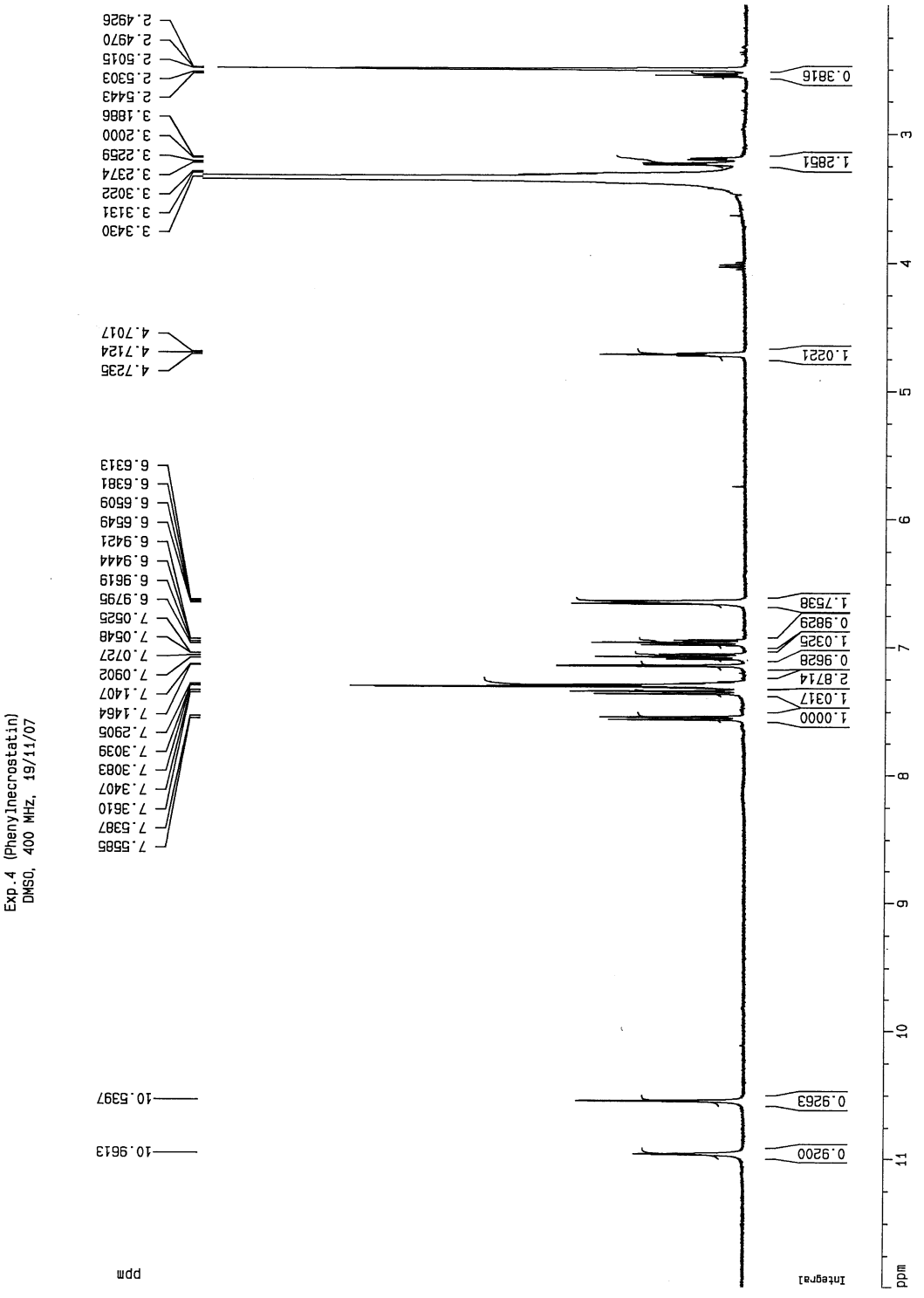


Figure 5.11: compound **24**

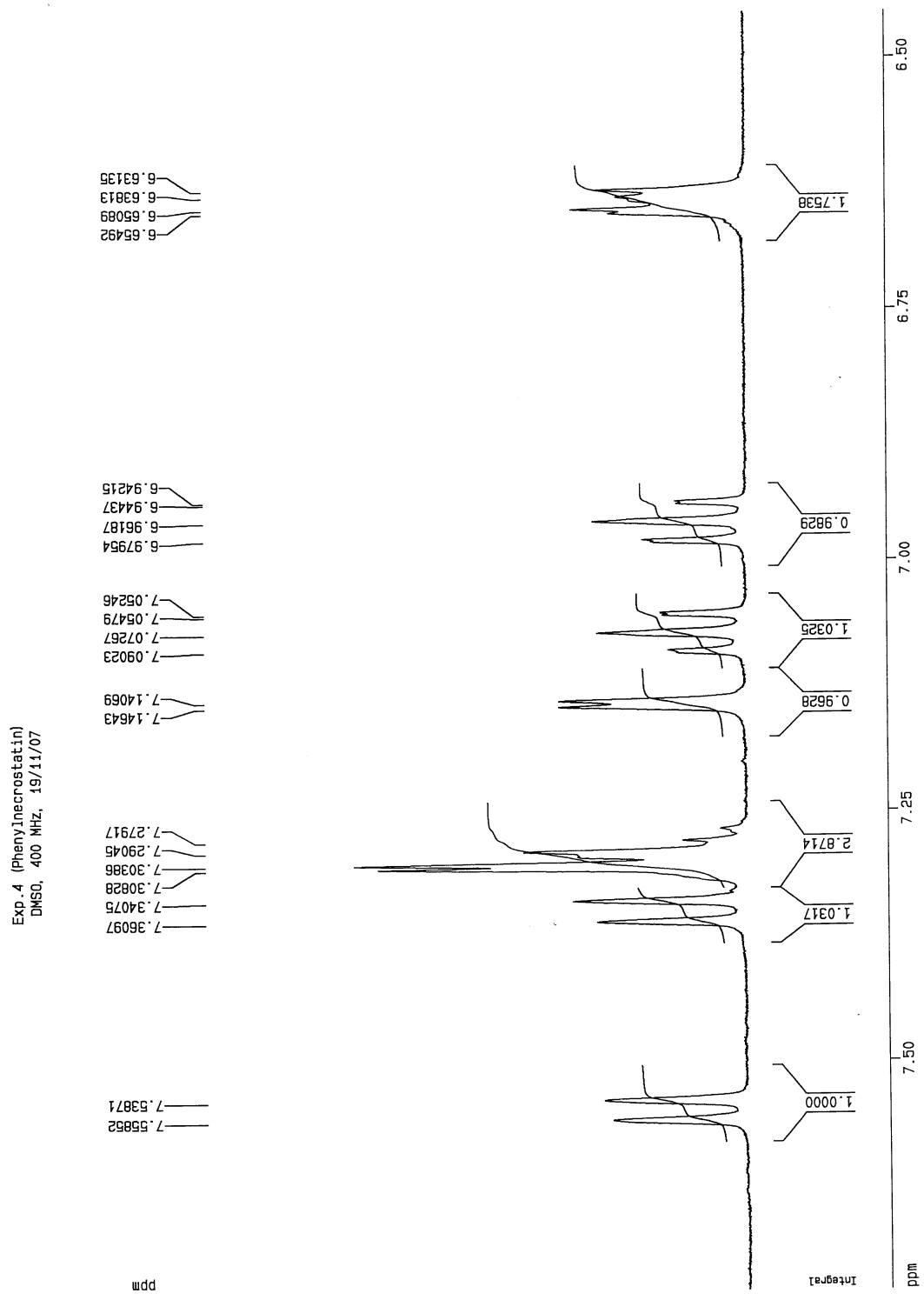


Figure 5.12: compound **24**

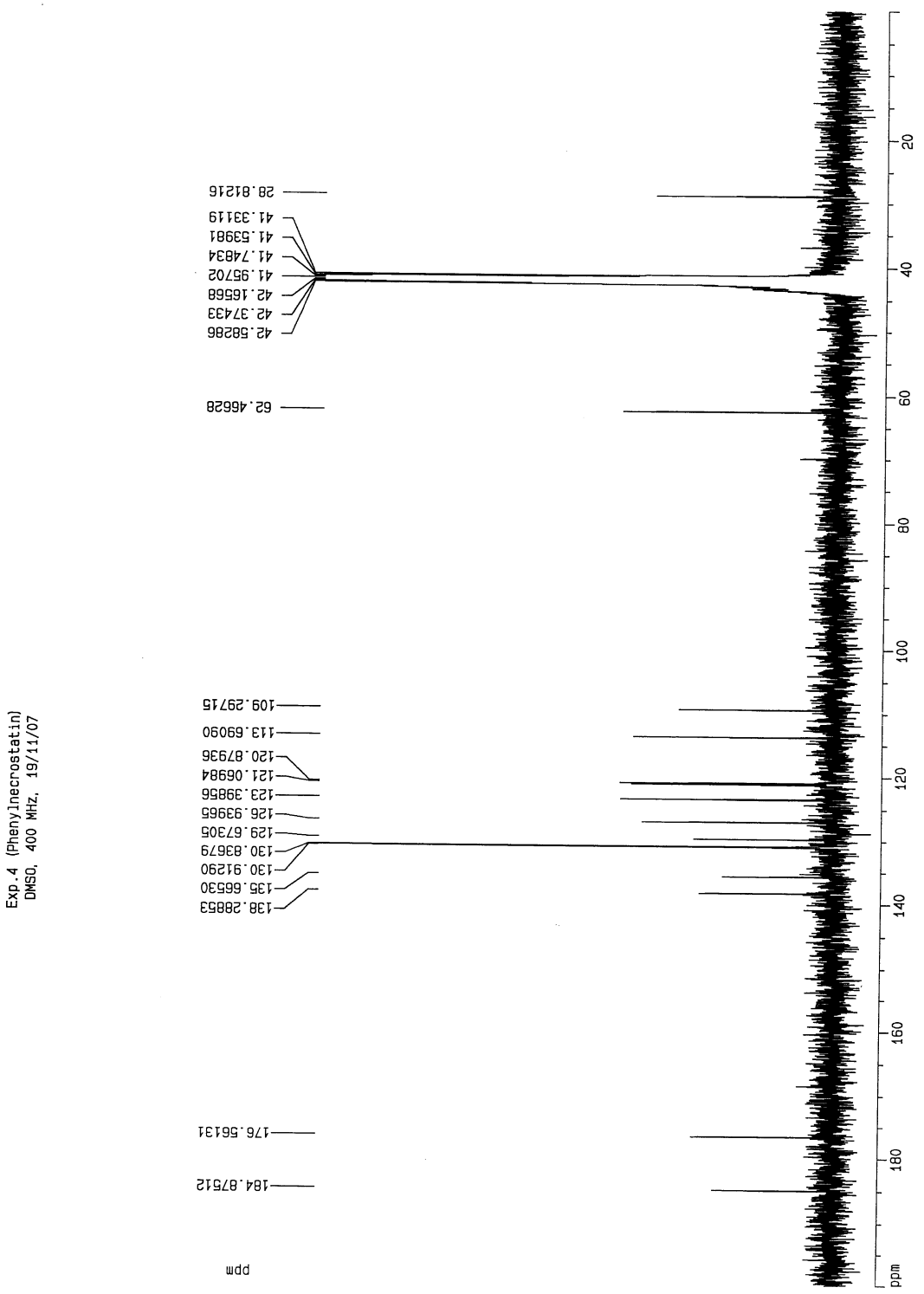


Figure 5.13: compound **24**

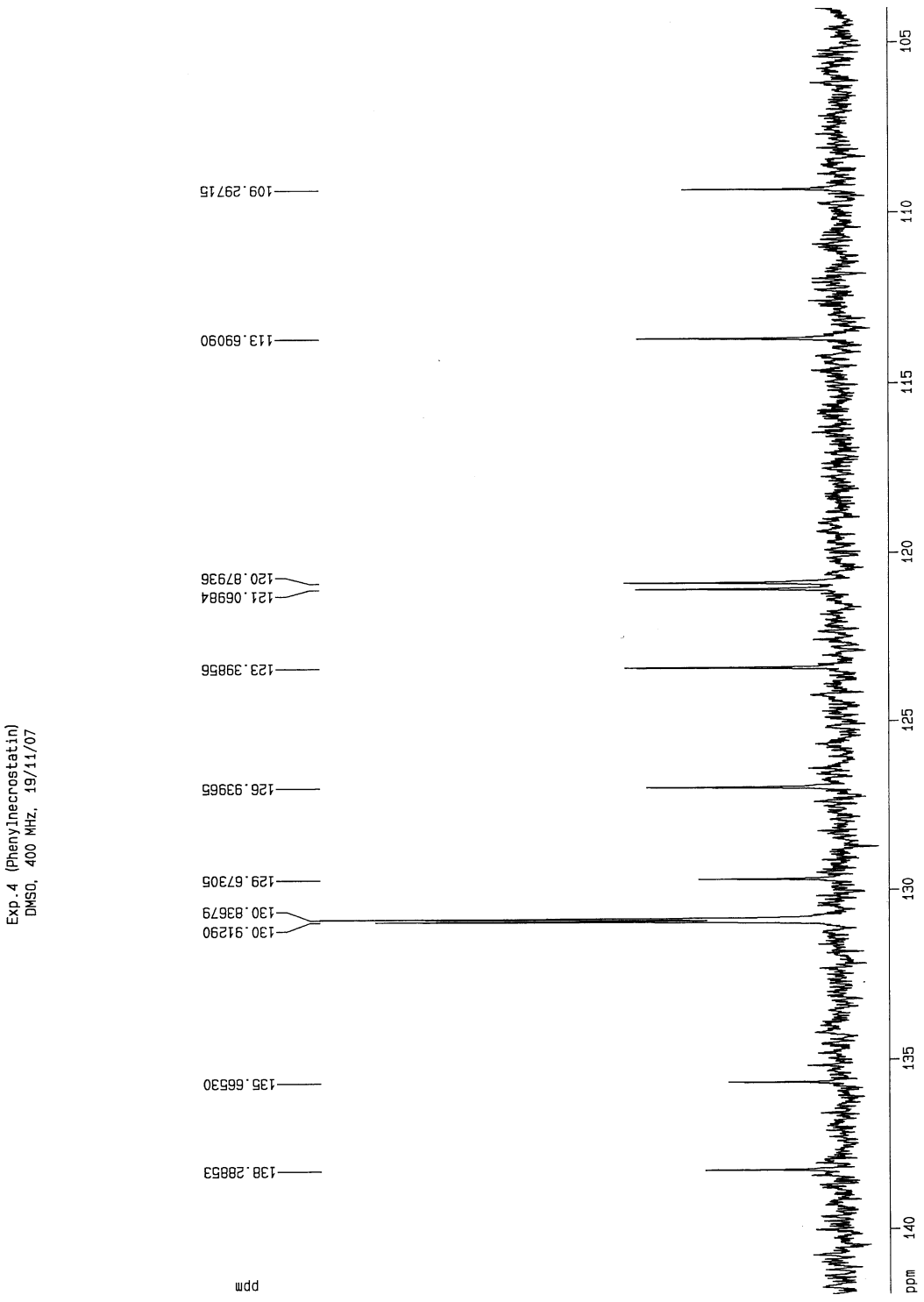


Figure 5.14: *(S)*-5-(1*H*-Indol-3-ylmethyl)-3-methyl-2-thiohydantoin (**25**)

