EFFECT OF ETHER GROUPS ON THE STRUCTURE ACTIVITY RELATIONSHIP

(SAR) OF NOVEL ANTIMICROBIAL COMPOUNDS

By

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DEDICATION

This project is dedicated to my Mommy who never failed to give me moral support, who taught me that even the largest task can be accomplished if it is done one step at a time. I love you dearly and will forever hold you close to my heart.

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ABSTRACT

Bacterial resistance to antibiotic treatment has become a major problem over the years originating from penicillin binding proteins, efflux pumps and the major contributor, β-lactamases. Therefore, a need arises for developing new antimicrobial drugs, with a different mechanism of action, in-order to delay further bacterial resistance. Examples of such compounds prepared in our research group comprise of monocyclic β-lactams with distinct substituent groups attached to an aromatic thiol, at the C4 of the β-lactam. These compounds, especially fluorinated thiophenols in the *ortho* position with the addition of benzyl isocyanate on the lactam nitrogen demonstrate activity against lipo-oliogosaccharide-containing bacteria, specifically *Mycobacterium tuberculosis* (Mtb). The focus of my research is to create a library of derivatives of these lactams with methoxy groups attached at various positions of the aromatic ring at C4 in-order to evaluate their activity against the aforementioned bacteria. SAR of these ether-containing-lactams against Mtb will be discussed.

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I thank God for making this phase in my life possible and for His continuous favor and blessings upon my life. It would not have been possible without the hand of God over my life. My parents deserve special mention for their inseparable support and prayers in sincerely raising me with their caring and gently love. To my three loving siblings, Jane, Benjie and Nyakeh, for being supportive and caring. Words fail me to express my appreciation to Alhassan Kabia. His love and persistent confidence in me, has taken the load off my shoulder. This degree would not have been easy without his genuine support and sacrifice. Furthermore, to the Kabia, Mooring and Jones families with their thoughtful support, thank you. I would like to acknowledge the advice and guidance of Dr. Monika Konaklieva, my thesis advisor. You are my inspiration and I wholeheartedly thank you for your patience and kindness through the program. You sincerely devoted your time and service to ensure my success, boosted my self esteem and taught me to be more responsible. I thank you for the love shown that made me feel more than just a student but a part of a family, your family. To Tim Beck and Dina Lloyd, thank you for your guidance. Many thanks to Dr Abigail Miller and Dr Hartings, for their constructive comments on this thesis in the midst of all their activity. I wish to thank the Department of Chemistry at American University, the Tuberculosis Research Section, LCID, NIAID, NIH in MD and the Naval Research Laboratory, Code 6030, in Washington, DC for their financial and logistical support of this research.

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CHAPTER 1

INTRODUCTION

The discovery of penicillin against the growth of infectious bacteria by Alexander Fleming was the gateway to the innovation of β-lactam antibiotics. [14, 6] A vast number of different β-lactams have been developed, which share a common core structure, the β-lactam ring. These include penicillins, cephalosporins, monobactams, carbapenems and penems (Fig. 1). [16]

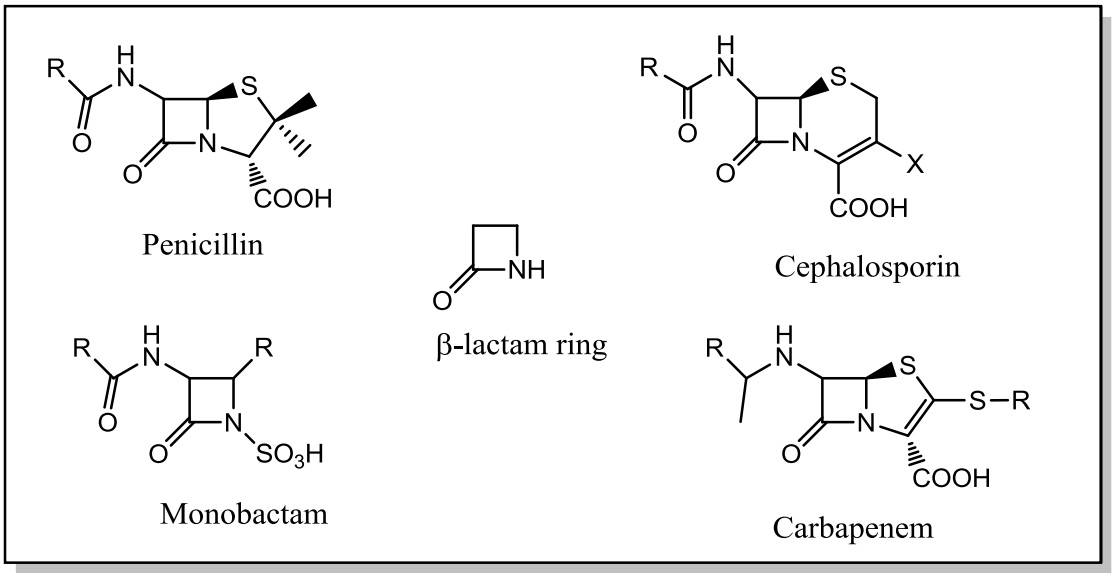


Figure 1: Existing lactam antibiotics.

The strain in the β-lactam four-membered ring increases the electrophilicity of the lactam carbonyl thereby increasing the amide's reactivity toward hydrolysis. The β-lactams shown in Fig.1 have antibacterial activities and presently remain one of the most widely

used set of antibiotics because of their effectiveness, low cost, ease of delivery and limited side effects.

β-Lactam antibiotics inactivate the enzyme, peptidoglycan D,D-transpeptidase, essential for bacterial growth. This enzyme catalyzes the cross-linking of peptidoglycan, which is necessary for the formation of the bacterial cell wall. Peptidoglycan is a branched polymer of alternating β-D-N-acetylglucosamine (NAG) and β-D-N-acetylmuramic acid (NAM) residues.[11] The terminal D-alanyl-D-alanine of the NAM side chain binds to the D,D-transpeptidase, which acts as a serine protease to make a serine ester. Cross-linking this ester to another peptidoglygan strand assembles the bacterial cell wall. β-Lactams mimic the D-alanyl-D-alanine side of the peptidoglycan, acting as suicide substrates of the D,D-transpeptidase.[1, 11]

β-lactam antibiotics have been very beneficial in treating infectious diseases over the years. However, the emergence of bacterial resistance has become a major problem with antibiotic treatment. [6, 13, 17] The mechanisms of resistance of the β-lactams originate from altered penicillin binding proteins, efflux pumps and β-lactamases.[16] β-lactam antibiotics' target are the penicillin binding proteins (PBP) and bind to PBPs due to the similarity in their chemical structure to the extremities that form peptidoglycan.[3, 9] When they irreversibly bind to penicillin, the β-lactam amide bond is hydrolyzed to form a covalent bond with the serine residue at the PBPs active site. Modification and alteration of PBP's by β-lactam resistant cell wall transpeptidation causes resistance as there becomes a decrease in the binding affinities for β-lactam antibiotics to execute their

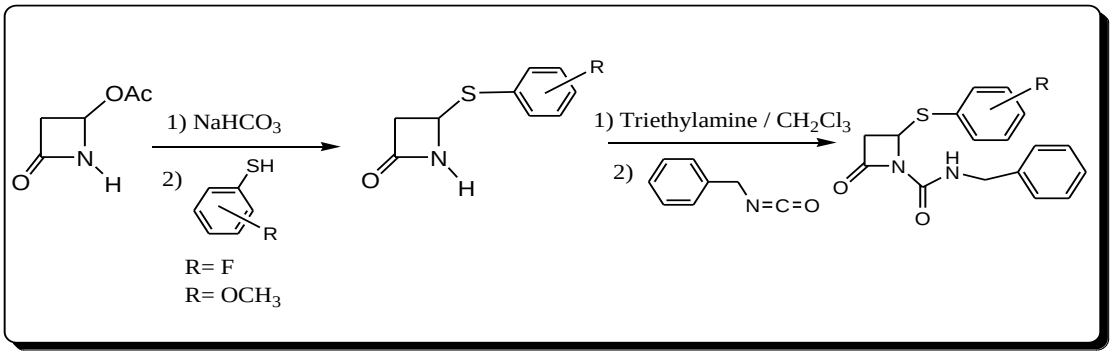
bacteriostatic effect.[1, 16] Recently, evidence suggests that the L,D transpeptidases are a possible target of resistance to the lactam antibiotics.[5, 8, 11]

Overcoming PBP-mediated resistance remains a challenge; therefore there is a need to find inhibitors of altered PBP's.[16] Efflux mechanisms have also been recognized as a means of resistance to β-lactams. Efflux pumps are transport proteins that eject toxic substrates, including antibiotics, from within the cell. Bacteria contain up-regulating pumps that emit antibiotics from the cell, thereby decreasing the drug's access to the bacterial target.[2, 16] The third mechanism, a major cause for resistance, is the production of β-lactamases. This is by far the main type of resistance towards β-lactams.[4, 5, 7, 16] β-Lactamases hydrolize the β-lactam ring, before it reaches its target – the PBPs. One strategy to avoid degradation from β-lactamases is to synthesize β-lactams to overcome resistance from being destroyed by β-lactamases. The latter has no antibiotic activity but is used to protect β-lactams from being destroyed prior to reaching the appropriate target site and killing the bacteria.[4] A combination of β-lactam antibiotics with a β-lactamase inhibitor is currently a temporary solution to resistance against β-lactamases. An example of a current drug synergy is combining meropenem, a β-lactam, with clavulanate, a β-lactamase inhibitor, in treating *Mycobacterium tuberculosis* (Mtb, the causative agent of tuberculosis).[4,10] Minimum inhibitory concentration (MIC) results show potent activity against laboratory strains of Mtb to be less than 1 microgram per milliliter.[4] Monocyclic β-lactam antibiotics (fig. 1) are also potent in inhibiting bacteria. For example, aztreonam, a monocyclic β-lactam is currently used in clinics against gram-negative bacteria.[16]

Extensive research has been done to determine the potential efficacy of monocyclic β-lactams as antimicrobial therapy. In addition, recent discoveries have shown other biological properties of these compounds apart from their antibacterial action.[1, 7, 12] These specific β-lactams are used as inhibitors of a range of enzymes containing a catalytic serine residue[12] including serine proteases [7, 12], such as human leukocyte elastase (HLE), an enzyme involved in the degradation of structural proteins such as collagen and elastin[7, 12] and as acyl-CoA cholesterol acyltransferase inhibitors and inhibitors of human cytomegalovirus protease.[1] There is a need for new antimicrobials to combat disease and restore drug activity in spite of resistant microorganisms.

Objective

Research in our group has shown that fluorinated thiophenols in the *ortho* position of the aromatic ring at the C4 of the lactam and having a carbamyl group at the lactam nitrogen (N1) as shown in Scheme 1 demonstrate a promising activity against lipo-oligosaccharide-containing bacteria, specifically *Moraxella catharralis* (M.cat) and *Mycobacterium tuberculosis* (Mtb).



Scheme 1: Synthesis of fluorinated and methoxylated β-lactams

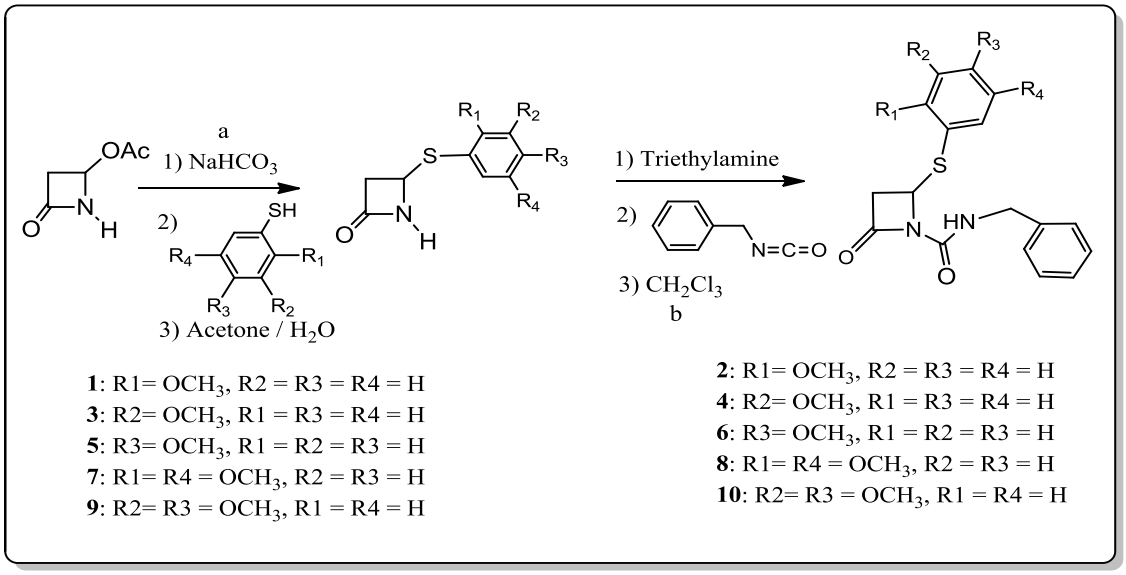
There was an interest as to how similar compounds like the ones described in Scheme 1 with an electron donating group as a substituent on the thiophenol will affect the drug's potency against Mtb. The focus of my research project was to synthesize a library of methoxy-substituted β-lactam derivatives (Scheme 2), where the methoxy group is attached at the *ortho-*, *meta-*, *para-* as well as *di*-substituted methoxy derivatives of the aromatic ring at C4. These compounds have been prepared as racemates and as pure isomers[15] (diastereomers, Scheme 3) in order to evaluate their activity against the aforementioned bacteria and test the hypothesis of whether a methoxy group would inhibit the growth of bacterial strains, Mtb. The structure activity relationships (SAR) of these ether-containing lactams against Mtb will be discussed.