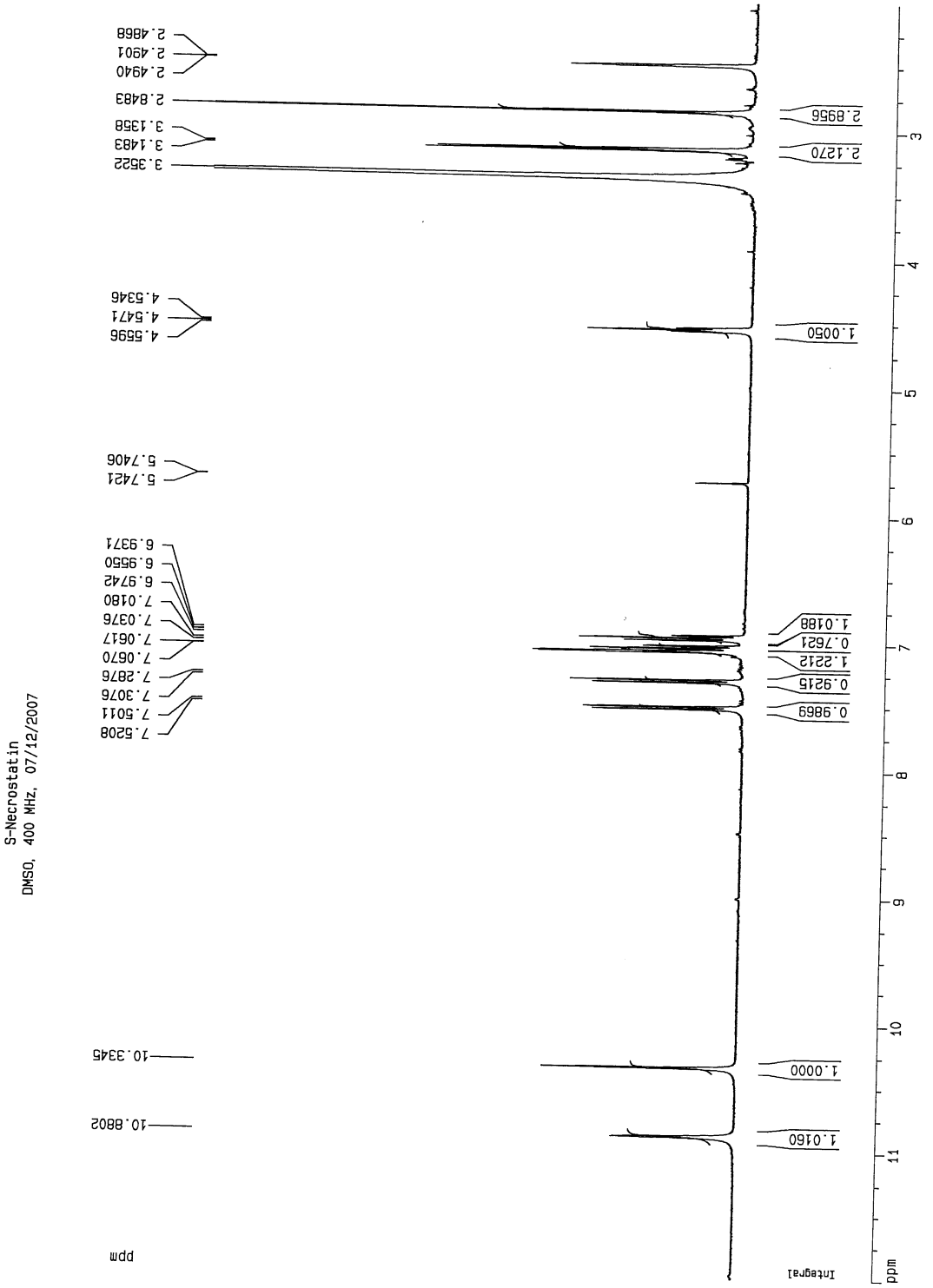


Figure 5.15: compound **25**

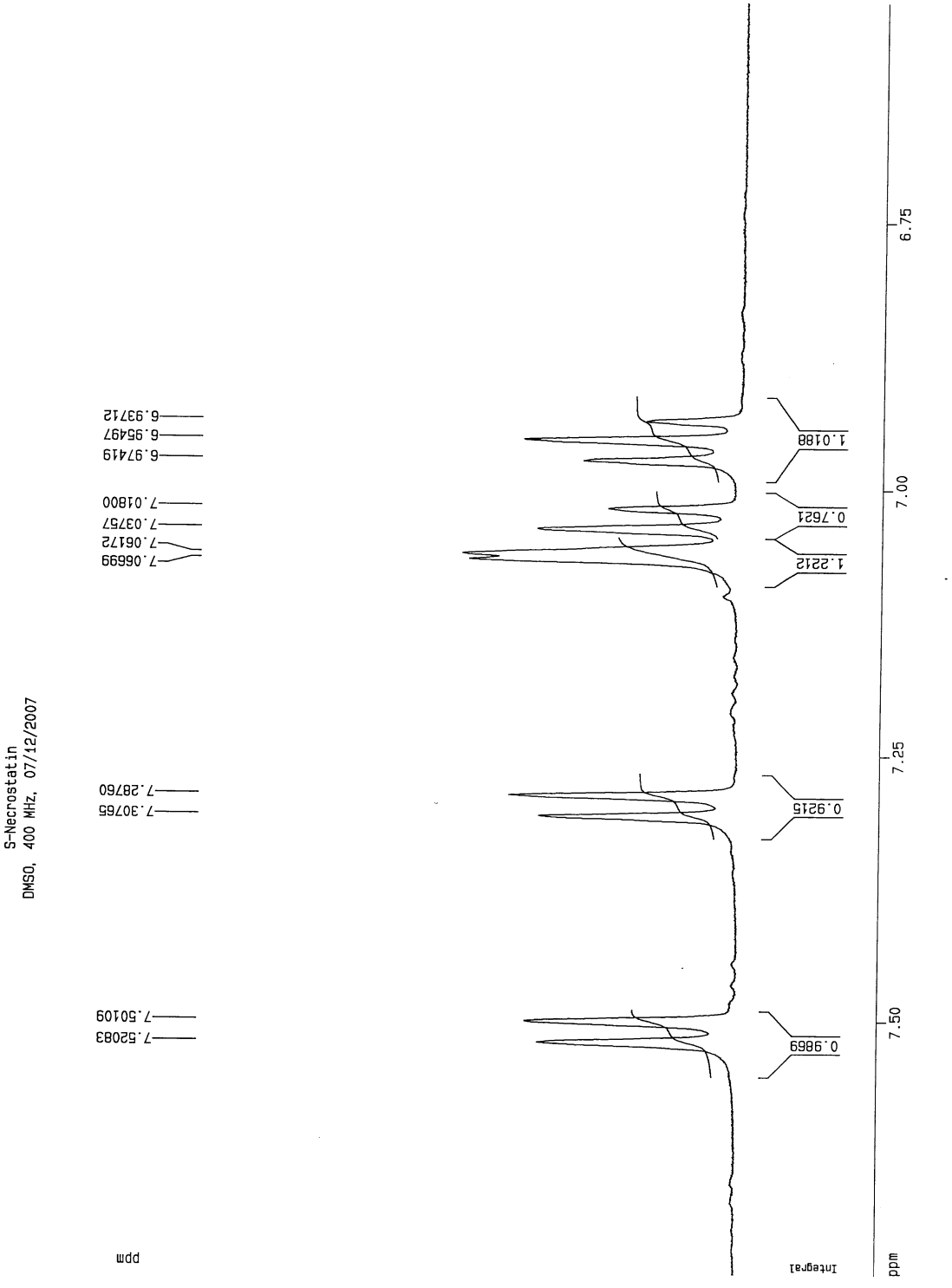


Figure 5.16: compound **25**

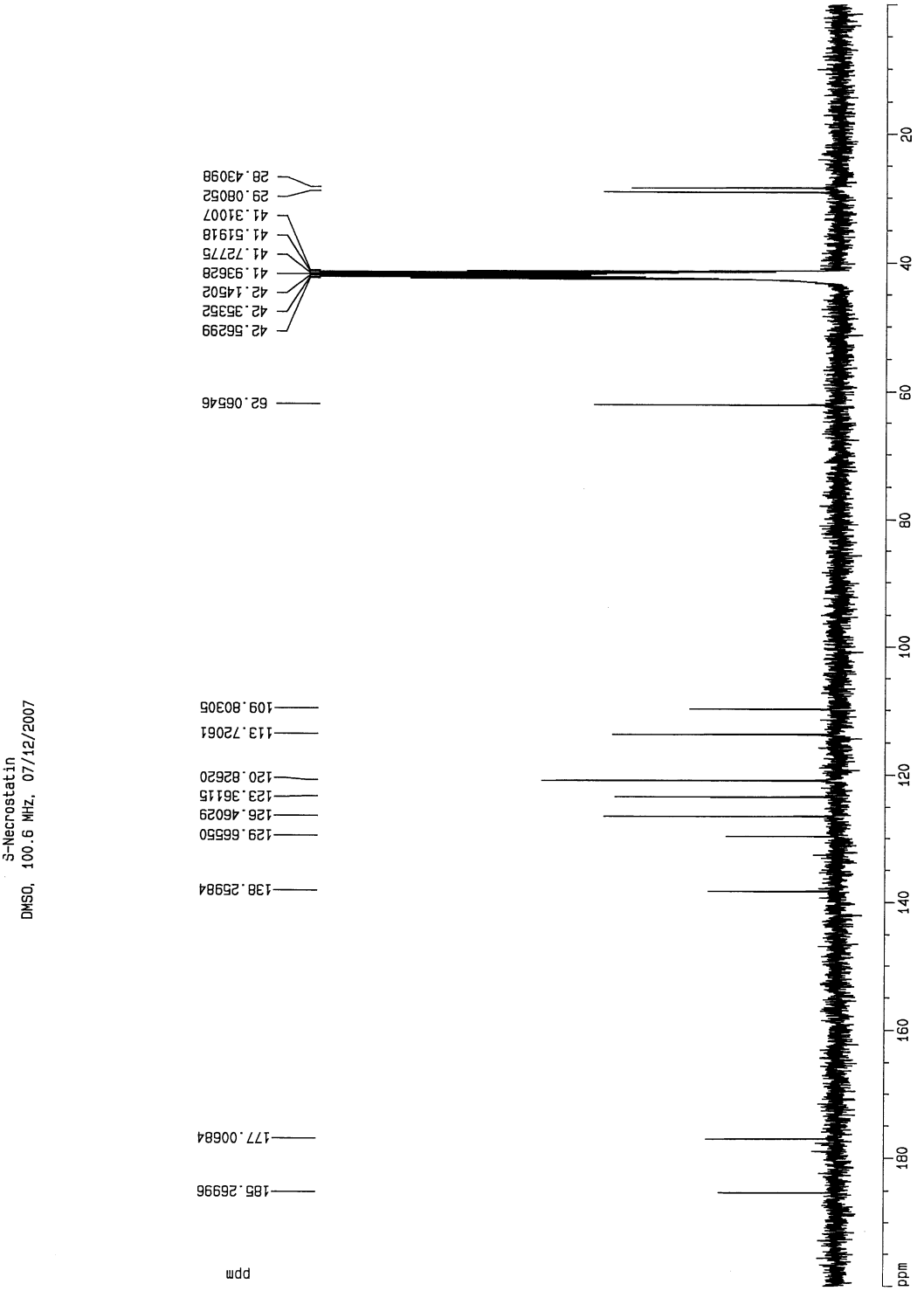


Figure 5.17: compound **25**

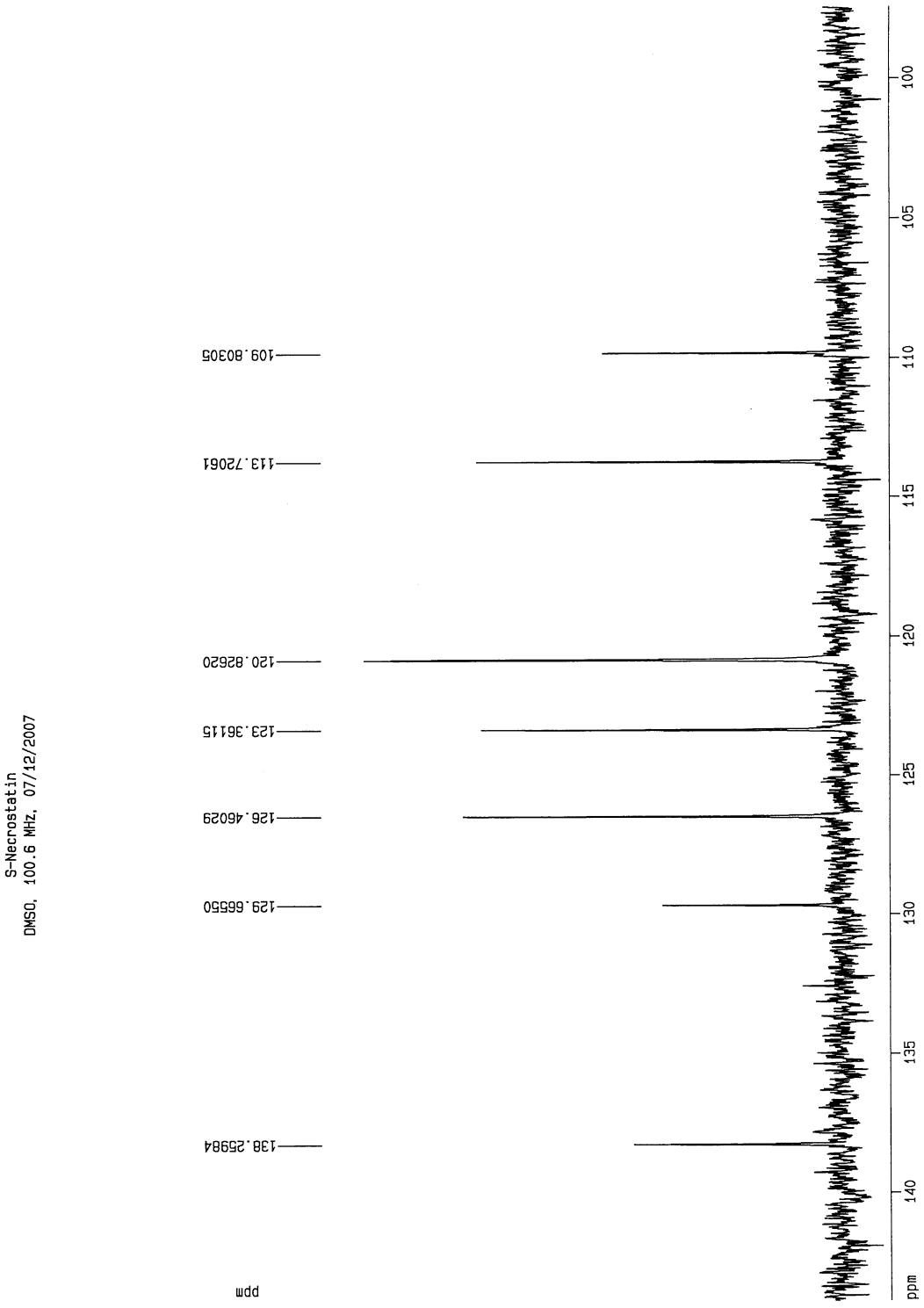


Figure 5.18: *(R)*-5-(1*H*-Indol-3-ylmethyl)-3-methyl-2-thiohydantoin (**26**)

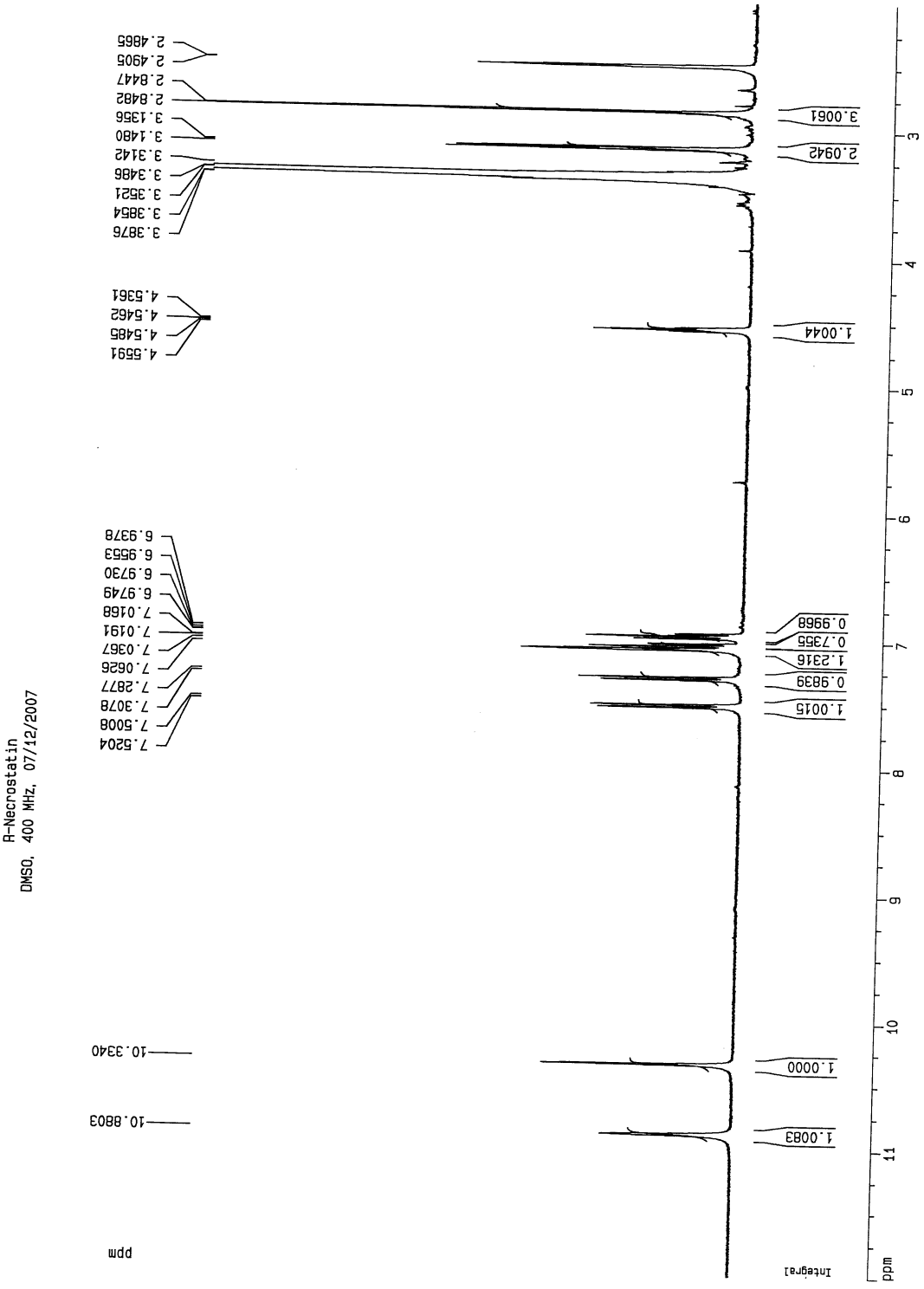


Figure 5.19: compound **26**

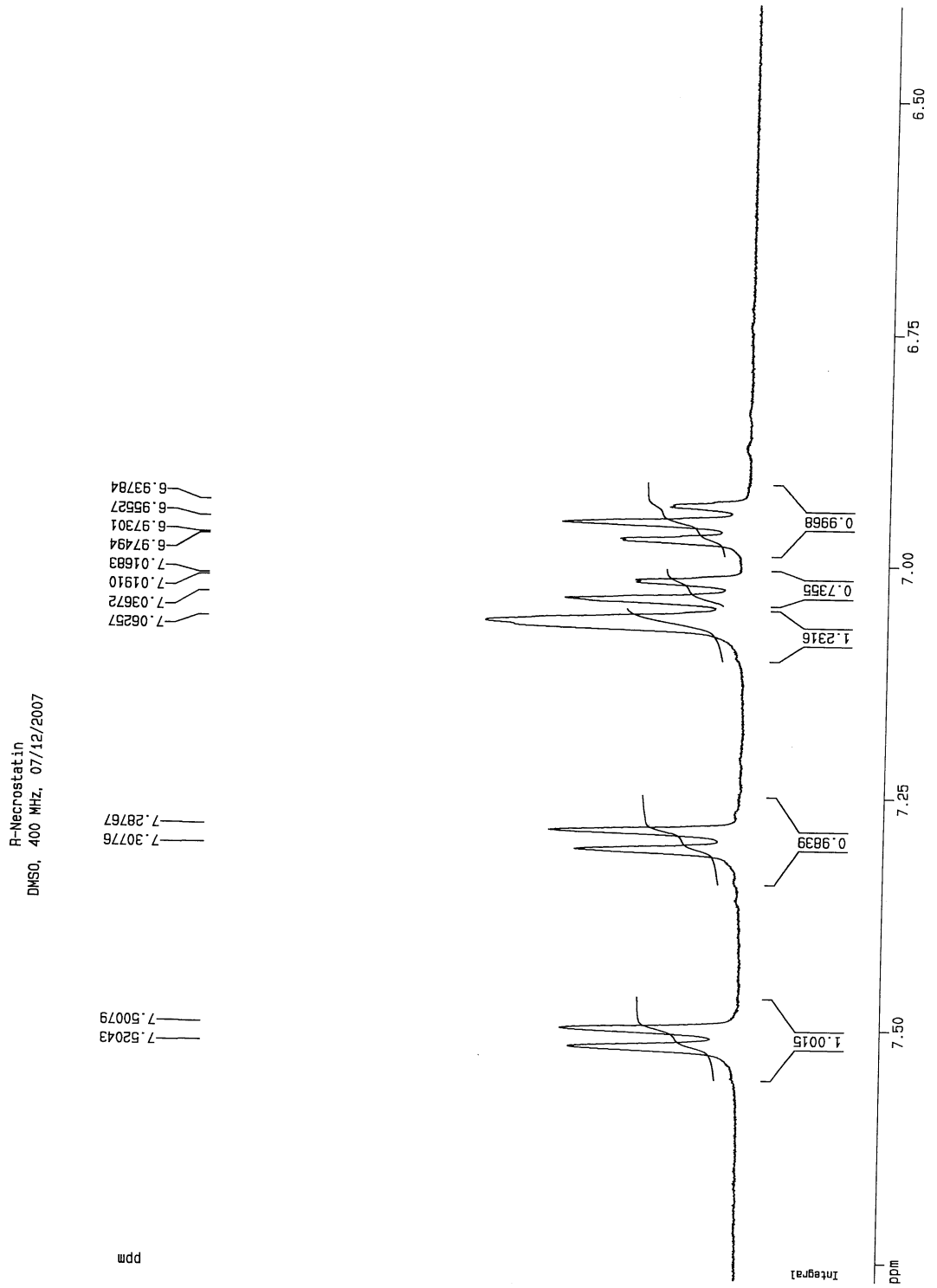


Figure 5.20: 5-(5-Fluoro-1*H*-indol-3-ylmethyl)-3-methyl-2-thiohydantoin (**29**)