CHAPTER 2

RESULTS AND DISCUSSION

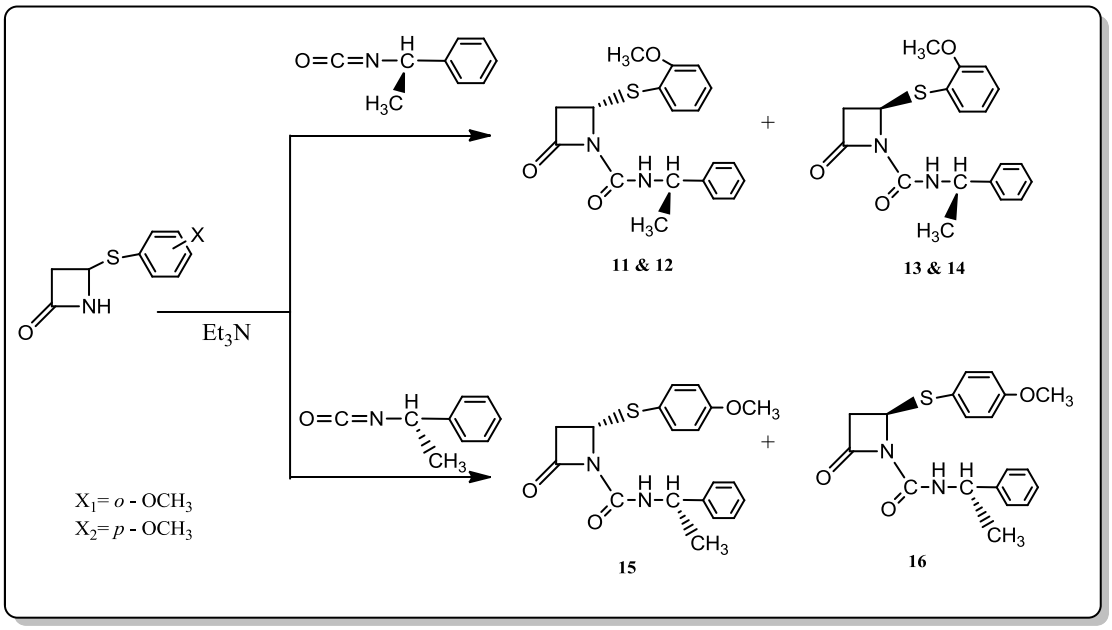
Minimum Inhibitory Concentration

Novel methoxy derivatives of monocyclic β-lactams have been designed, synthesized, and tested using the H37Rv bacterial strain of Mtb, with penicillin (100 μg/mL) as the standard. Testing against the strain of Mtb was done by the Tuberculosis Research Section, LCID, NIAID at the National Institute of Health, Bethesda, MD. Several of these compounds, **8**, **14** and **15** (scheme 2 and 3) with minimum inhibitory concentrations (MIC) of 50, 25 and 12.5 μg/ mL, respectively (Table 1), have shown activity against serine β-lactamase producing *Mycobacterium tuberculosis* wild type strain (Mtb).



Reagents and conditions: (a) $NaHCO_3$ in acetone/water, RT, 5h, **YIELD**; (b) Triethylamine in methylene chloride, RT, 12 hours, **YIELD**

Scheme 2: Synthesis of methoxy substituted derivatives of β-lactam.



Reagents and conditions: (a) Et_3N in CH_2Cl_2, RT, 12h, **YIELD**

Scheme 3: Synthesis of pure isomeric methoxy substituted derivatives of β-lactam.

The methoxy-substituted β-lactams were prepared both as racemates (Scheme 2) and as pure isomers (Scheme 3) which were then tested for their Mtb activity in the absence of clavulanic acid, a β-lactamase inhibitor. Previous results have demonstrated that the activity of our lactams is not altered by the presence of clavulanic acid, which suggests that these compounds are not hydrolized by β-lactamases, the major cause of bacterial resistance. Therefore, my compounds were not tested in the presence of clavulanic acid.

Compounds **14** and **15**, diastereomers of **2** and **6** lactams, respectively, are the most active β-lactams against *M.tuberculosis,* from the methoxy-substituted β-lactam library. The MICs of the racemic mixtures of lactams, **2** and **6** against Mtb are 125-250

μg/mL and 125 μg/mL, respectively, whereas, the activities of **14** and **15** lower with 25 μg/mL and 12.5 μg/mL respectively (Table 1). Compounds **14** and **15** have a *S*-methyl group on the benzylic carbon of the carbamyl group at N1 and a methoxy group at the *ortho*- (**14**) and *para*-(**15**) positions at the phenyl ring at C4 (Scheme 3). The MIC data of lactams **14** and **15** suggest that the diastereomers having a *S*-methyl group at the benzylic carbamyl carbon improves the activity of these type of compounds at least five to ten-fold as compared to their racemic counterparts – lactams **2** and **6**. The effect of the presence of *R*-methyl group at the benzylic carbamyl carbon, compounds **11**, **12** and **17** is to be determined. The presence of a methoxy group at the *meta*- position of the arylthio group at C4 does not appear to improve the activity of the compounds, e.g. the activities of lactam **3** and lactam **4**. As mentioned earlier, the most active compounds tested so far are lactams **14** and **15**.

Prior to the attachment of the isomeric substituents, compounds without a substituent (Scheme 2) at the lactam nitrogen (**1**, **3**, **5**, **7**, **9**) and those with the racemic substituent, benzyl isocyanate (**2**, **4**, **6**, **8**, **10**) at the lactam nitrogen were tested against Mtb. The better activities for the most part were seen in the racemic compounds with the benzyl isocyanate substituent. This result confirms the hypothesis that the MICs of these compounds are lower when in their respective isomeric forms. Based on the antimicrobial data in table 1, it is conceivable to expect that the di-substituted methoxy compounds, **8** and **10**, will have improved MICs when the β-lactam nitrogen is carbamylated with the chiral benzyl isocyanate.

Conclusion

In summary, 16 different lactams of methoxy-derivatives have been prepared, of which, all showed promising activities against H37Rv strains of Mtb bacteria. The compounds with the lowest MICs are **14** and **15**. The mechanism of action and molecular target of the methoxy synthesized β-lactams, having activity against β-lactamase producing Mtb strains, could be different from the current β-lactams used for bacterial therapy (Fig. 1) based on the fact that they are not hydrolyzed by β-lactamases as in the case with the aforementioned lactams in figure 1. Current studies in our laboratories are directed towards a better understanding of the structure-activity relationships and the molecular target of these promising new anti-bacterial β-lactam compounds.

CHAPTER 3

Experimental

Equipment and Materials

All reagents and solvents were obtained from commercial suppliers and used without any further purification or distillation. All other starting materials were purchased from Acros, Aldrich, and Fisher. Unless otherwise noted, reaction mixtures were magnetically stirred and reactions were monitored by thin layer chromatography (TLC) using glass-backed sorbent layer, 250μm (0.25 mm) analytical TLC plates coated with silica gel 60 with F254 indicator. TLC preparation plates (silica G prep TLC plates with UV254 glass backed, 500 μm – 20 x 20 cm) were used to purify and separate diastereomers. The chromatograms were visualized under ultraviolet light and/or by staining with an iodine chamber. All solvents were evaporated using the Buchi rotavapor R-205 with an attached Buchi heating bath B-490. ^{1}H NMR spectra were recorded using Bruker 400 MHz instrument at 400 MHz in $CDCl_3$. Chemical shifts are reported in δ (ppm) units (downfield to $\delta_{TMS} = 0$ ppm), with the signal multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad) by reference to Me_4Si with coupling constants (J in Hz) and integration area in parentheses. ^{13}C NMR spectra were recorded using Bruker 400 MHz instrument at 125 MHz in $CDCl_3$. Infrared (IR) spectra were recorded using Shimadzu FTIR – 8300 instrument. All Melting Points are

uncorrected and reported using Thomas Hoover Unimelt Capillary melting point apparatus (Authur H. Thomas Company, Philadelphia, PA, US).

Reaction and Synthesis

General procedure for the preparation of methoxy-phenyl sulfanyl)-azetidin-2-one derivatives (Compounds **1**, **3**, **5**, **7** and **9** from Scheme 2)

All general procedures for the preparation of Methoxy-phenyl sulfanyl)-azetidin-2-one derivatives were adopted from Wasserman et al. and modified to suit our purposes. 0.500 g of 4-acetoxy-2-azetidinone (1.0 mol. equivalent) was added to a solution of 2-methoxythophenol (0.559 g) in acetone (25 mL), and water (15 mL). After stirring for a minute, 4.0 molar equivalence of $NaHCO_3$ (1.30 g) was added. The reaction was stirred for five hours at room temperature, after which, a teaspoon of rock salt was added and stirred for another minute. The mixture was filtered using a whatman-grade 595 1/2 prefolded, diam. 90 mm filter paper and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried with magnesium sulfate (a teaspoon, $MgSO_4$), and concentrated in vacuo via a rotavaporator.

Compound **1**: The crude product was purified by recrystallization overnight with 15 mL hexane after being dissolved in 10 mL of ethyl acetate to give a white solid (a 87 % yield) with a melting point of 106 – 107^o. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.80 (3H, s); 2.93 (1H, dq, J=1.36, 1.41, 11.78), 3.28 (1H, ddd, J=1.82, 1.81, 8.53); 4.86 (1H, dd, J=2.23, 2.60); 6.75 (1H, brs); 7.43-6.88 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 44.99, 54.90, 55.54, 114.96, 120.82, 136.93, 160.74, 166.56. IR (neat) ν_{max}, C=O; 1760 cm^{-1}. *Anal.* calcd for $C_{10}H_{11}NO_2S$; C, 57.39; H, 5.30; N, 6.69. Found: C, 57.37; H, 5.19; N, 6.67.

Compound **3**: Purification of the crude product (yellow oil with a 75 % yield) was not needed. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.81 (3H, s); 2.91 (1H, dd, J=1.46, 13.75), 3.39 (1H, ddd, J=1.01, 1.27, 9.00); 5.03 (1H, dd, J=2.31, 2.64); 6.07 (1H, brs); 7.29-6.87 (4H, m).^{13}C NMR (125 MHz, $CDCl_3$): δ 45.41, 54.22, 55.46, 114.22, 118.24, 125.02, 130.30, 133.17, 160.01, 169.99. IR (neat) ν_{max}, C=O; 1756 cm^{-1}. *Anal.* calcd for $C_{10}H_{11}NO_2S$; C, 57.39; H, 5.30; N, 6.69. Found: C, 57.25; H, 5.34; N, 6.52.

Compound **5**: The crude product was purified by recrystallization overnight with 10 mL of hexane after being dissolved in 5 mL of ethyl acetate to give a white solid with a 57 % yield and a melting point of 83 - 85^{o}. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.89 (3H, s); 2.93 (1H, dt, J=1.89, 11.44), 3.37 (1H, ddd, J=1.45, 1.45, 8.82); 4.97 (1H, dd, J=2.30, 2.62); 6.81 (1H, brs); 7.40-6.86 (4H, m).^{13}C NMR (125 MHz, $CDCl_3$): δ 45.87, 54.06, 56.06, 111.47, 121.44, 130.47, 134.93, 159.16, 169.69. IR (neat) ν_{max}, C=O; 1756 cm^{-1}. *Anal.* calcd for $C_{10}H_{11}NO_2S$; C, 57.39; H, 5.30; N, 6.69. Found: C, 57.20; H, 5.22; N, 6.58.

Compound **7**: White solid with a 51 % yield and a melting point of 116 - 118^{o}. The crude product was purified by recrystallization overnight with 15 mL of hexane after being dissolved in 10 mL of ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.86 (3H, s), 3.78 (3H, s); 2.98 (1H, dt, J=2.04, 11.17), 3.41 (1H, ddd J=1.41, 1.40, 8.87); 5.01 (1H, dd, J=2.34, 2.61); 6.46 (1H, brs); 7.28-6.87 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 45.73, 53.88, 55.83, 56.44, 112.22, 114.78, 120.13, 120.93, 153.19, 153.59, 166.26. IR (neat) ν_{max}, C=O; 1760 cm^{-1}. *Anal.* calcd for $C_{11}H_{13}NO_3S$; C, 55.21; H, 5.48; N, 5.85. Found: C, 54.92; H, 5.36; N, 5.81.

Compound **9**: White powder with a 63 % yield 0.876 g and a melting point of 79 - 80^{o}. The crude product was purified by recrystallization overnight with 15 mL of hexane after being dissolved in a 10 mL of ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.68 (3H, s), 3.67 (3H, s); 2.64 (1H, dq, J=1.40, 1.41, 11.60), 3.10 (1H, ddd J=1.86, 1.92, 8.38); 4.71 (1H, dd, J=2.28, 2.64); 6.36 (1H, brs); 7.07-6.34 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.19, 21.06, 44.89, 54.78, 55.91, 56.02, 60.41, 111.59, 117.74, 121.20, 128.05, 149.15, 150.12, 166.21, 171.20. IR (neat) ν_{max}, C=O; 1760 cm^{-1}. *Anal.* calcd for $C_{11}H_{13}NO_3S$; C, 55.21; H, 5.48; N, 5.85. Found: C, 55.03; H, 5.40; N, 5.78.

General procedures for the methoxy-phenyl sulfanyl-4-oxo-azetidine-1-carboxylic acid benzylamide derivatives (Compounds **2**, **4**, **6**, **8** and **10** from Scheme 2)

All general procedures for the methoxy-phenyl sulfanyl-4-oxo-azetidine-1-carboxylic acid benzylamide derivatives were adopted from Mulchande et al. and modified to suit our purposes. 3.5 mmol equivalent of **1** (0.43 g) was added to a solution of benzyl isocyanate (0.305 mL) in dimethylchloride (8 mL). After stirring for a minute on a stir plate, 4.2 mmol equivalence of triethylamine (0.344 mL) was added and left to stir overnight at room temperature. The reaction mixture was evaporated under vacuum.

Compound **2**: Prepared from lactam **1** to yield 90 % with a melting point of 105 - 107^{o}. The crude product was purified via recrystallization overnight with 10 mL of hexane after being dissolved in 8 mL of ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.86 (3H, s); 2.94 (1H, dd, *J*=2.62, 13.75), 3.40 (1H, dd, *J*=5.62, 10.71); 5.38 (1H, dd, *J*=2.62, 2.96); 7.55-6.81 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.65, 43.94, 55.81, 111.22, 117.37, 121.25, 127.64, 127.75, 128.74, 131.25, 137.28, 137.88, 149.61, 159.93,

165.70. IR (neat) ν_{max}, C=O; 1627 cm^{-1}, 1576 cm^{-1}. *Anal.* calcd for $C_{18}H_{18}N_2O_3S$; C, 63.14; H, 5.30; N, 8.18. Found: C, 63.40; H, 5.28; N, 8.32.

Compound **4**: Synthesized using **3** to produce a colorless oil with a 57 % yield. The reaction mixture was purified via a flash column chromatography with a ratio gradient of 3:1 hexane and dichloromethane (TLC mobile phase was 100% dicloromethane). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.68 (3H, s); 2.78 (1H, dt, *J*=1.32, 13.74), 3.22 (1H, dd, *J*=1.84, 3.09); 5.19 (1H, dd, *J*=2.70, 2.93); 7.25-6.74 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.69, 44.01, 55.35, 56.60, 115.34, 120.01, 127.13, 127.74, 128.78, 130.04, 130.47, 137.79, 149.63, 159.87, 165.53. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1700 cm^{-1}. *Anal.* calcd for $C_{18}H_{18}N_2O_3S$; C, 63.14; H, 5.30; N, 8.18. Found: C, 63.57; H, 5.51; N, 8.05.