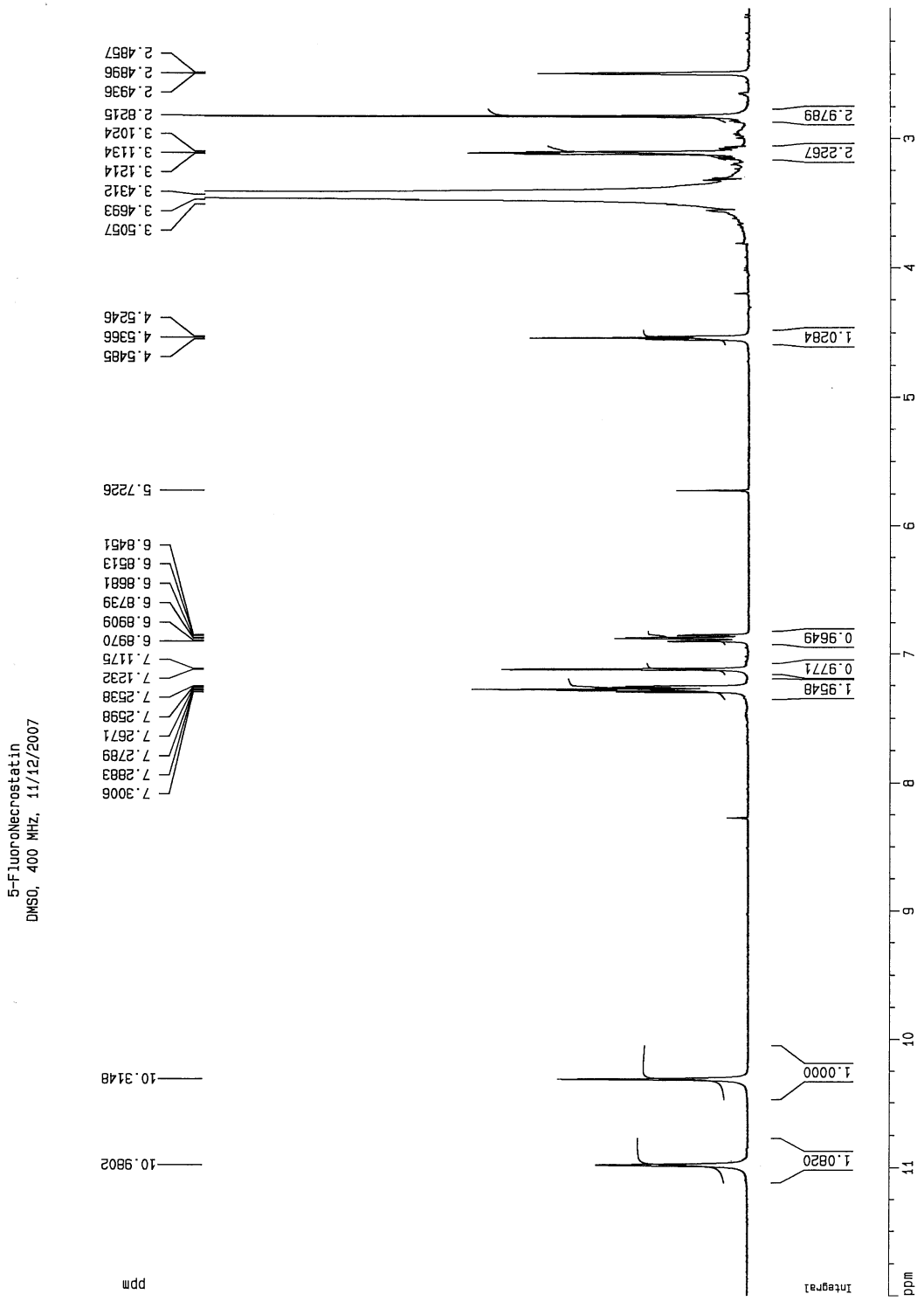


Figure 5.21: compound **29**

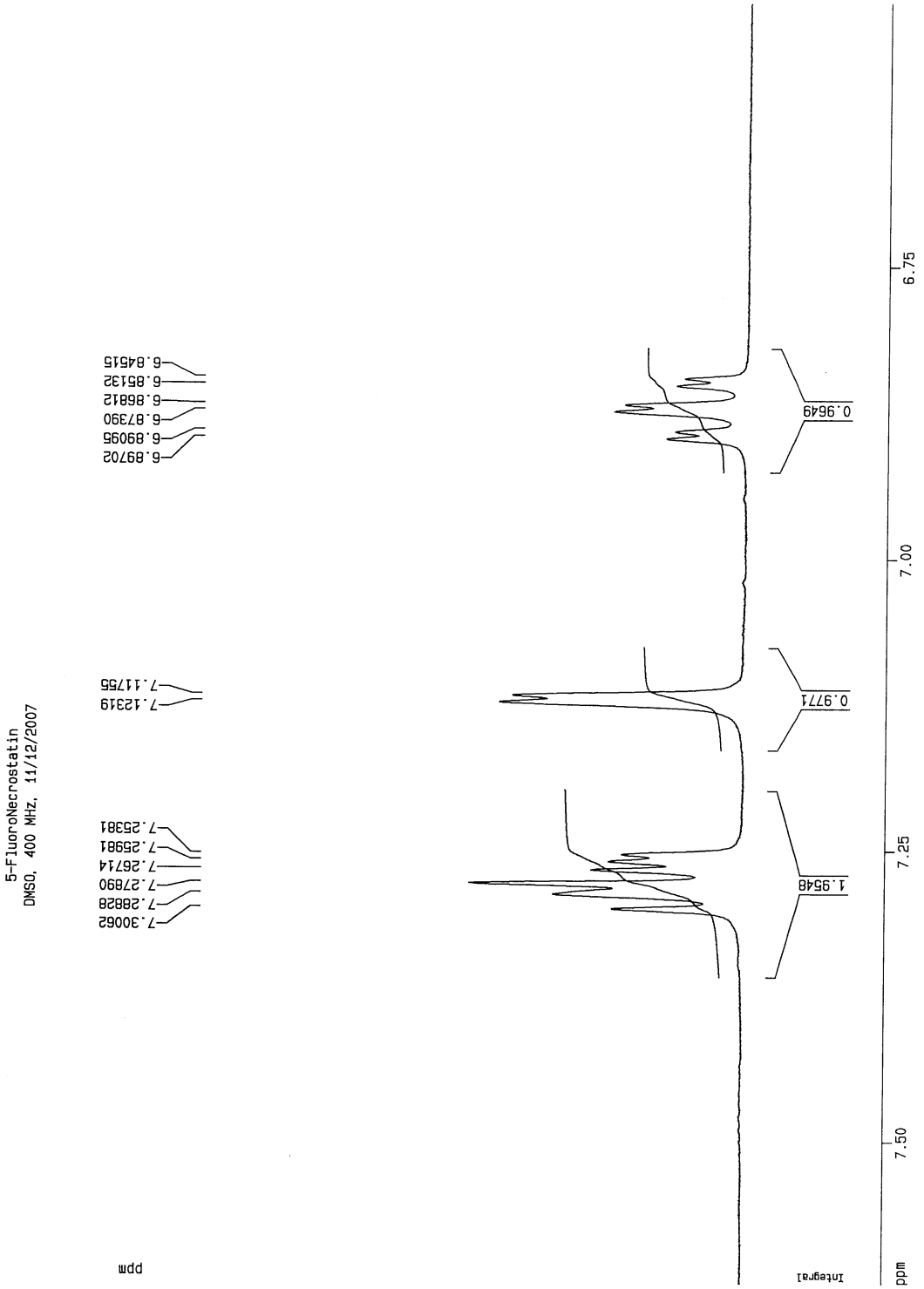


Figure 5.22: compound **29**

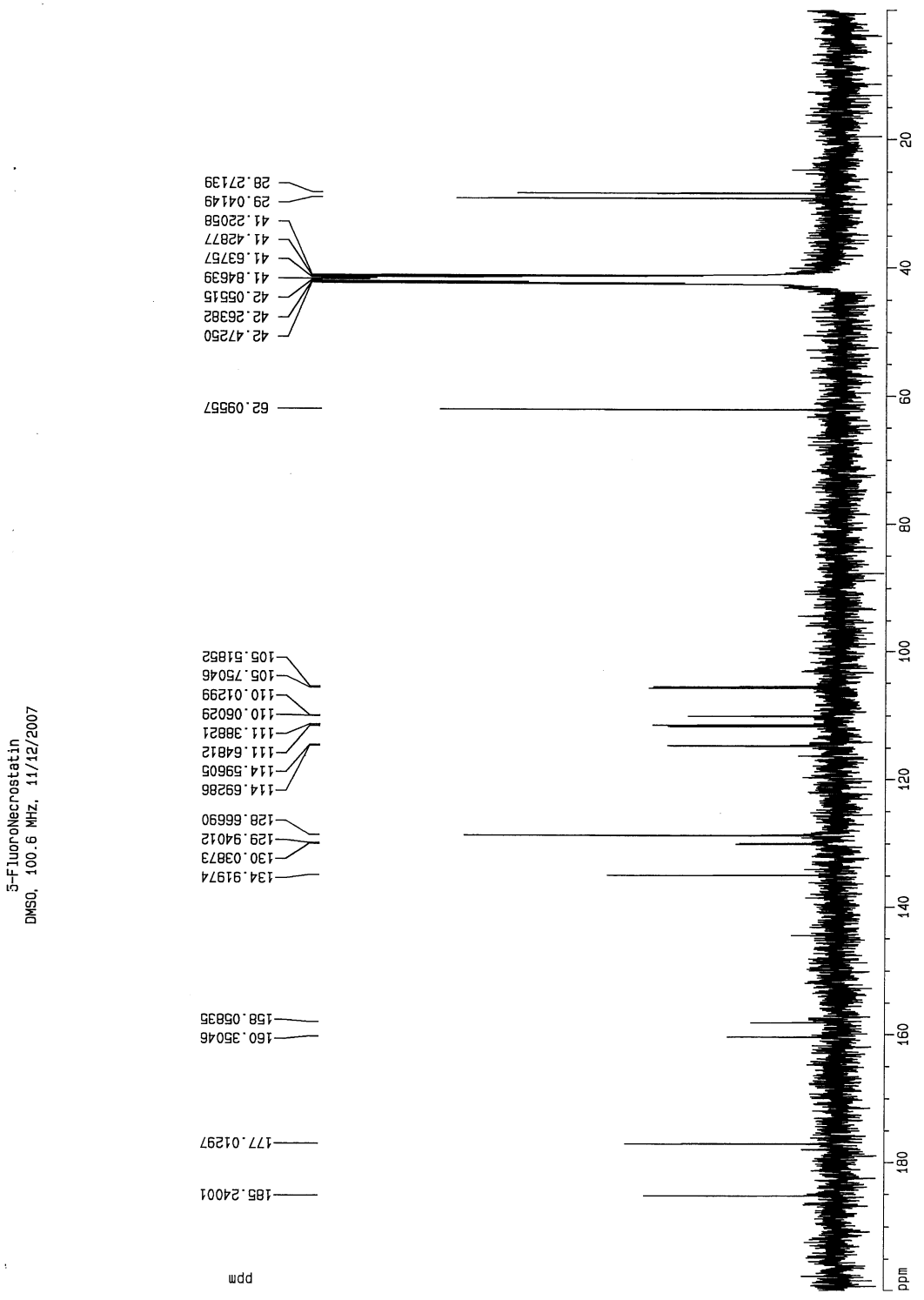


Figure 5.23: compound **29**

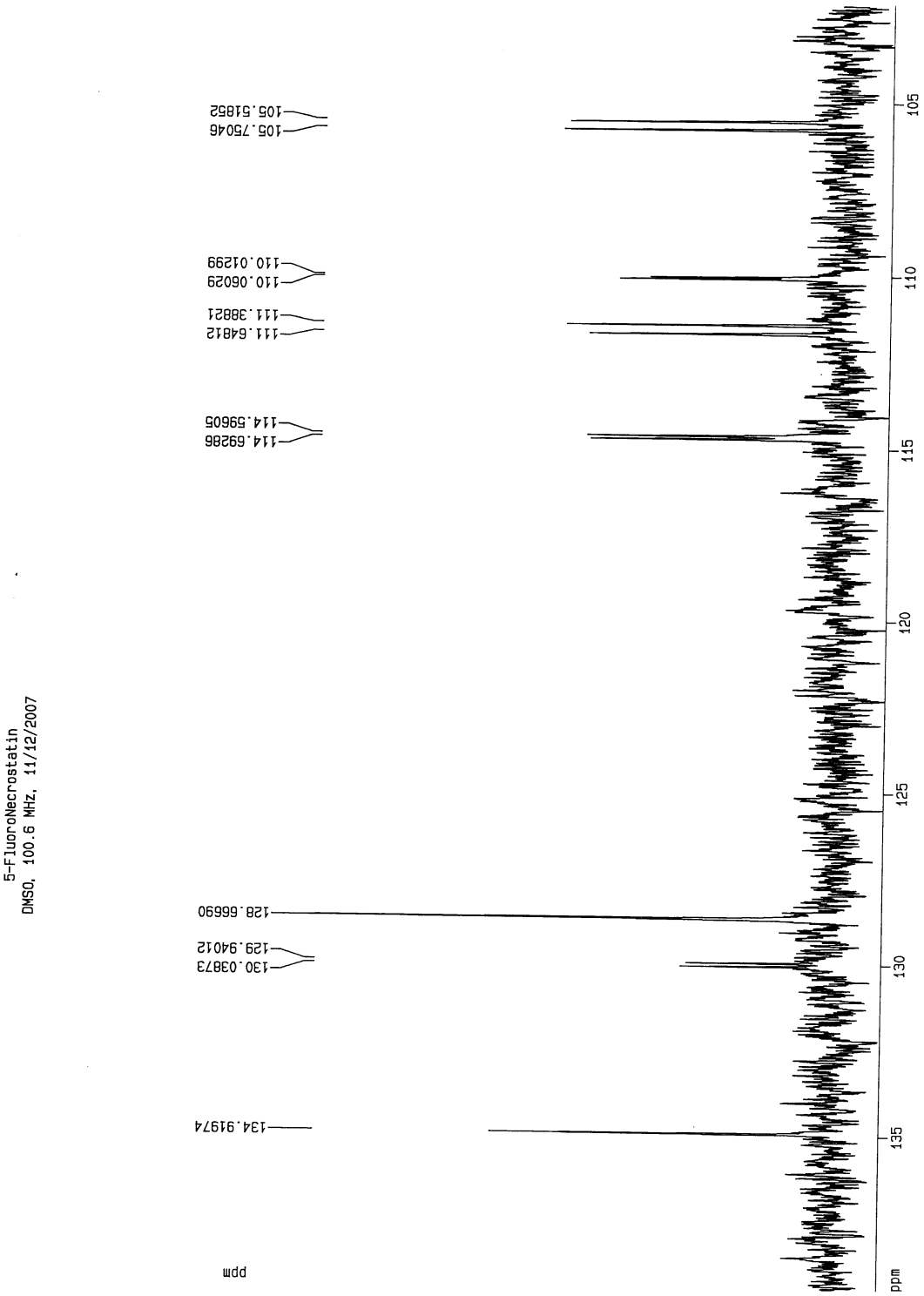


Figure 5.24: 5-(1-*N*-Methyl-1*H*-Indol-3-ylmethyl)-3-methyl-2-thiohydantoin (**32**)

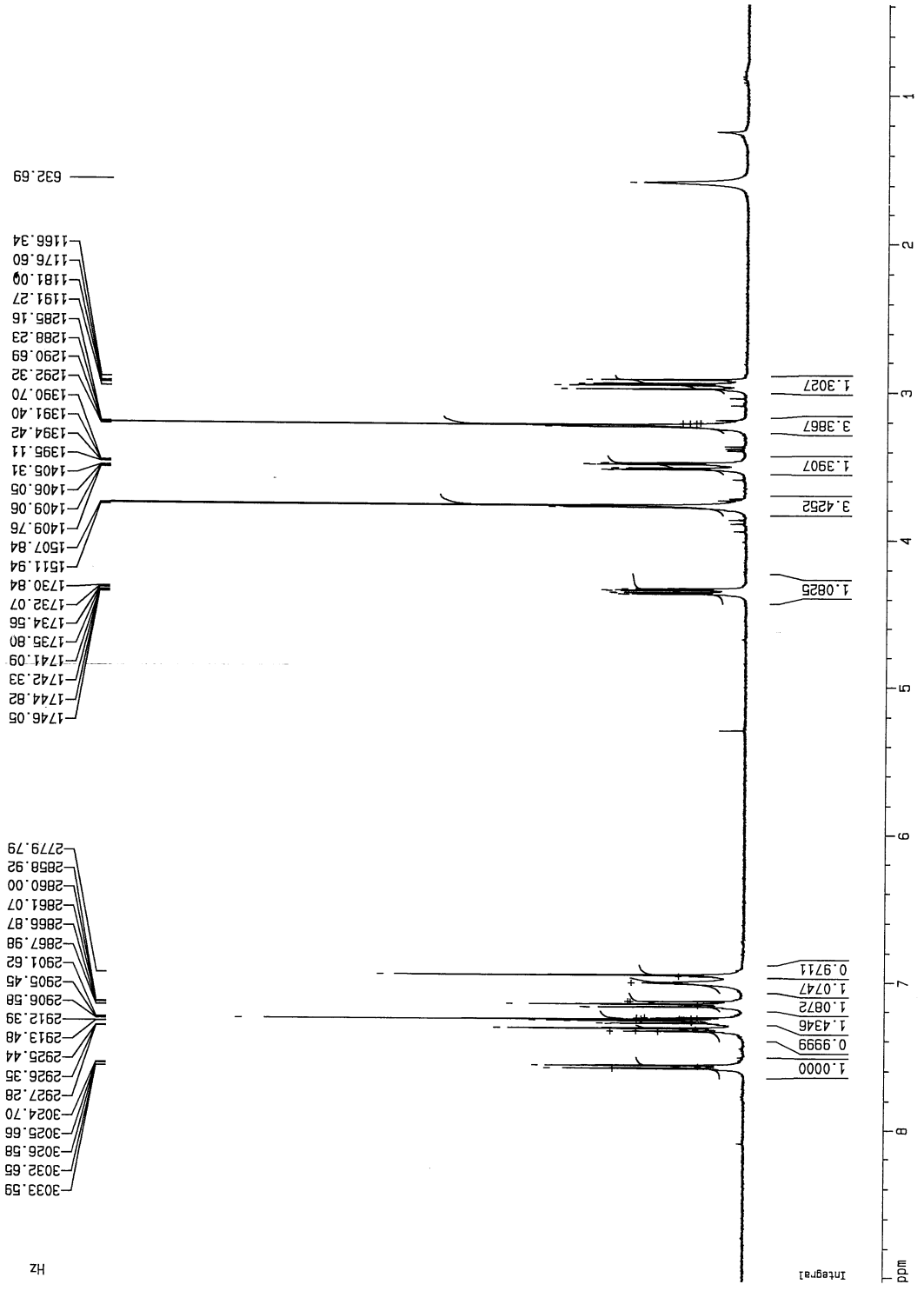


Figure 5.25: compound **32**

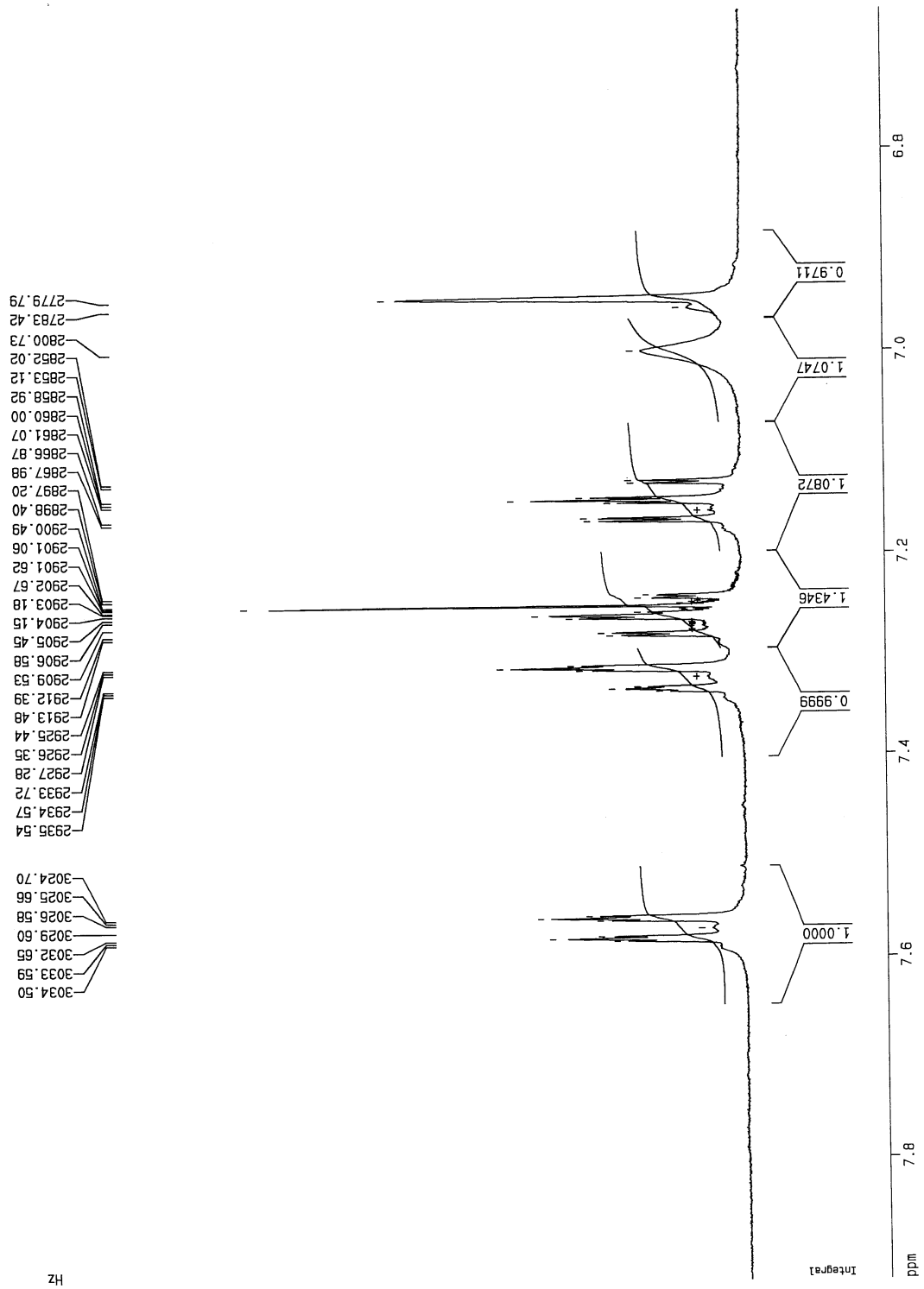


Figure 5.26: compound **32**

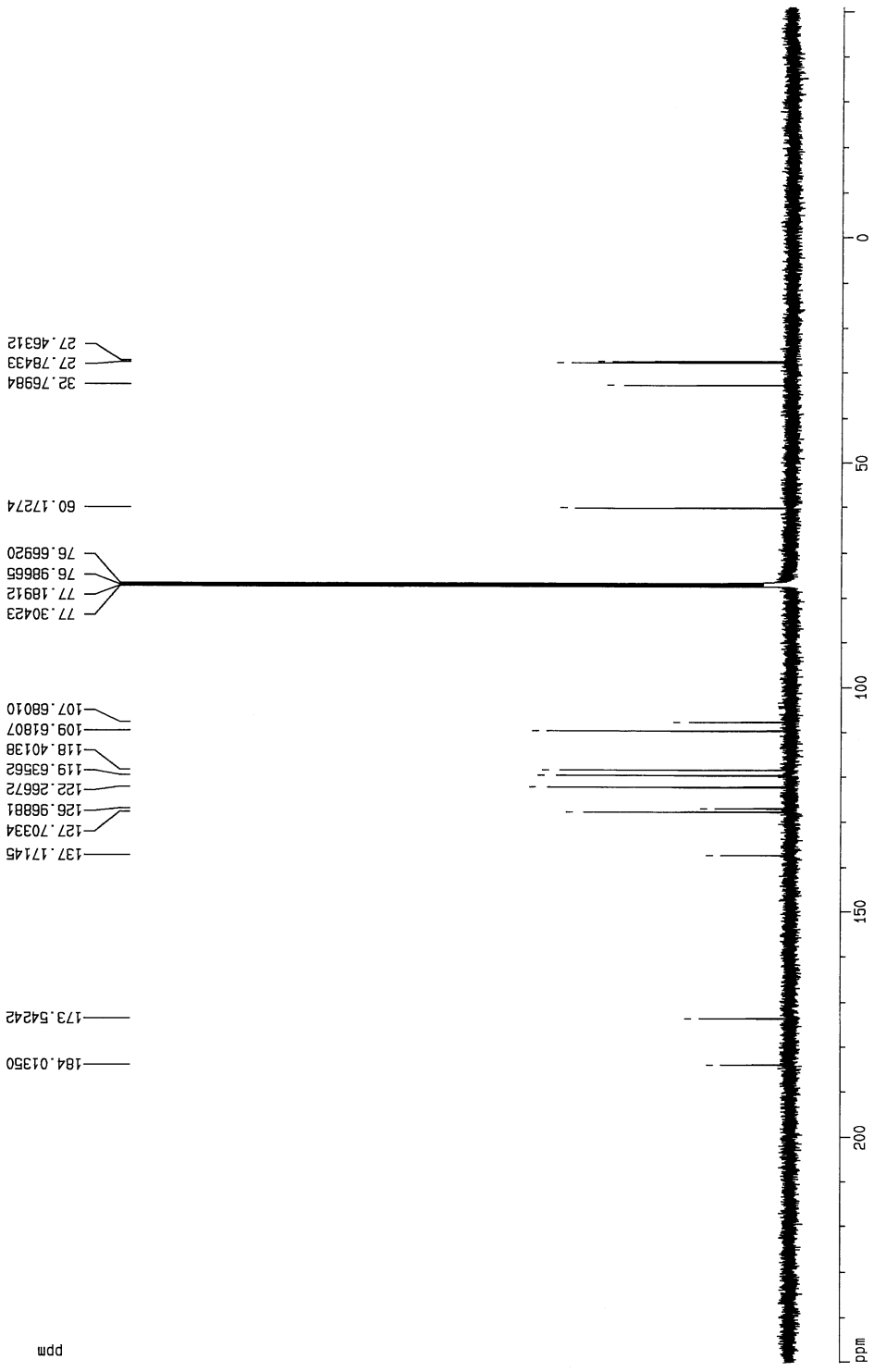


Figure 5.27: compound **32**

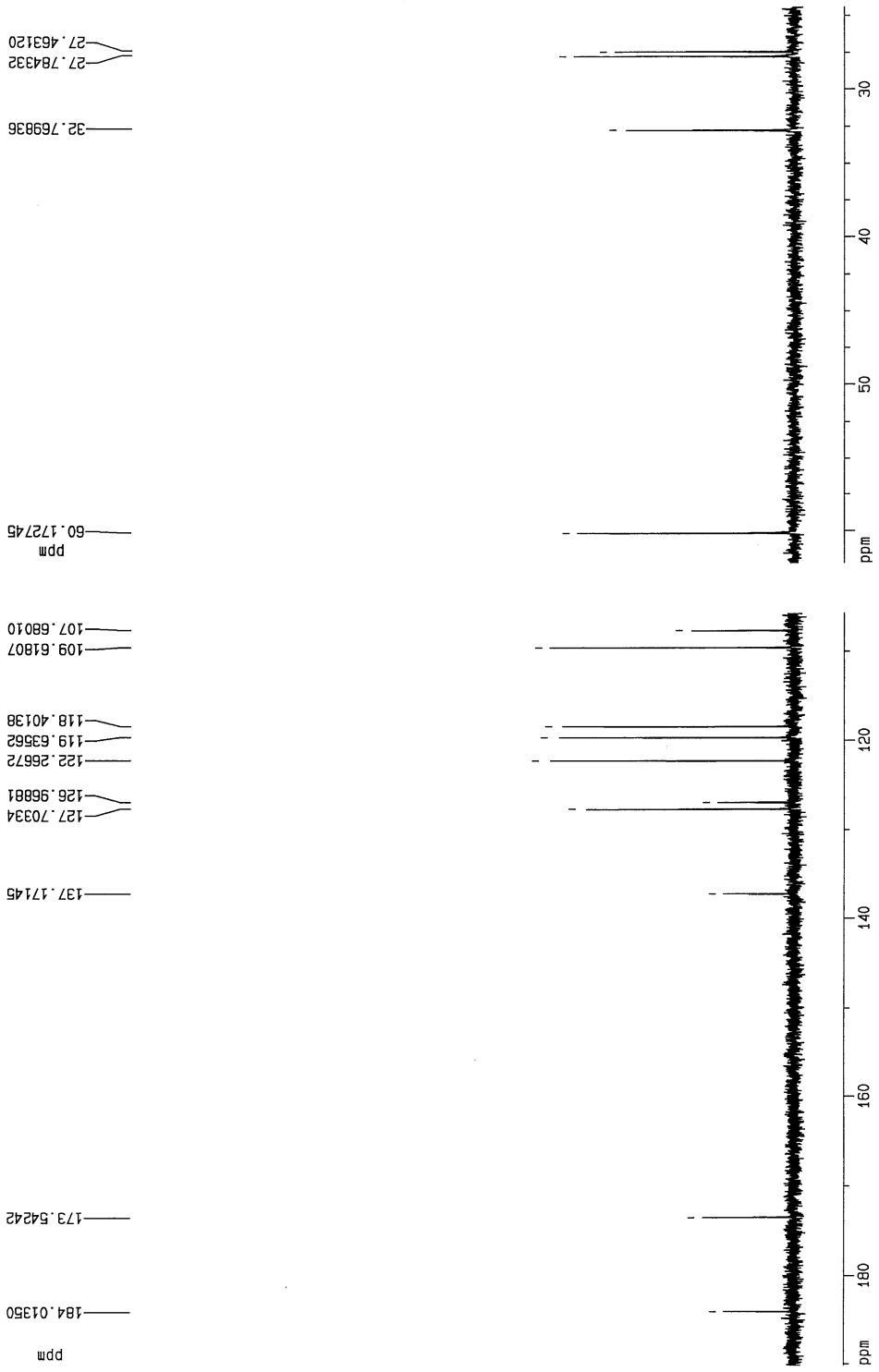


Figure 5.28: Tryptophan methyl amide (**33**)