Compound **6**: Reaction using compound **5** produced a white powder with a 58 % yield and a melting point of 105 - 110^o. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.82 (3H, s); 2.86 (1H, dd, *J*=2.67, 13.65), 3.36 (1H, dd, *J*=5.61, 10.69); 5.21 (1H, dd, *J*=2.65, 2.91); 7.48-6.84 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.39, 43.64, 55.35, 56.72, 114.83, 118.74, 127.67, 127.78, 128.76, 137.95, 194.66, 160.88, 165.53. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1700 cm^{-1}. *Anal.* calcd for $C_{18}H_{18}N_2O_3S$; C, 63.14; H, 5.30; N, 8.18. Found: C, 63.34; H, 5.13; N, 8.18.

Compound **8**: A white solid prepared from **7** with an 88 % yield and a melting point of 87.5 – 88.5^o. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.82 (3H, s); 2.86 (1H, dd, *J*=2.67, 13.65), 3.36 (1H, dd, *J*=5.61, 10.69); 5.21 (1H, dd, *J*=2.65, 2.91); 7.48-6.84 (4H, m).^{13}C NMR (125 MHz, $CDCl_3$): δ 43.67, 44.04, 55.77, 56.35, 112.16, 116.30, 121.90, 127.49,

127.64, 127.74, 128.63, 128.75. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_4S$; C, 61.27; H, 5.41; N, 7.52. Found: C, 61.04; H, 5.39; N, 7.47.

Compound **10**: Clear oil prepared from **9** with a 63 % yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.82 (3H, s); 2.86 (1H, dd, *J*=2.67, 13.65), 3.36 (1H, dd, *J*=5.61, 10.69); 5.21 (1H, dd, *J*=2.65, 2.91); 7.48-6.84 (4H, m).^{13}C NMR (125 MHz, $CDCl_3$): δ 43.37, 43.58, 55.88, 56.74, 111.37, 118.77, 118.98, 119.13, 127.64, 127.75, 128.73, 129.29, 137.93, 149.03, 149.68, 150.38, 165.59. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_4S$; C, 61.27; H, 5.41; N, 7.52. Found: C, 61.54; H, 5.56; N, 7.43.

General procedure for methoxy-phenyl sulfanyl-4-oxo-azetidine-1-carboxylic acid benzylamide derivatives as pure isomers (Compounds **11** and **12** from Scheme 3)

3.5 mmol equivalent of compound **1** (0.498 g) was added to a solution of R-(+)-α-methyl benzyl isocyanate (0.403 mL) in dimethylchloride (5 mL). After stirring for a minute, 4.2 mmol equivalence of triethylamine (0.398 mL) was added and left to stir overnight at room temperature. The yellow viscous reaction mixture was evaporated under vacuum to yield 0.340 g and purified via thin layer chromatography on a silica gel prep plate (Hexane/EtoAc 3:1) to yield compound **11** (46 %, top layer) and compound **12** (50 %, bottom layer).

Compound **11**: Top layer (diastereomer 1) from compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.75 (3H, s); 1.53 (1H, d, J= 6.95); 2.92 (1H, dd, J=2.61, 13.73), 3.39 (1H, dd, J=5.61, 10.73); 5.11 (1H, quin, J= 7.11, 7.41); 5.34 (1H, q, J=2.64, 2.64); 7.42-6.75 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.63, 44.10, 49.46, 55.80, 55.85, 60.44, 111.19, 117.76, 121.26, 126.02, 127.45, 128.71, 131.12, 136.96,

137.66, 142.96, 148.77, 159.80, 165.81. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_3S$; C, 64.02; H, 5.66; N, 7.86. Found: C, 64.10; H, 5.66; N, 7.77.

Compound **12**: Bottom layer (diastereomer 2) from compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.75 (3H, s); 1.53 (1H, d, J= 6.95); 2.92 (1H, dd, J=2.61, 13.73), 3.39 (1H, dd, J=5.61, 10.73); 5.11 (1H, quin, J= 7.11, 7.41); 5.34 (1H, q, J=2.64, 2.64); 7.42-6.75 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ14.24, 43.81, 49.31, 55.72, 55.79, 60.43, 111.21, 116.90, 121.20, 126.17, 127.49, 128.73, 131.33, 137.65, 143.00, 148.76, 160.10, 165.77. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_3S$; C, 64.02; H, 5.66; N, 7.86. Found: C, 63.30; H, 5.75; N, 7.61.

General procedure for methoxy-phenyl sulfanyl-4-oxo-azetidine-1-carboxylic acid benzylamide derivatives as pure isomers (Compounds **13** and **14** from Scheme 3)

3.5 mmol equivalent of compound **1** (0.498 g) was added to a solution of S-(+)-α-methyl benzyl isocyanate (0.403 mL) in dimethylchloride (5 mL). After stirring for a minute, 4.2 mmol equivalence of triethylamine (0.398 mL) was added and left to stir overnight at room temperature. The yellow oil reaction mixture was evaporated under vacuum to yield 0.327 g. The crude product was purified via thin layer chromatography on a silica gel prep plate (Hexane/EtoAc 8:1) to yield **13** (34 %, top layer) and **14** (56 %, bottom layer).

Compound **13**: Top layer (diastereomer 1) from compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.81 (3H, s); 1.53 (1H, d, *J*= 6.93); 2.86 (1H, dd, *J*=2.59, 13.76), 3.29 (1H, dd, *J*=5.61, 10.73); 5.00 (1H, quin, *J*= 7.09, 7.38); 5.23 (1H, q, *J*=2.68, 2.60); 7.49-6.68 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ22.65, 44.09, 49.47, 55.79, 55.85, 111.22, 117.76, 121.27, 126.02, 127.46, 128.72, 131.12, 136.93, 142.99, 148.78, 159.80, 165.81.

IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_3S$; C, 64.02; H, 5.66; N, 7.86. Found: C, 64.23; H, 5.60; N, 7.71.

Compound **14**: Bottom layer (diastereomer 2) from compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.74 (3H, s); 1.53 (1H, d, *J*= 6.96); 2.92 (1H, dd, *J*=2.63, 13.71), 3.38 (1H, dd, *J*=5.59, 10.73); 5.10 (1H, quin, *J*= 7.11, 7.39); 5.34 (1H, q, *J*=2.96, 2.59); 7.42-6.75 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ22.63, 44.10, 49.46, 55.80, 55.85, 60.44, 111.19, 117.76, 121.26, 126.02, 127.45, 128.71, 131.12, 136.96, 137.66, 142.96, 148.77, 159.80, 165.81. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_3S$; C, 64.02; H, 5.66; N, 7.86. Found: C, 64.61; H, 5.88; N, 7.96.

Compounds **15** and **16**

General procedures for the preparation of compounds **15** and **16** were adopted from the abovementioned "General procedure for methoxy-phenyl sulfanyl-4-oxo-azetidine-1-carboxylic acid benzylamide derivatives as pure isomers," using compounds **13**, and **5**, respectively. The reaction mixture was evaporated under vacuum to yield 0.428 g. The crude product was purified via thin layer chromatography on a silica gel prep plate (Hexane/EtoAc 3:1) to yield **15** (66 %, top layer) and **16** (51 %, bottom layer).

Compound **15**: Top layer (diastereomer 1) from compound **5**. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.84 (3H, s); 1.59 (1H, d, *J*= 6.97); 2.86 (1H, dd, *J*=2.72, 13.60), 3.34 (1H, dd, *J*=5.60, 10.72); 5.12 (1H, sex, *J*= 5.03, 5.43); 5.15 (1H, q, *J*=2.80, 2.57); 7.53-6.90 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ22.62, 43.52, 50.03, 55.38, 56.86, 114.85, 119.23, 126.05, 127.50, 128.74, 137.74, 142.88, 148.83, 160.85, 165.66. IR (neat) ν_{max}, C=O; 1775 cm^{-1}, 1703 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_3S$; C, 64.02; H, 5.66; N, 7.86. Found: C, 64.19; H, 5.82; N, 7.74.

Compound **16**: Bottom layer (diastereomer 2) from compound **5**. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.80 (3H, s); 1.54 (1H, d, *J*= 6.98); 2.83 (1H, dd, *J*=2.65, 13.64), 3.34 (1H, dd, *J*=5.55, 10.74); 6.0 (1H, sex, *J*= 5.29, 5.59); 5.18 (1H, q, *J*=2.53, 2.76); 7.45-6.74 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ22.35, 43.29, 49.30, 55.33, 56.45, 114.77, 118.19, 119.23, 126.16, 127.52, 128.74, 138.13, 143.16, 148.82, 160.88, 165.57. IR (neat) ν_{max}, C=O; 1774 cm^{-1}, 1705 cm^{-1}. *Anal.* calcd for $C_{19}H_{20}N_2O_3S$; C, 64.02; H, 5.66; N, 7.86. Found: C, 63.54; H, 5.71; N, 7.13.

Table 1. Minimum inhibitory concentration of methoxy-derivative compounds

Compounds	Mtb H37Rv μg/mL
1	>250
2	125-250
3	>100
4	100
5	100
6	125
7	>250
8	50
9	>250
10	100
11	ND
12	ND
13	ND
14	25
15	12.5
Penicillin	100

[a] ND: Not determined.

APPENDIX A

^{1}H-NMR SPECTRUM OF COMPOUNDS

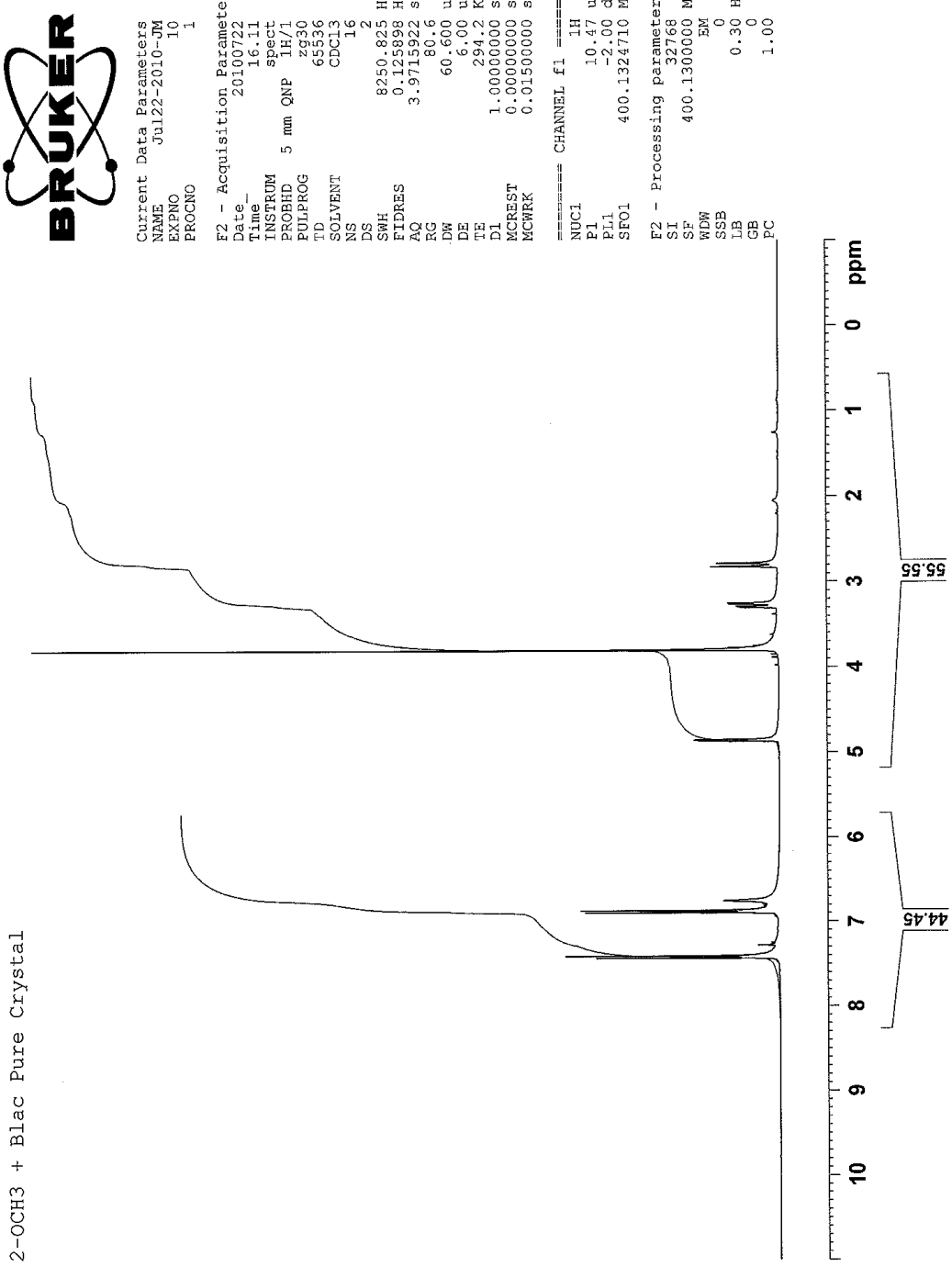


FIGURE 2: ^{1}H-NMR Spectrum of compound **1**.

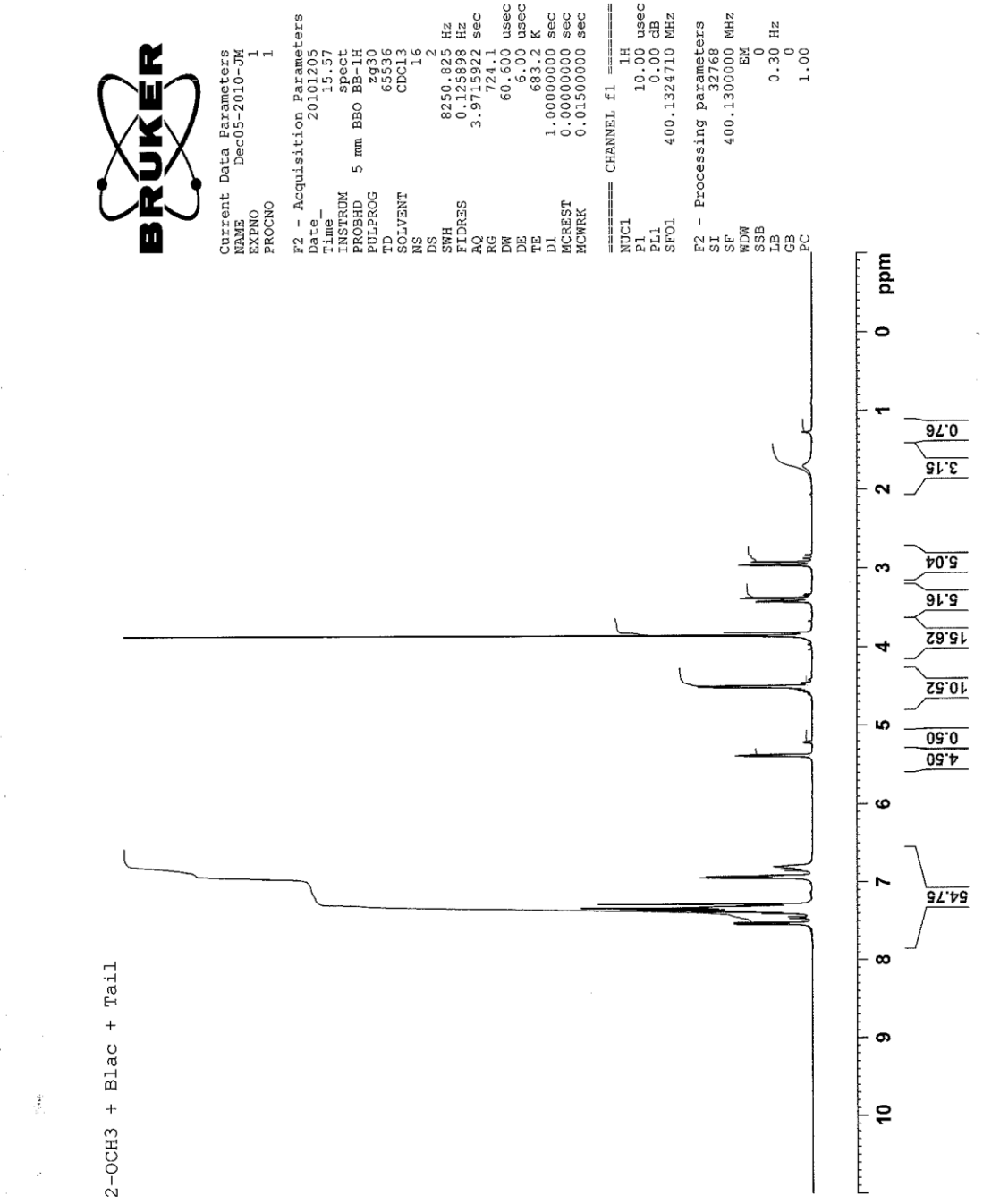


FIGURE 3: ^{1}H-NMR Spectrum of compound **2**.

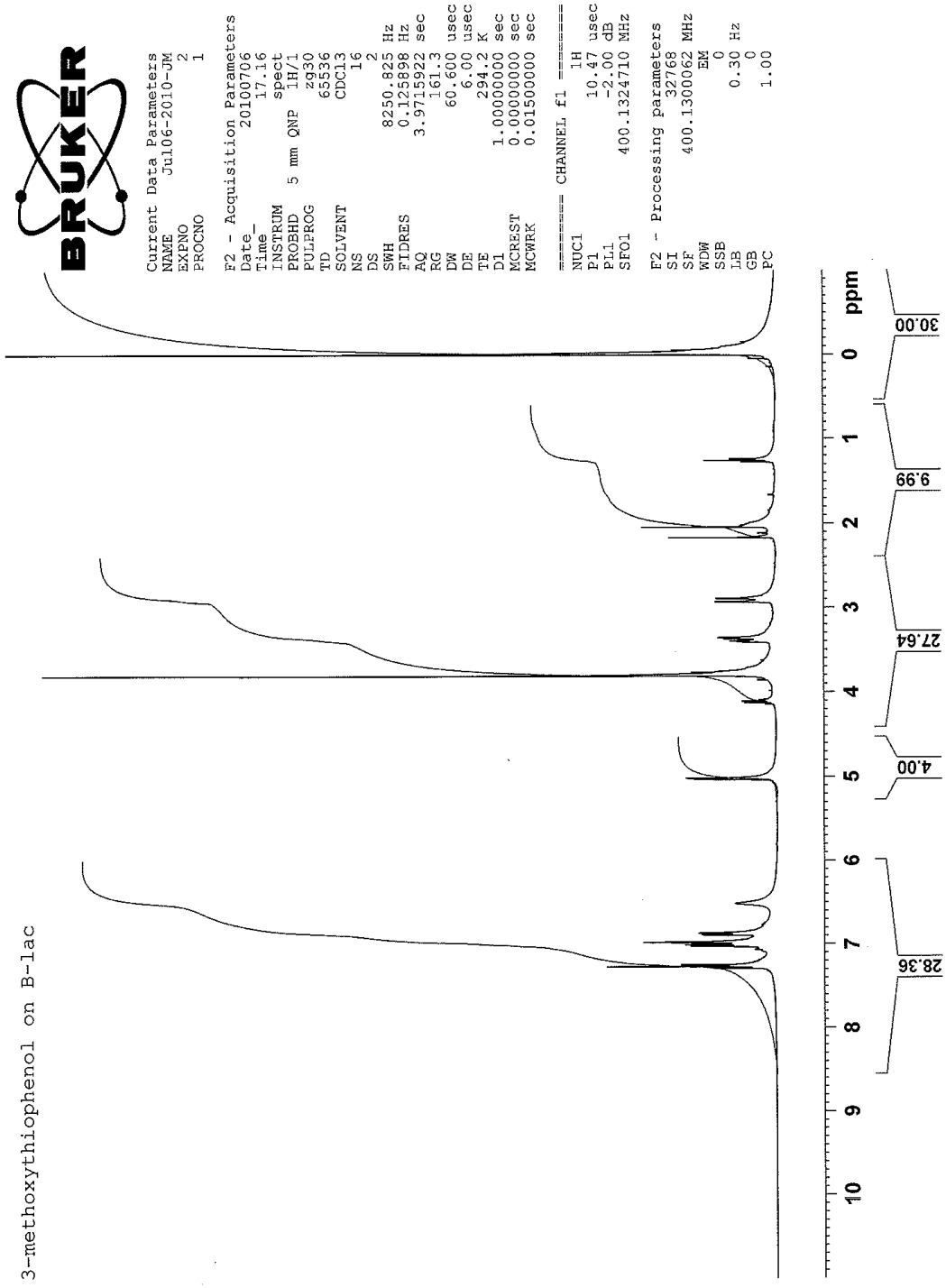


FIGURE 4: ^{1}H-NMR Spectrum of compound **3**.

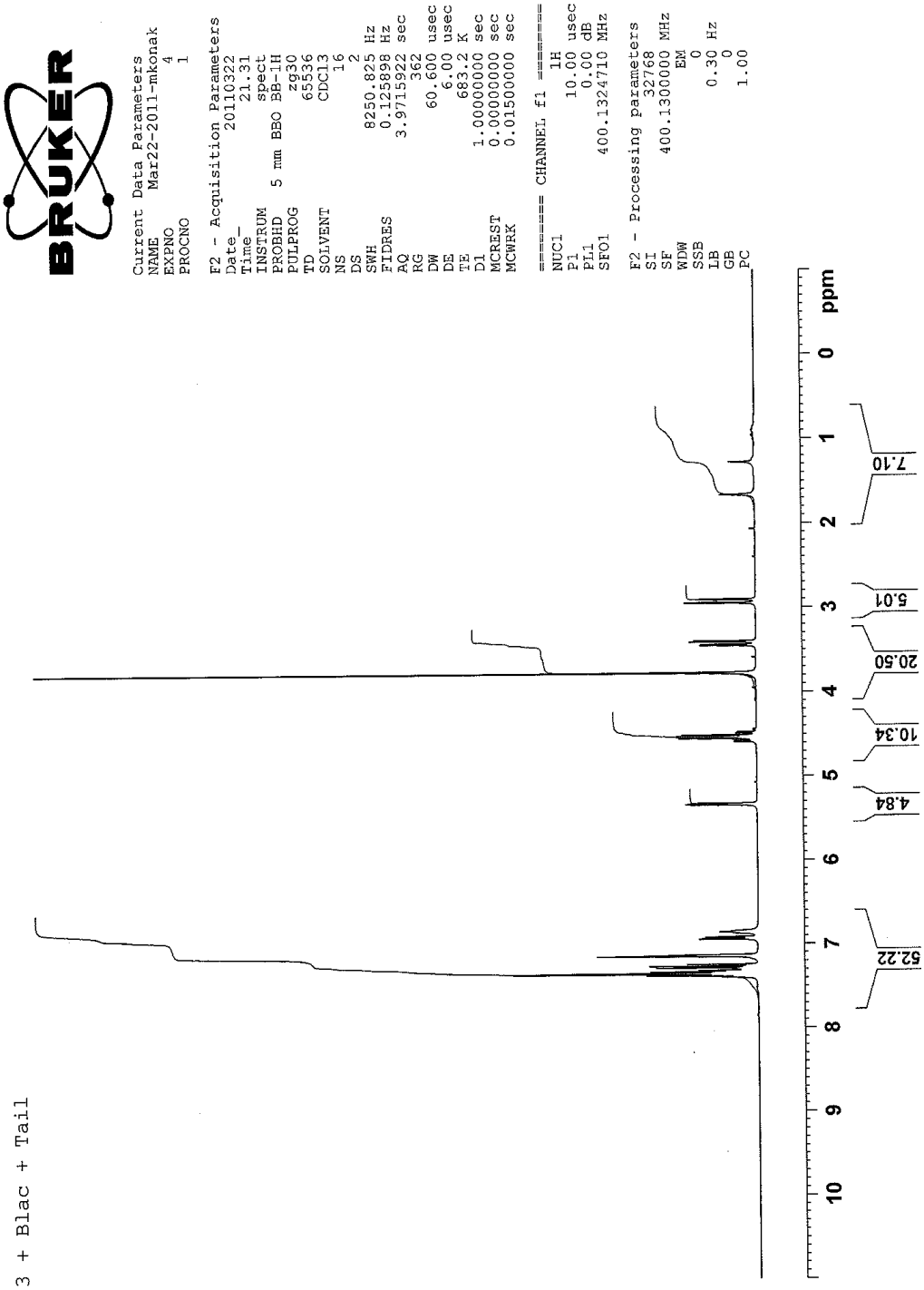


FIGURE 5: ^{1}H-NMR Spectrum of compound **4**.

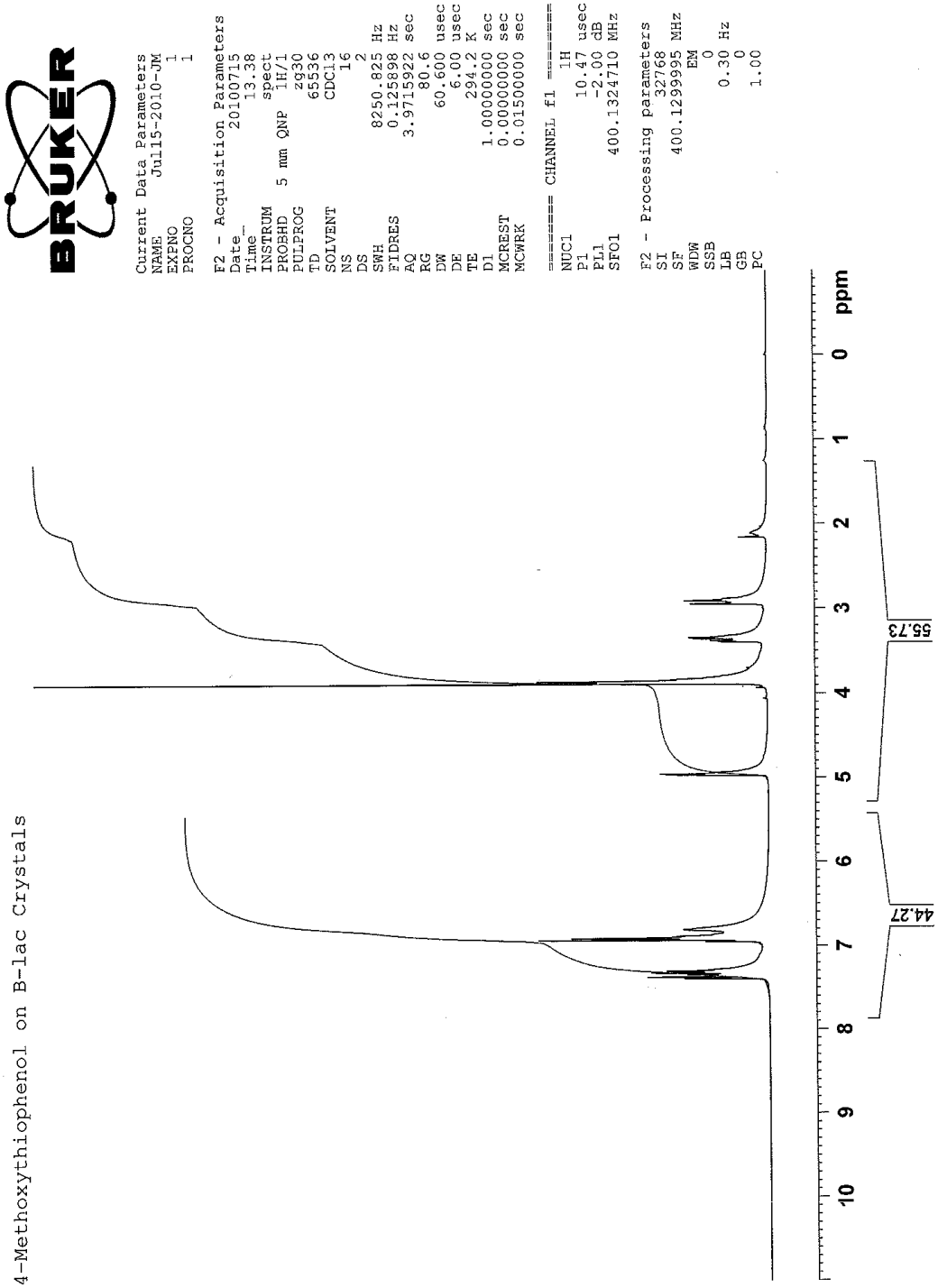


FIGURE 6: ^{1}H-NMR Spectrum of compound **5**.