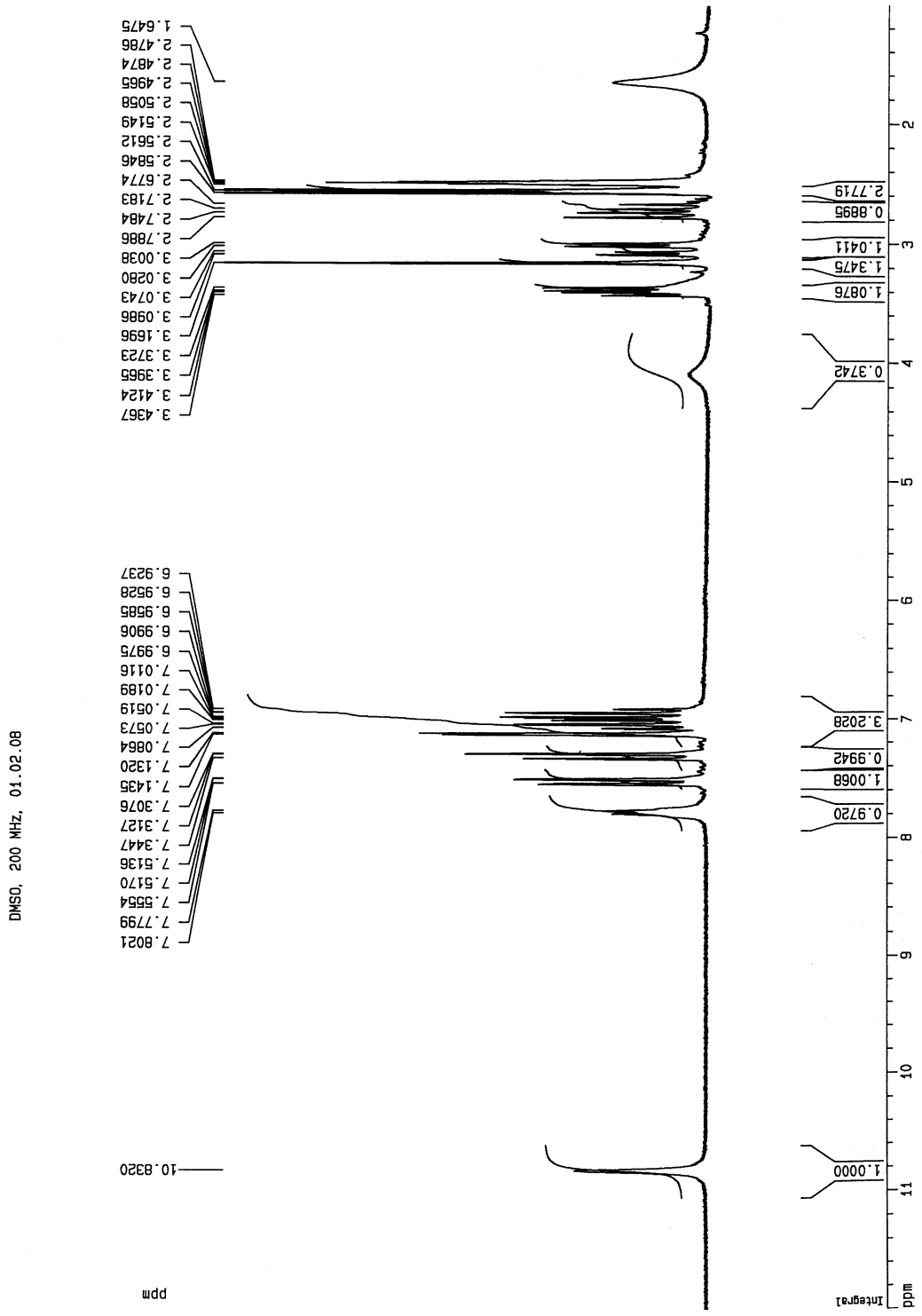


Figure 5.29: compound **33**

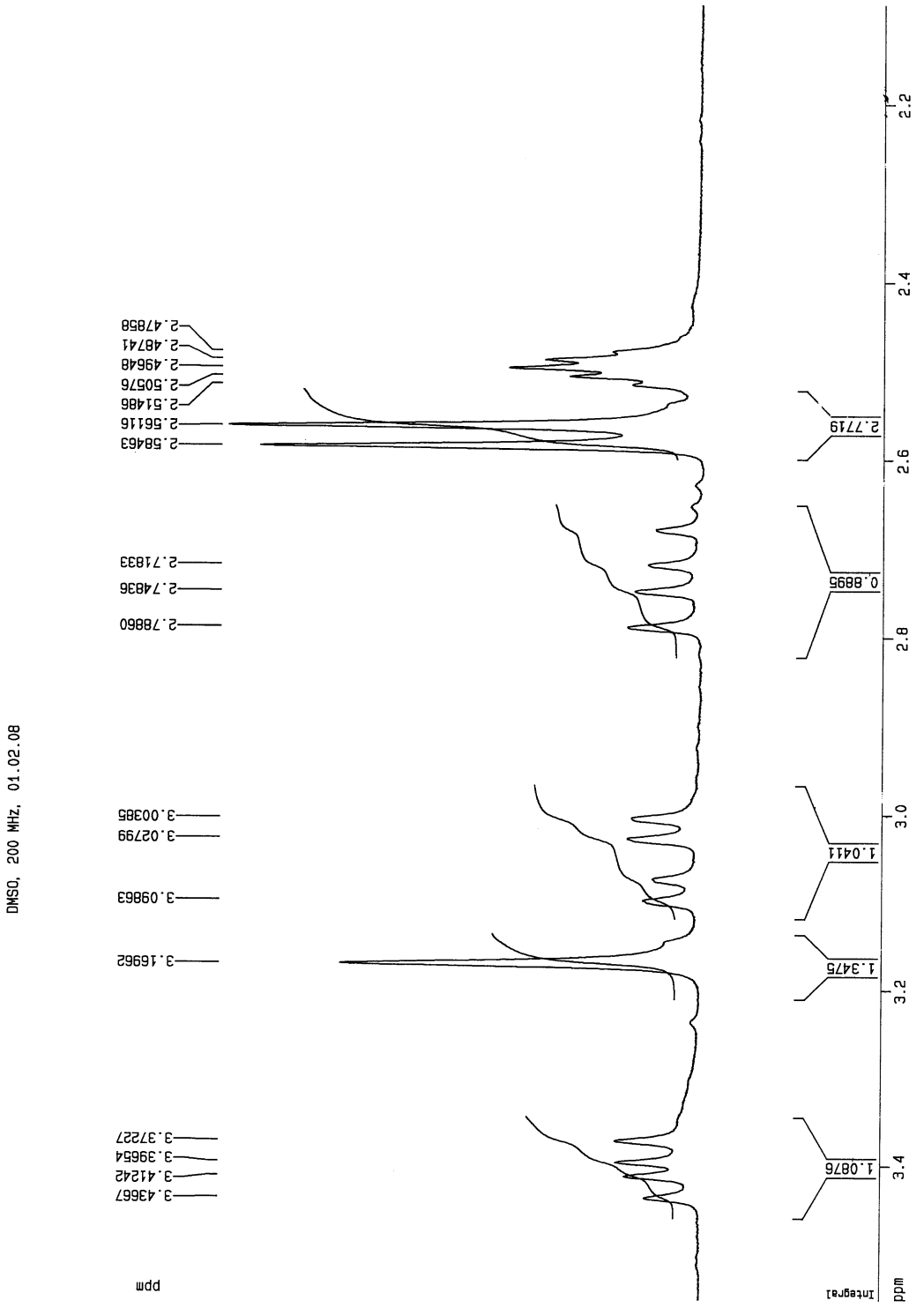


Figure 5.30: compound **33**

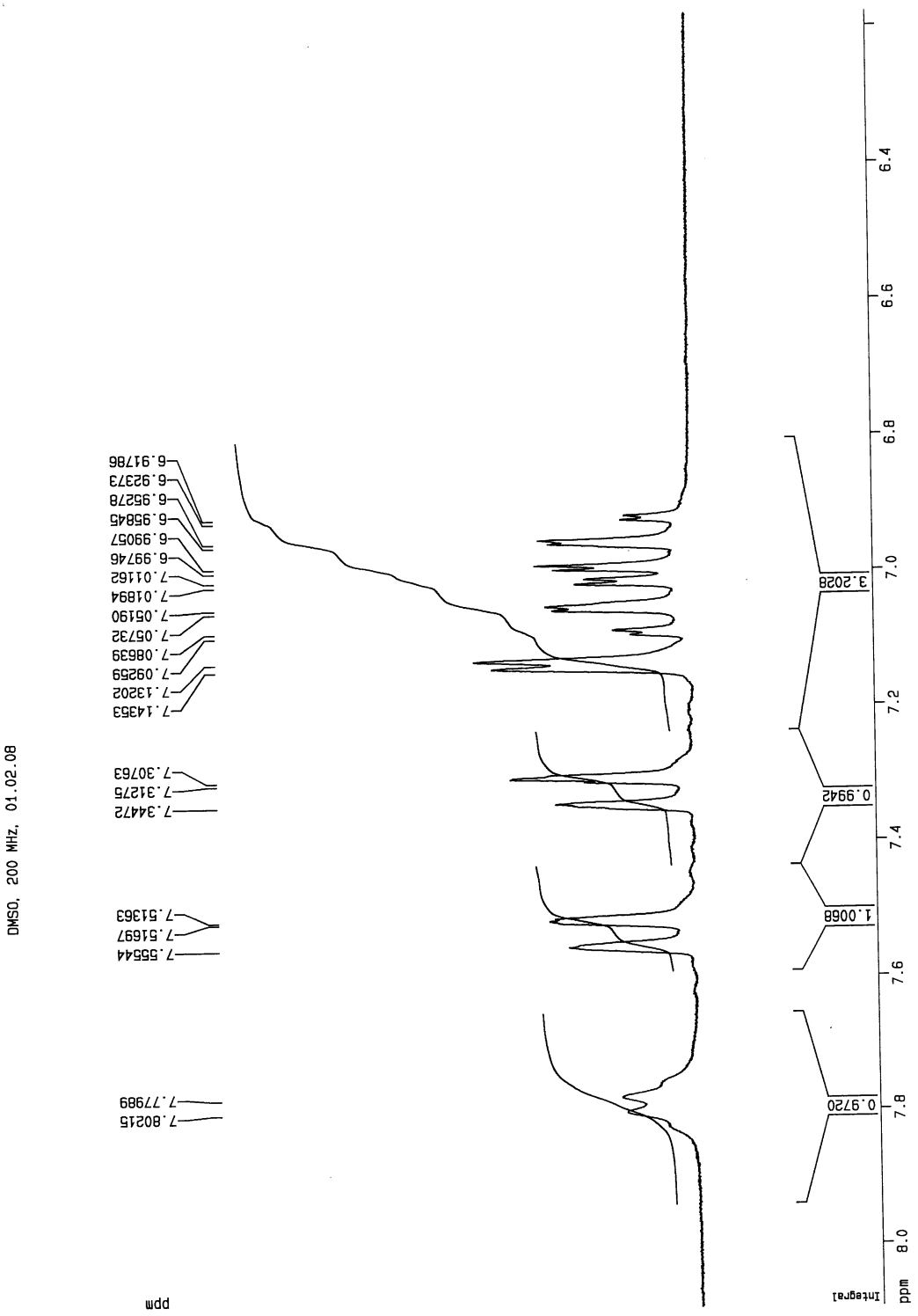


Figure 5.31: 5-(1*H*-Indol-3-ylmethyl)-3-methyl-hydantoin (**34**)