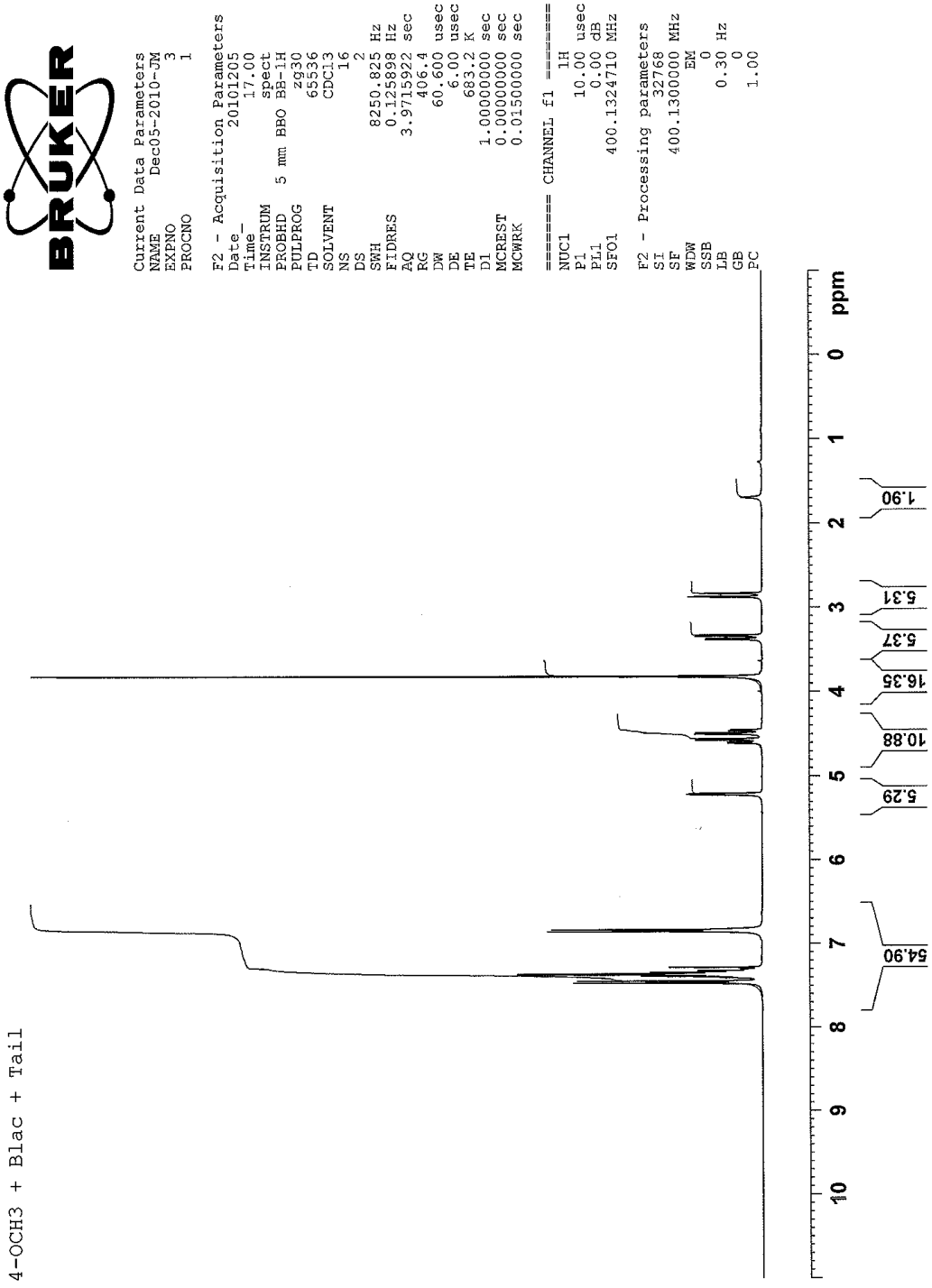


FIGURE 7: ^{1}H-NMR Spectrum of compound **6**.

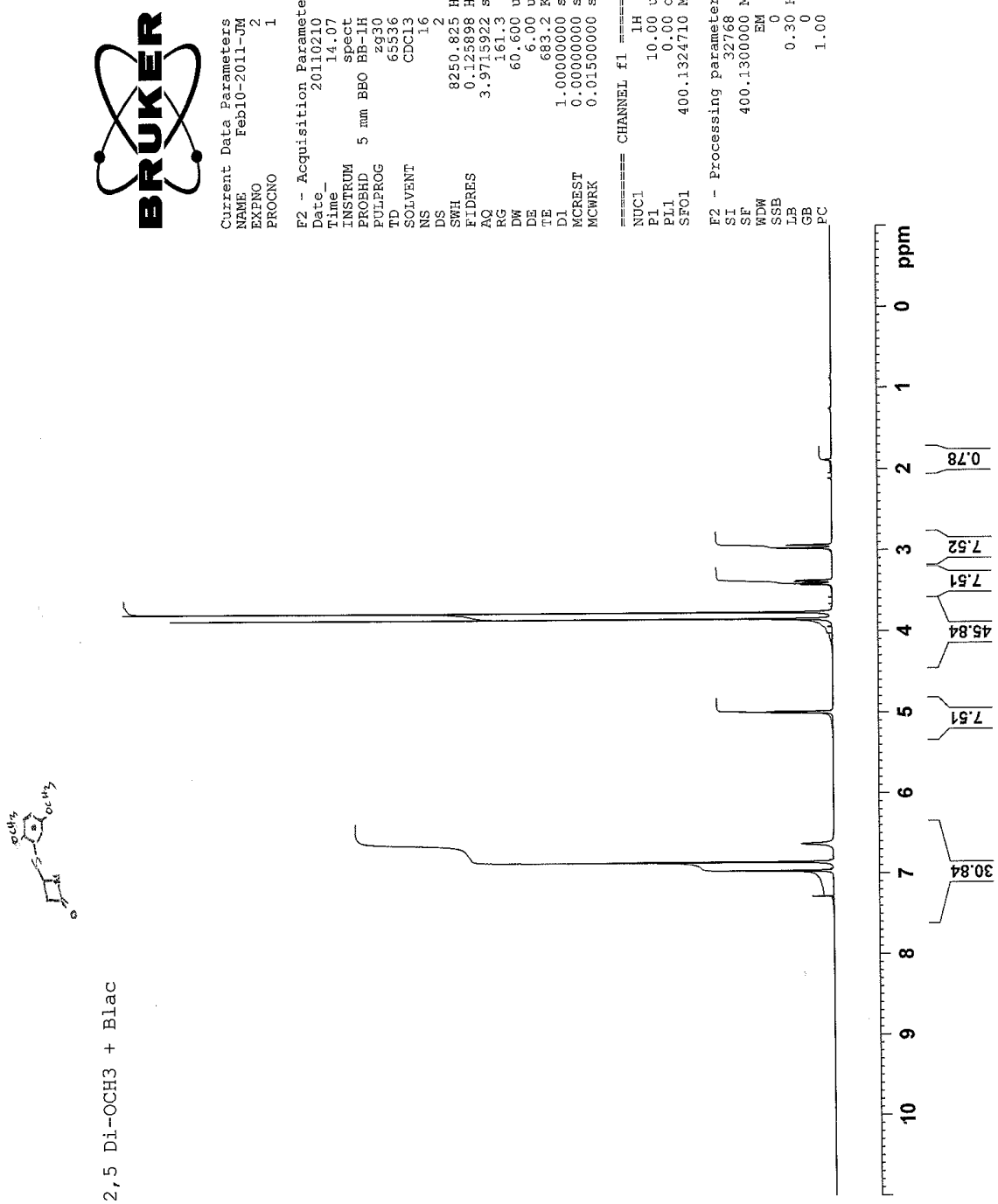


FIGURE 8: ^{1}H-NMR Spectrum of compound **7**.

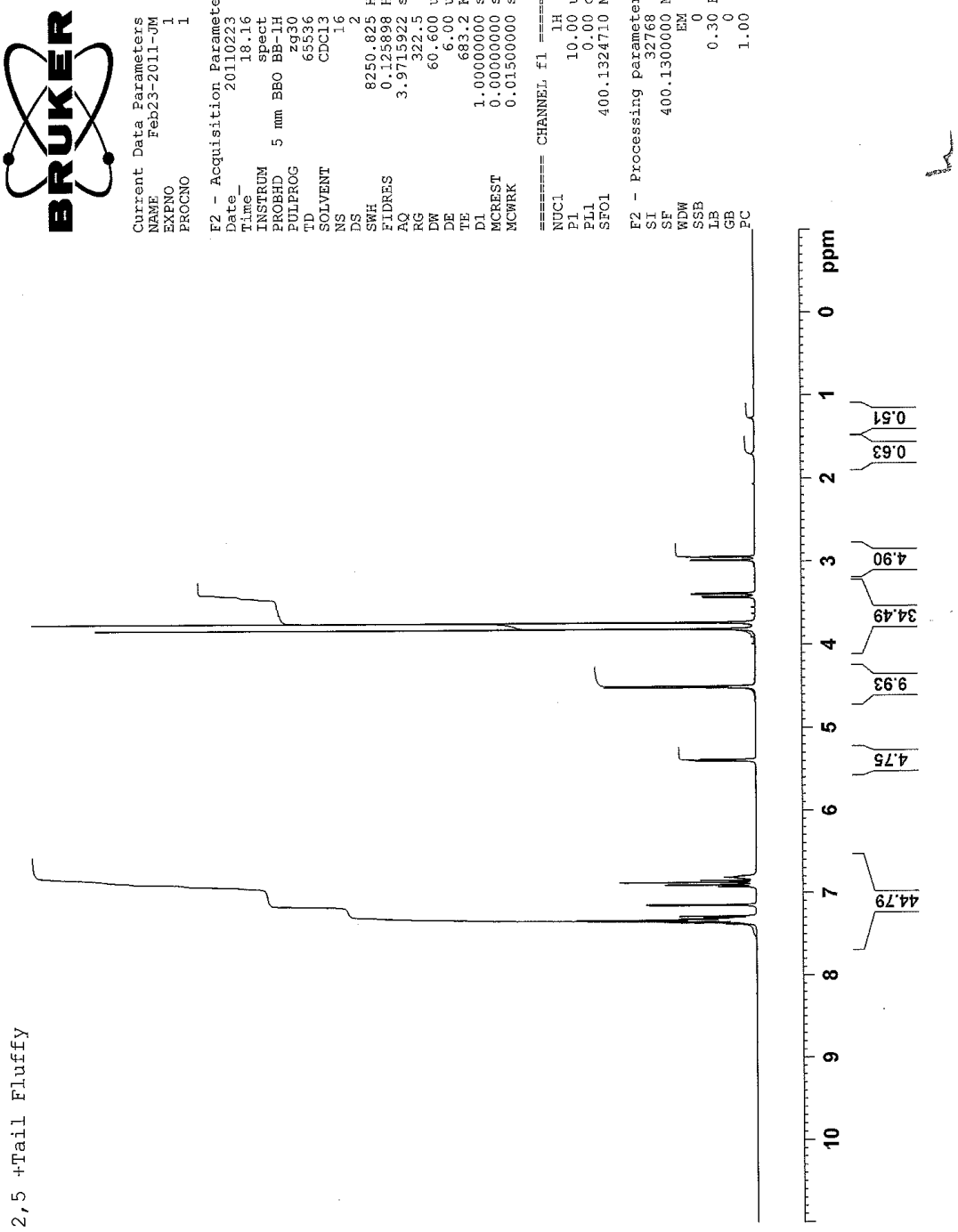


FIGURE 9: ^{1}H-NMR Spectrum of compound **8**.

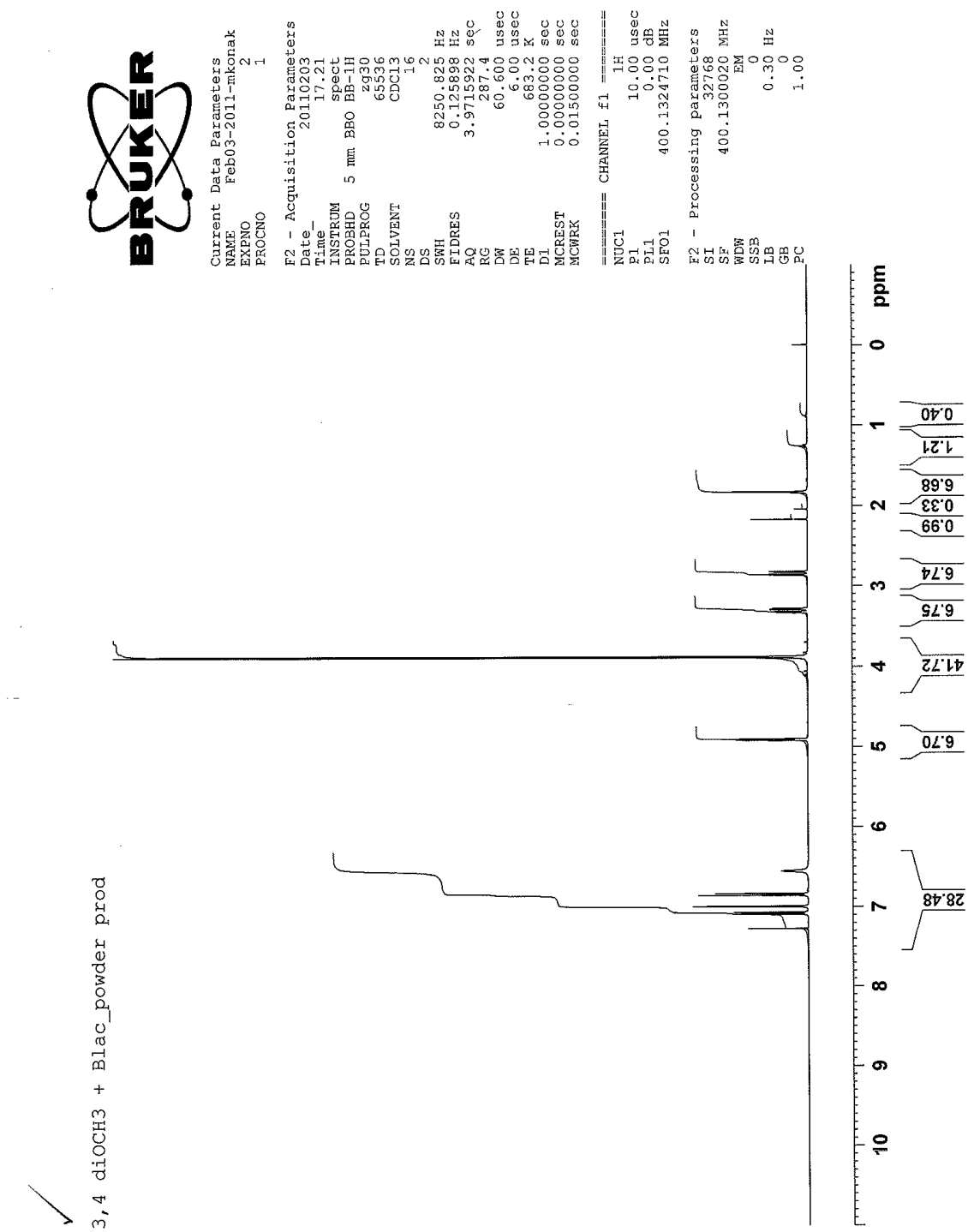


FIGURE 10: ^{1}H-NMR Spectrum of compound **9**.

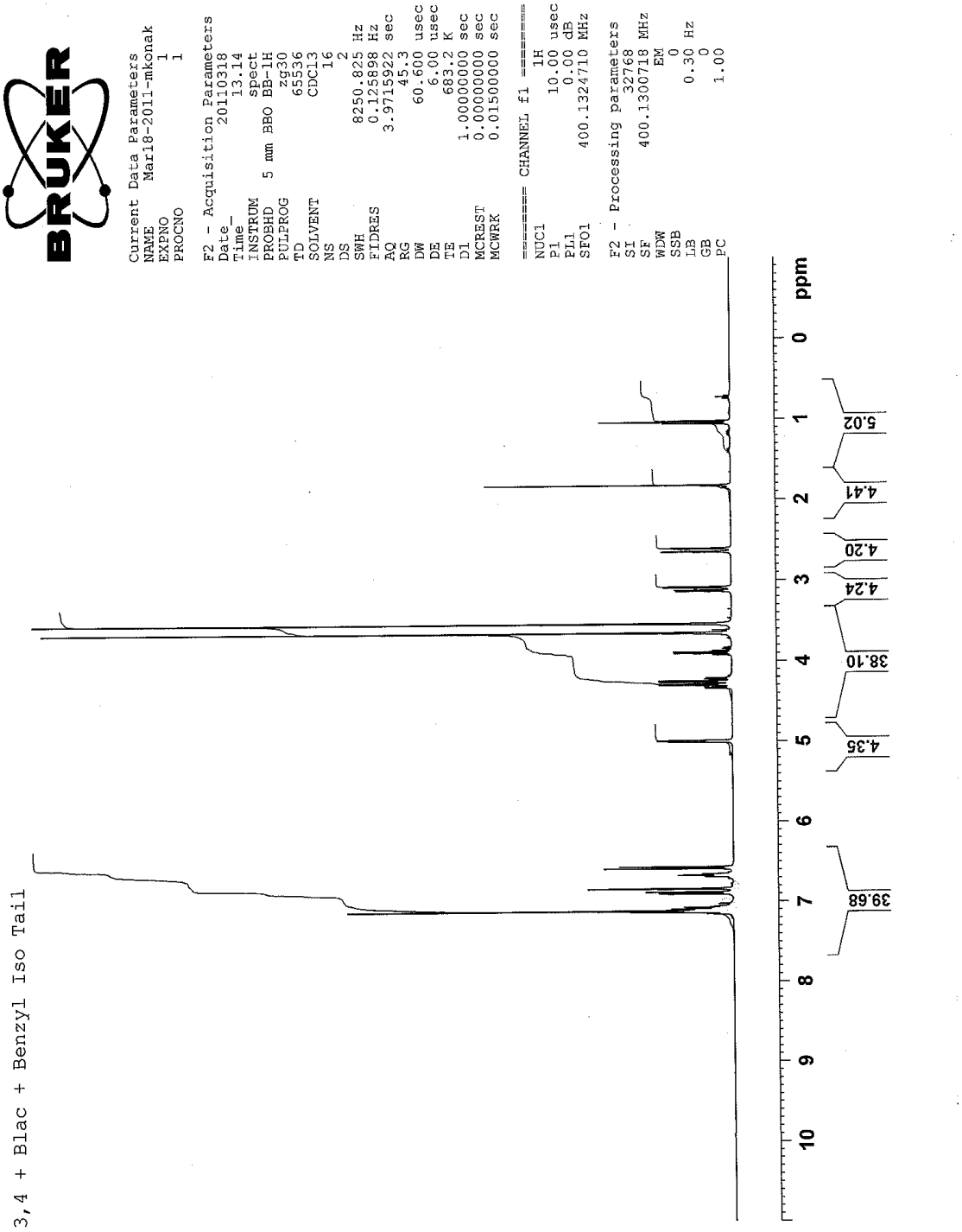


FIGURE 11: ^{1}H-NMR Spectrum of compound **10**.

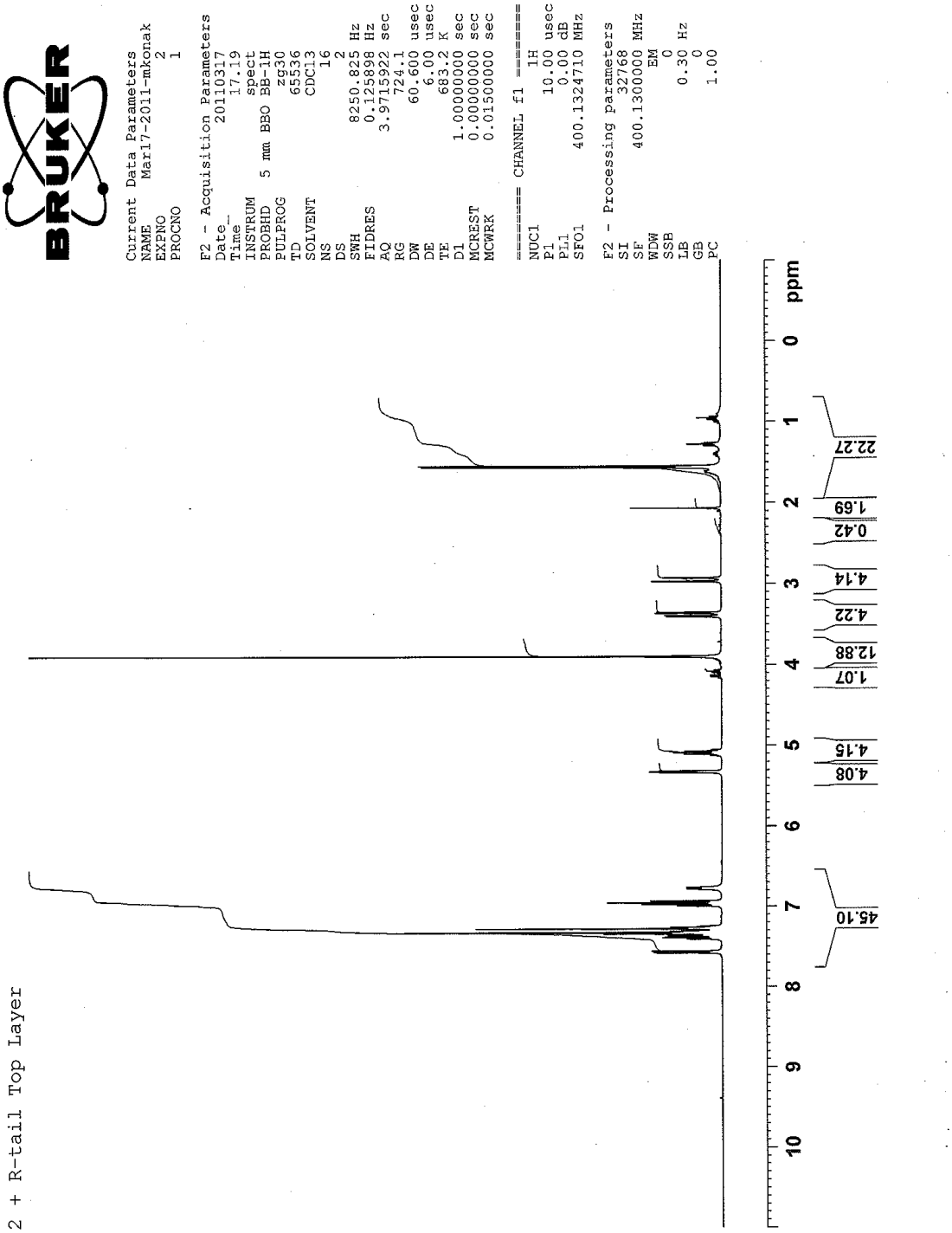


FIGURE 12: ^{1}H-NMR Spectrum of compound **11**.

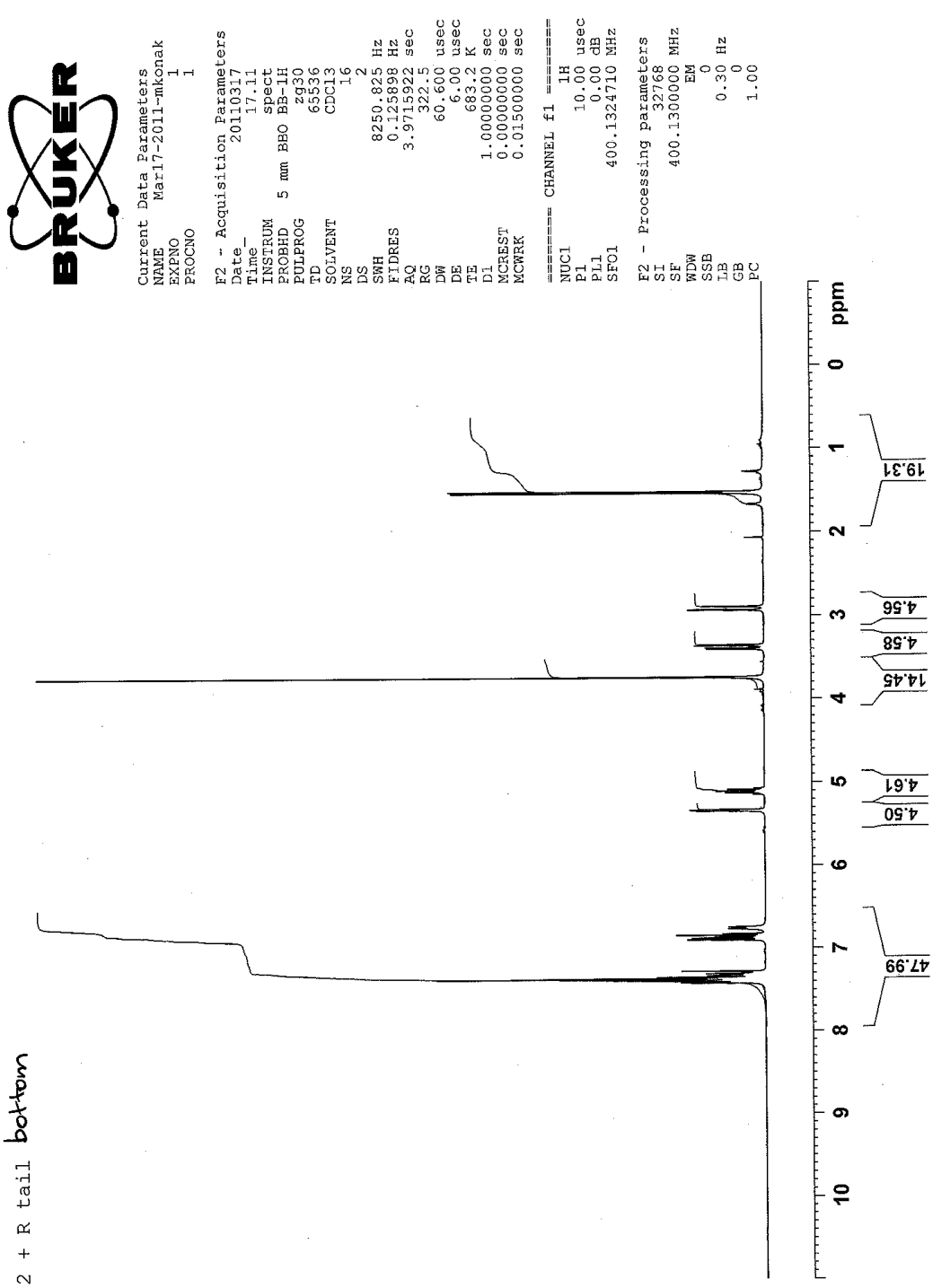


FIGURE 13: ^{1}H-NMR Spectrum of compound **12**.

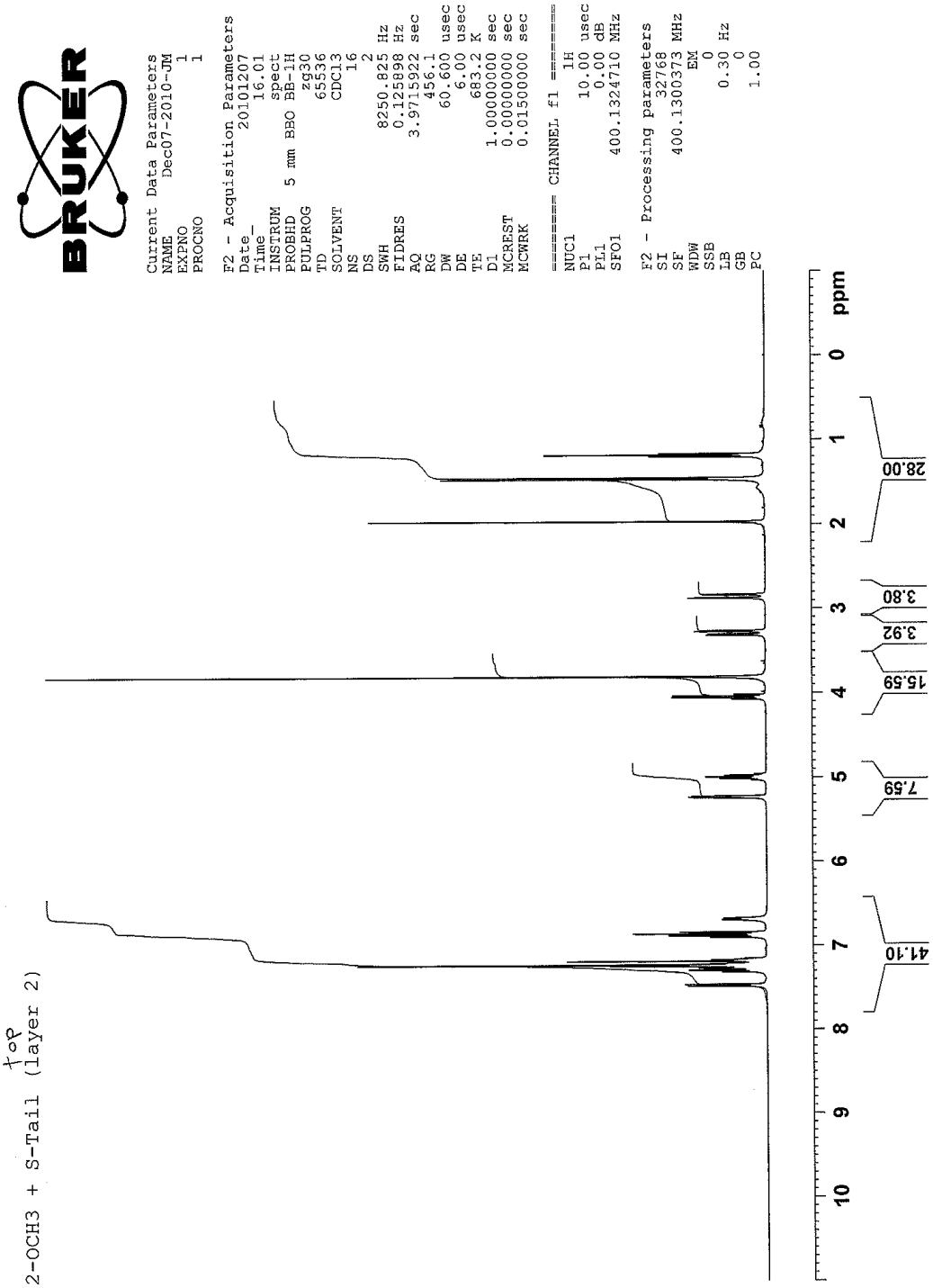


FIGURE 14: ^{1}H-NMR Spectrum of compound **13**.