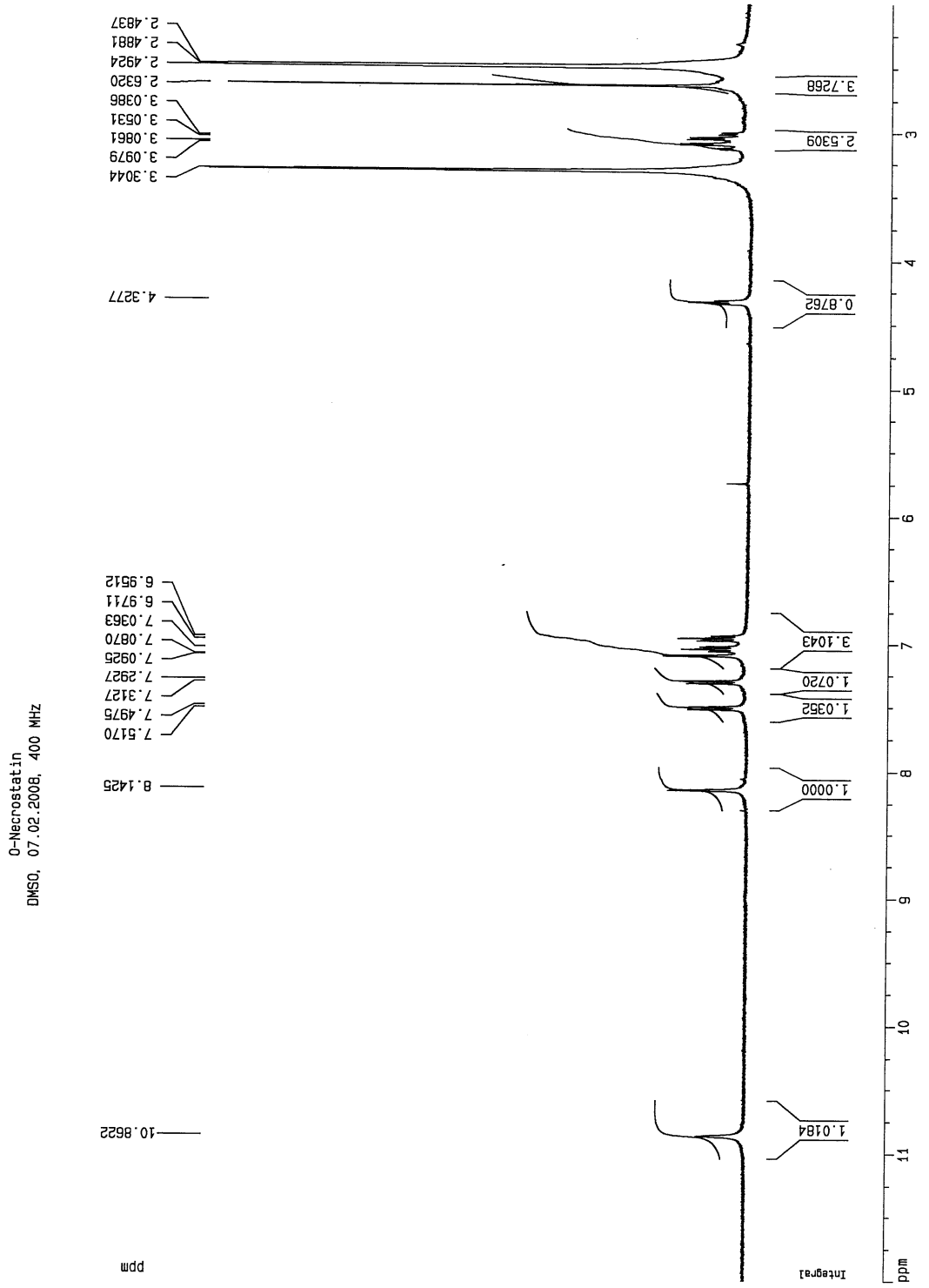


Figure 5.32: compound **34**

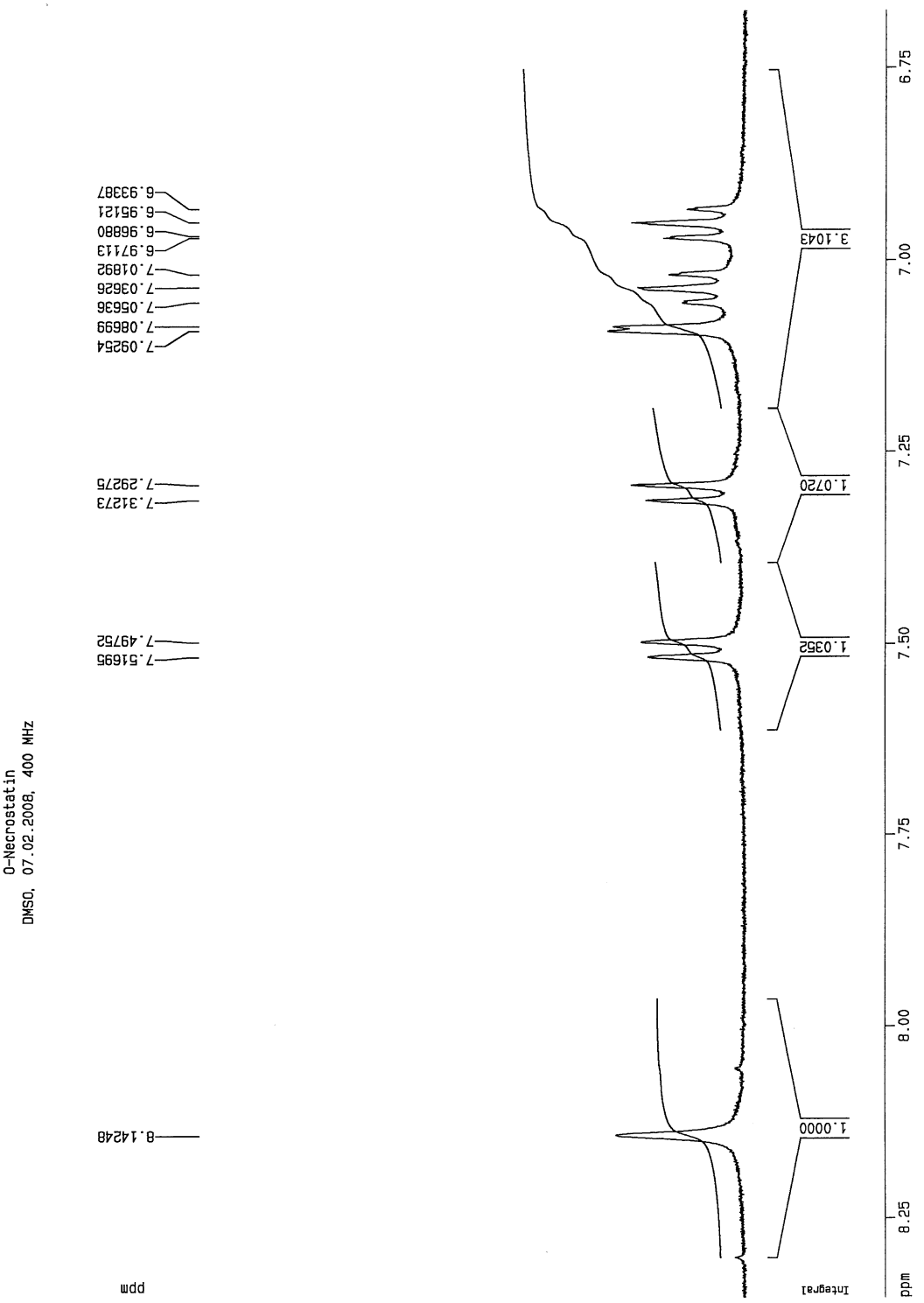


Figure 5.33: compound **34**

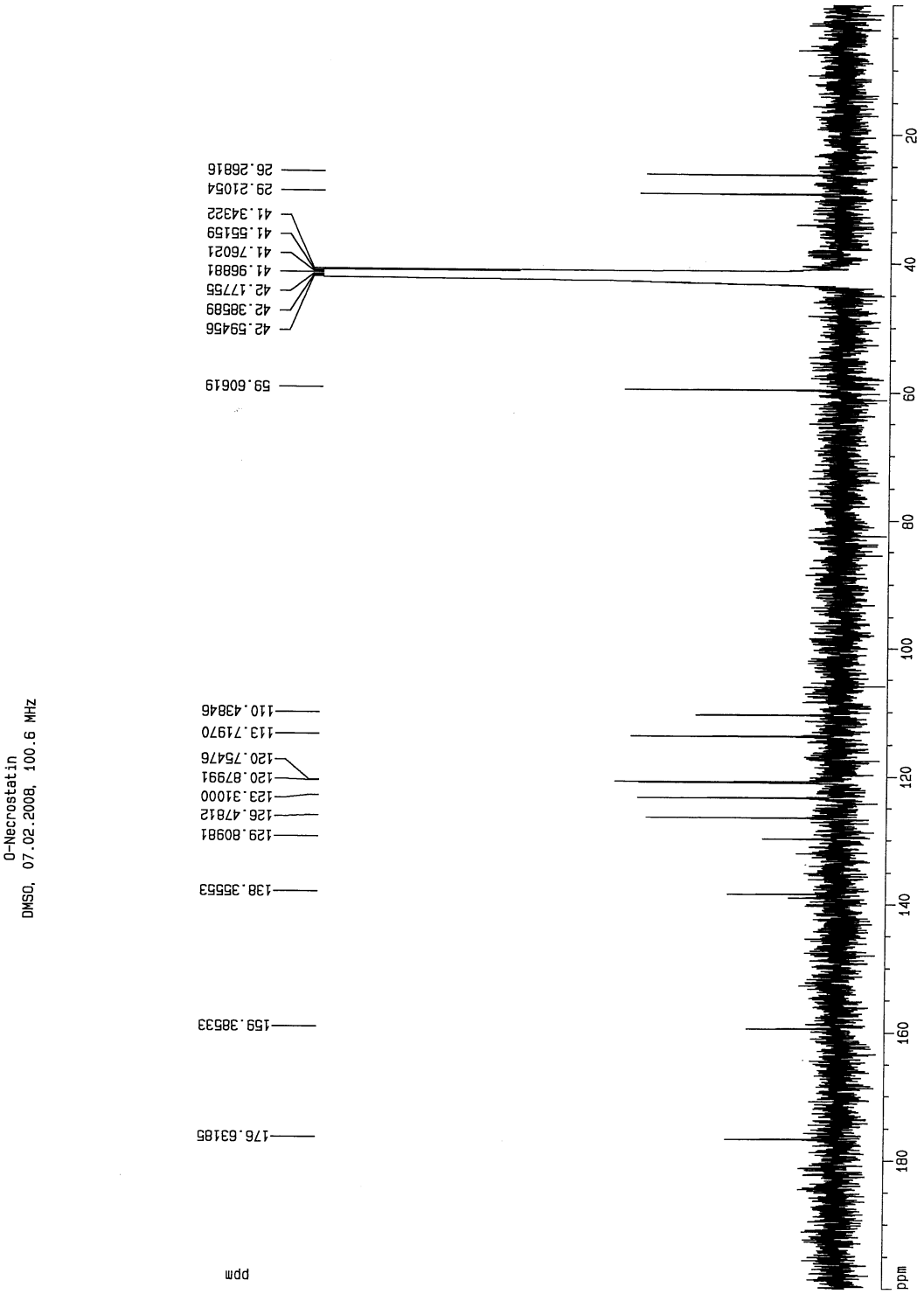
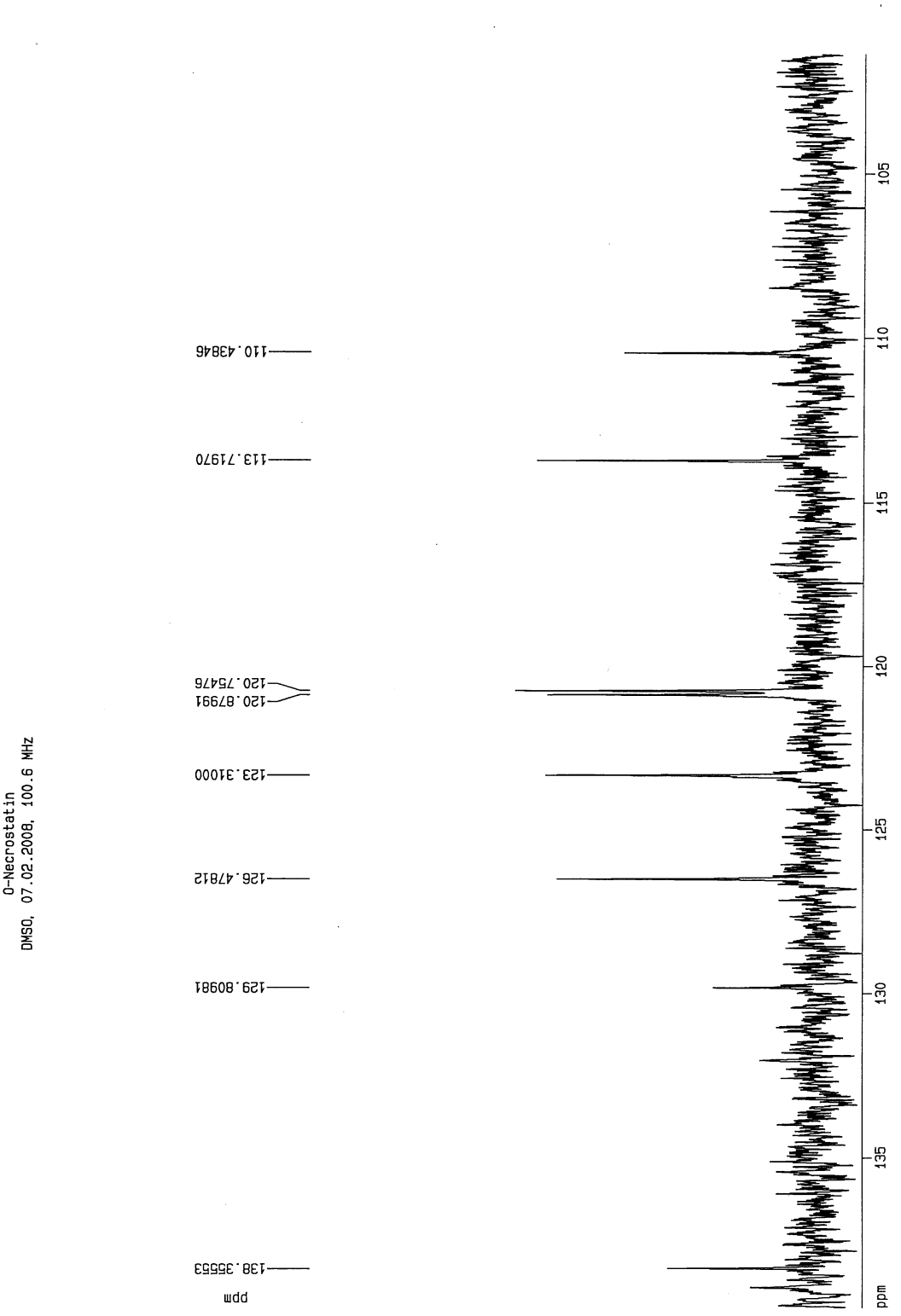


Figure 5.34: compound **34**



B List of abbrevations

1-MT	1-Methyl-tryptophan
5-HT	5-Hydroxy-tryptamine, serotonine
APC	antigen presenting cells
CD_3OD	deuterated methanol
DC	dendritic cells
DCM	dichloromethane
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EtOAc	ethylacetate
EtOH	ethanol
HPLC	high performance liqid chromatography
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon-γ
IL-1	interleukin-1
K_1	inhibitory constante
KP	kynurenine pathway
LPS	lipopolysaccharide
***L*-trp**	*L*-tryptophan
mAU	milli absorbance unit
MeOH	methanol
MTH	Methyl-thiohydantoin-tryptophan
NAD	nicotineamide adeninedinucleotide
PDA	photodiode array (detector)
SAR	structure activity relationship
TDO	tryptophan 2,3-dioxygenase
TLC	thin layer chromatography
TNF	tumour necrosis factor

C Zusammenfassung

Das immunsuppressive Enzym Indoleamine 2,3-dioxygenase, das eine Schlüsselfunktion im Tryptophan- Metabolismus einnimmt, wird seit geraumer Zeit mit Immuntoleranz gegenüber Tumorgewebe in Verbindung gebracht. Es wird in einigen Krebsarten überexprimiert und ist im Stande, durch lokalen Tryptophan-Abbau und die Akkumulierung der dadurch entstandenen cytotoxischen Metaboliten, die Proliferation der T-Zellen zu unterbinden und somit eine Immunantwort zu unterdrücken. Dieser Toleranzzustand verhindert zusätzlich eine erfolgreiche medikamentöse Therapie.