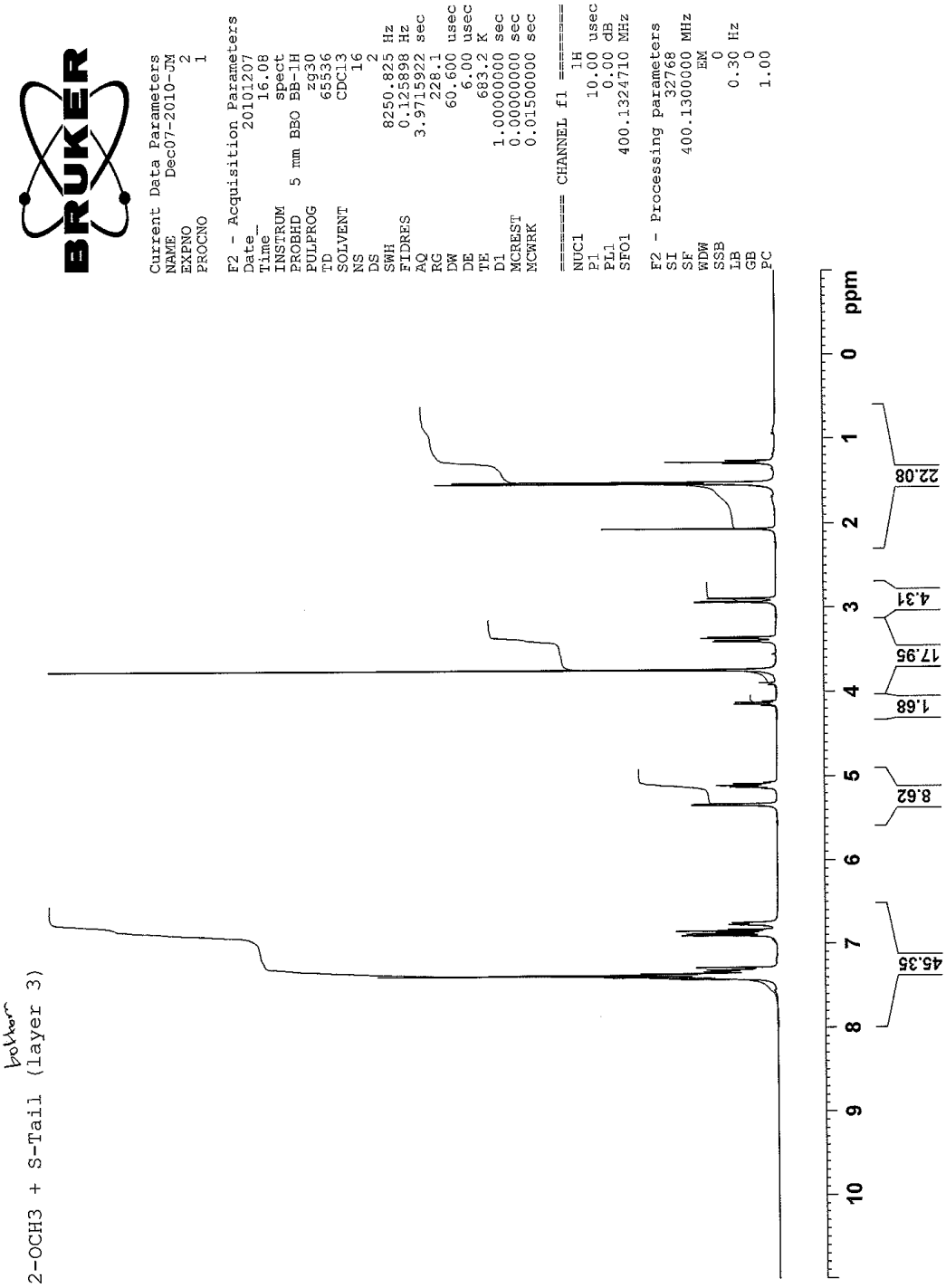


FIGURE 15: ^{1}H-NMR Spectrum of compound **14**.

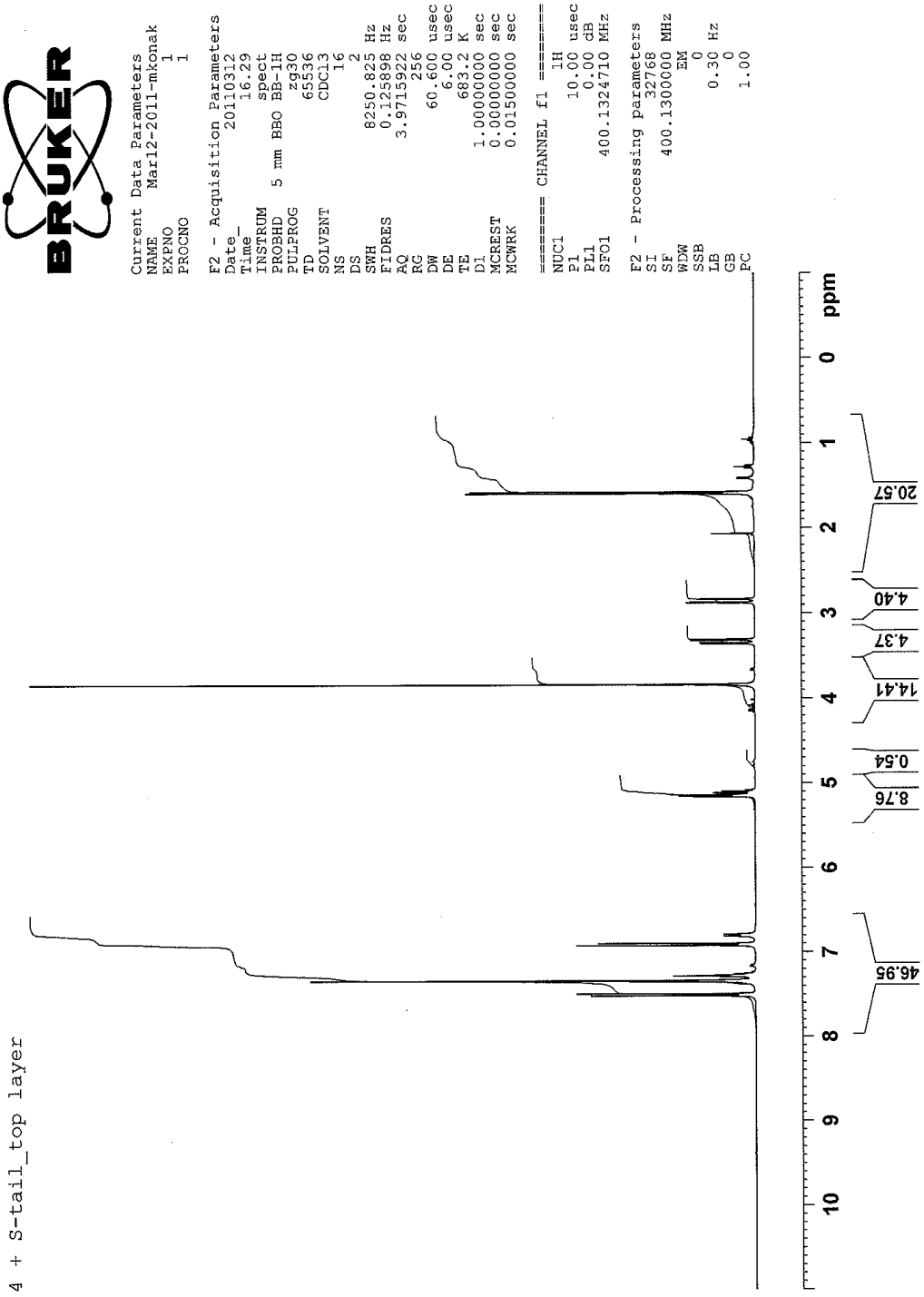


FIGURE 16: ^{1}H-NMR Spectrum of compound **15**.

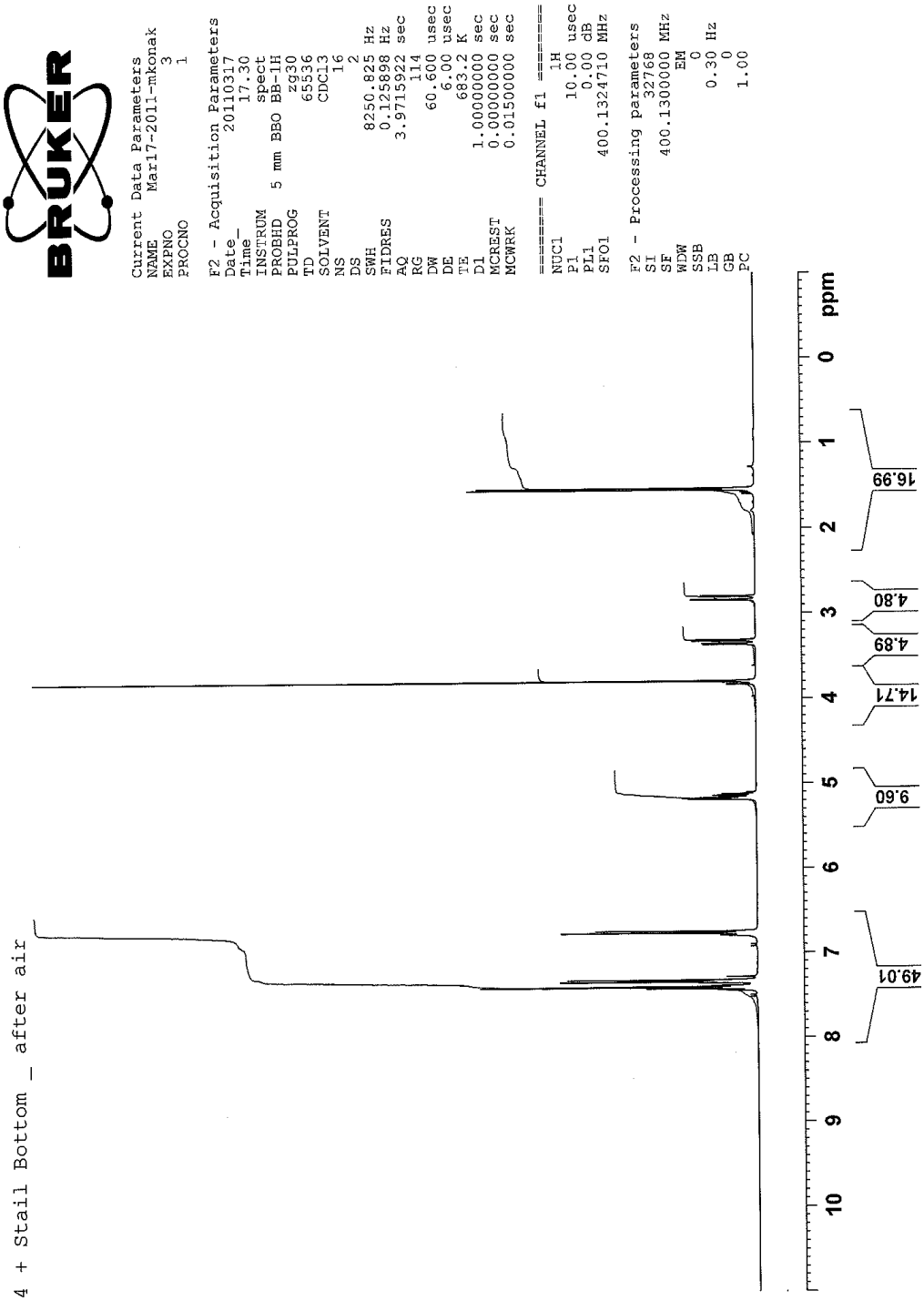


FIGURE 17: ^{1}H-NMR Spectrum of compound **16**.