Sowohl natürliche als auch synthetische Substanzen wurden als Hemmstoffe für das neu etablierte Zielmolekül entdeckt und entwickelt. Bisher wurden nur zwei davon direkt am Tumorgewebe getestet: 1-Methyl-tryptophan und Methyl-thiohydantoin-tryptophan. Tatsächlich zeigten beide in Kombination mit Paclitaxel eine signifikante Tumorregression.
Das Ziel der vorliegenden Arbeit war es, neue Derivate von Methyl-thiohydantoin-tryptophan zu synthetisieren und sie sogleich auf ihre Enzym-Wirkung zu untersuchen. Die Syntheseprodukte zeigten jedoch eine geringere Inhibitionsaktivität am isolierten humanen IDO als ihre Muttersubstanz.

D Curriculum Vitae

Susanne Moroder

Geburtsdatum:	07.03.1984
Geburtsort:	Bozen, Südtirol
Nationalität:	Italien

Ausbildung

1995 – 1998	Mittelschule „Adalbert Stifter“ in Bozen
1998 – 2003	Staatliches Realgymnasium Bozen in deutscher Sprache
30.06.2003	Staatliche Reifeprüfung
Seit Okt 03	Studium der Pharmazie an der Universität Wien
Sep 07 – Feb 08	Praktischer Teil der vorliegenden Diplomarbeit am „Dipartimento di Chimica e Tecnologia del Farmaco“, Universitá degli Studi di Perugia, unter der Leitung von Prof. Roberto Pellicciari im Rahmen des ERASMUS- Austauschprogramms
SS 08 – WS 09/10	Tutorin für die Übungen „Arzneistoffanalytik“ am Department für Medizinische/ Pharmazeutische Chemie

Studienrelevante Ferialpraktika

Aug 2004	Praktikum in der Apotheke „S. Quirino“, Bozen
Juli – Aug 2005	Praktikum in der Apotheke „St. Anna“, Bozen
Juli 2006	Praktikum in der „Alpha-Apotheke“, Wien 22
Juli – Aug 2007	Praktikum in der Krankenhausapotheke im Krankenhaus „Franz Tappeiner“, Meran