APPENDIX B

^{13}C-NMR SPECTRUM OF COMPOUNDS

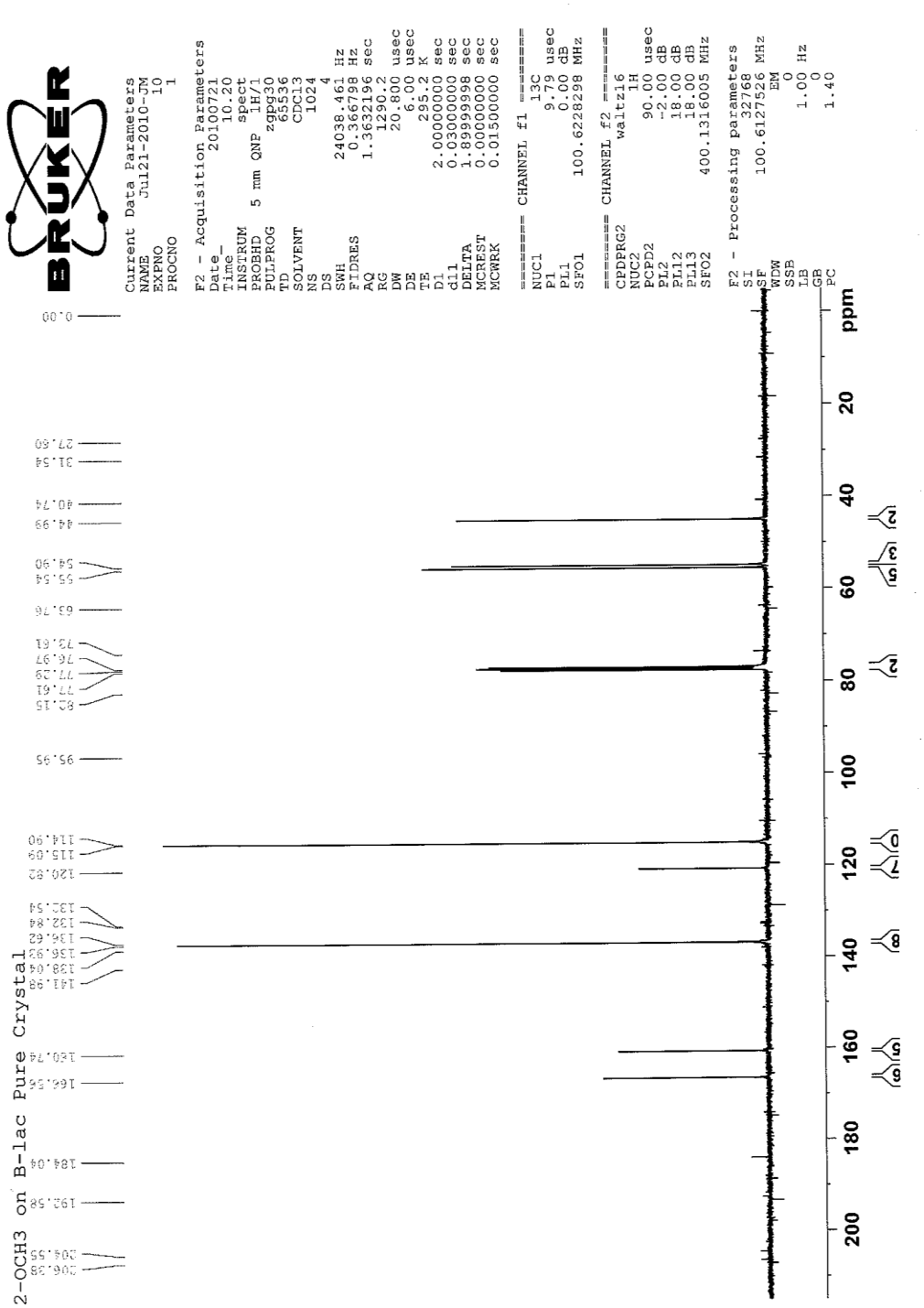


FIGURE 18: ^{13}C-NMR Spectrum of compound **1**.

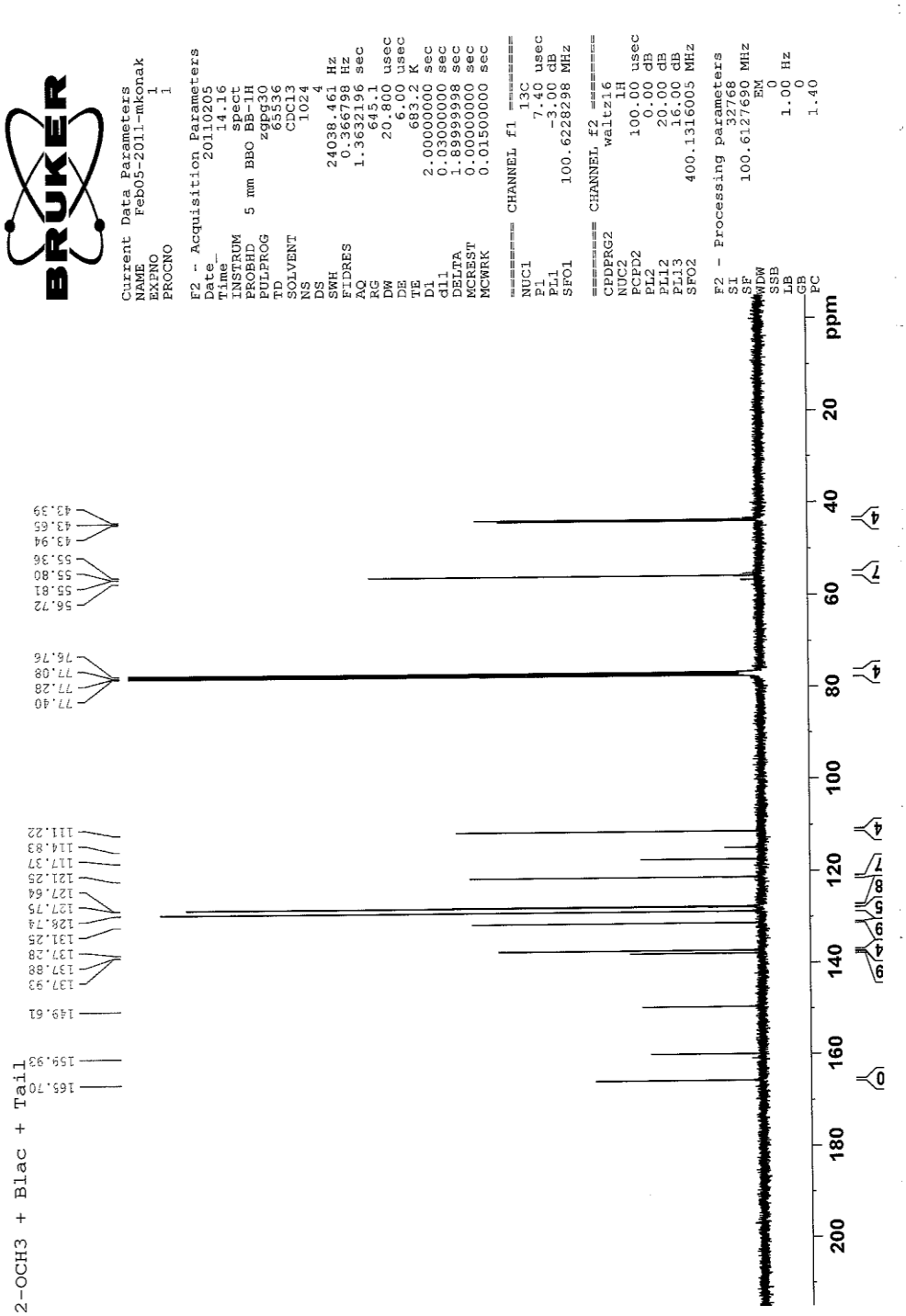


FIGURE 19: ^{13}C-NMR Spectrum of compound **2**.

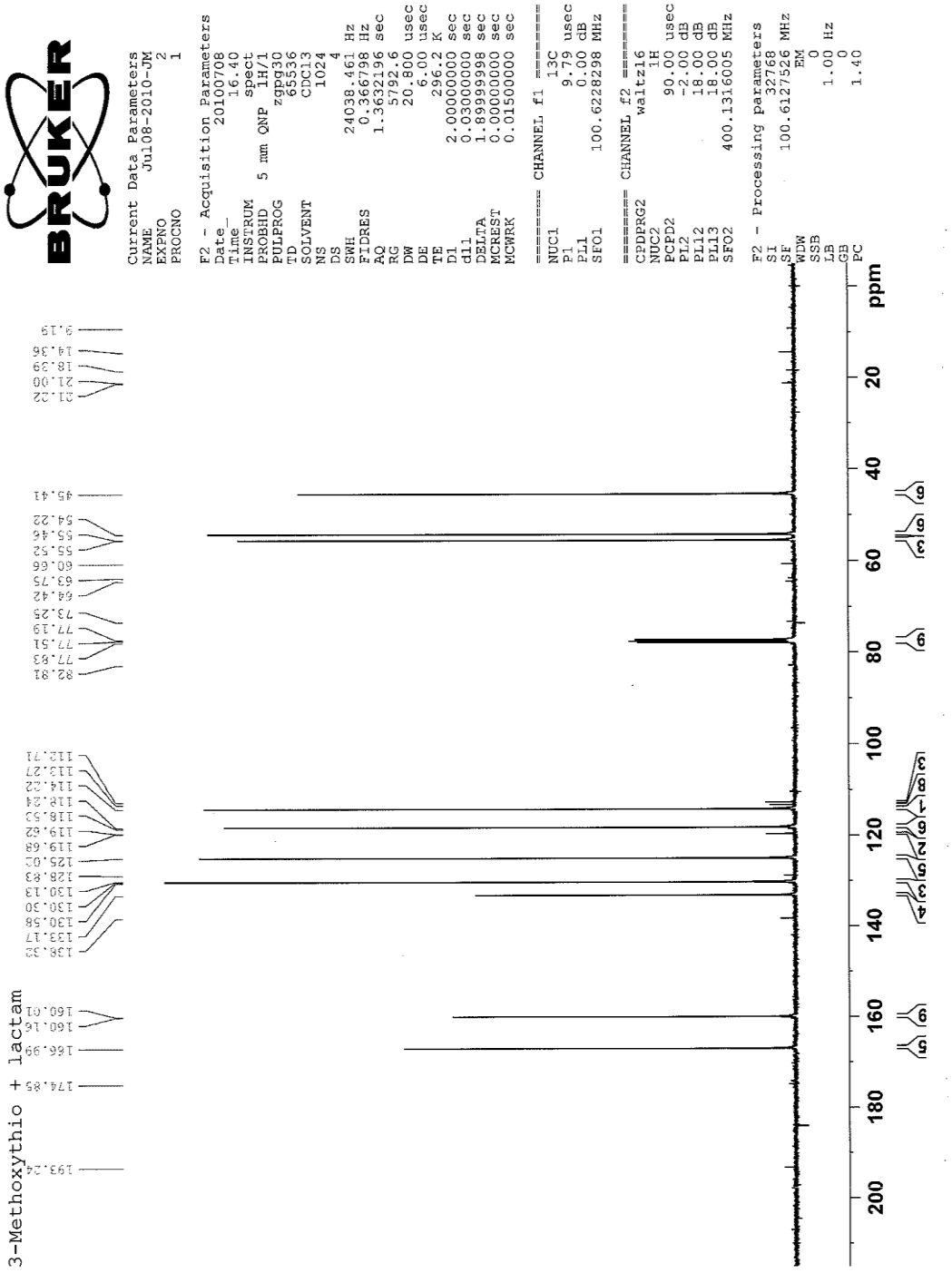


FIGURE 20: ^{13}C-NMR Spectrum of compound **3**.

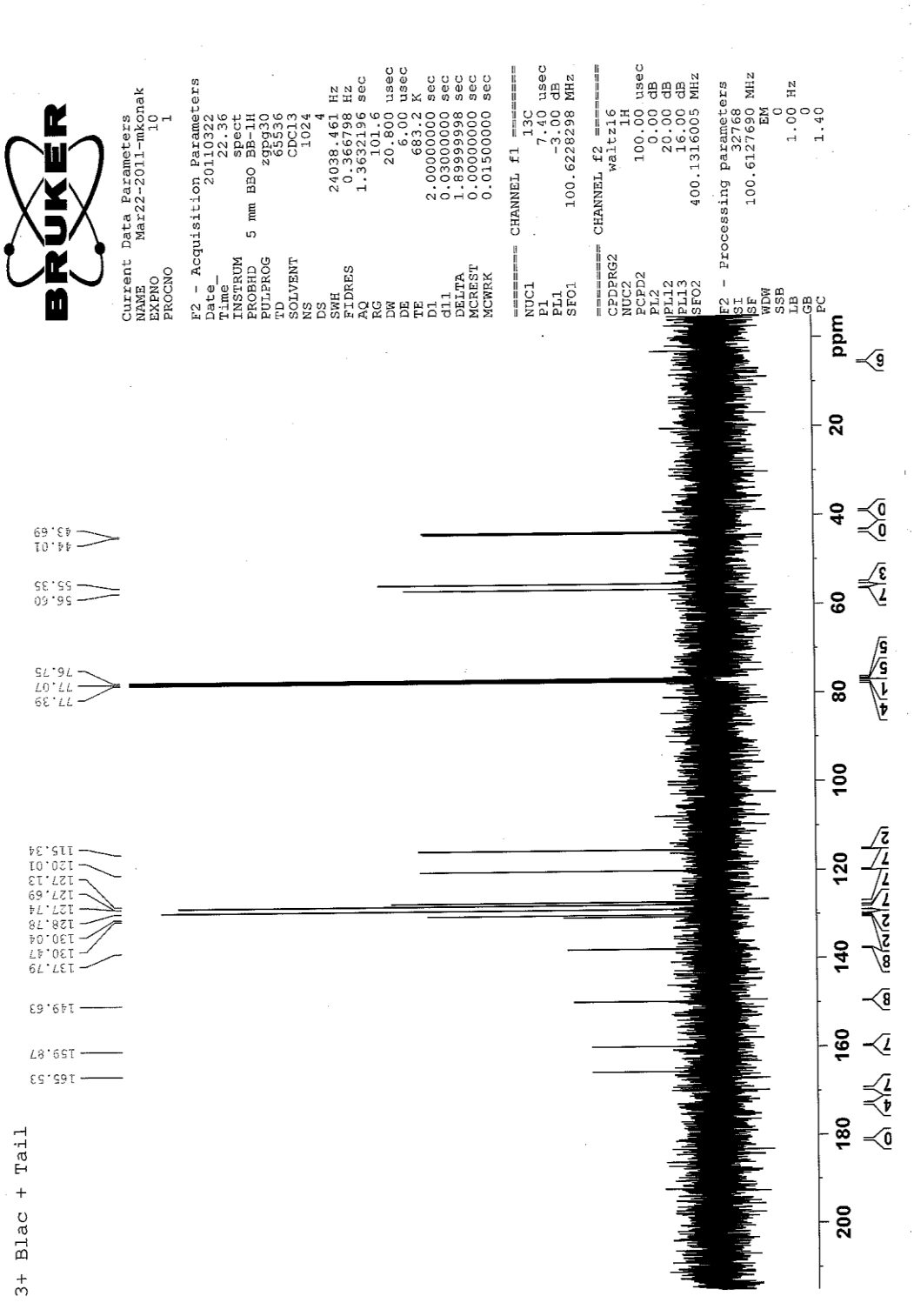


FIGURE 21: ^{13}C-NMR Spectrum of compound **4**.

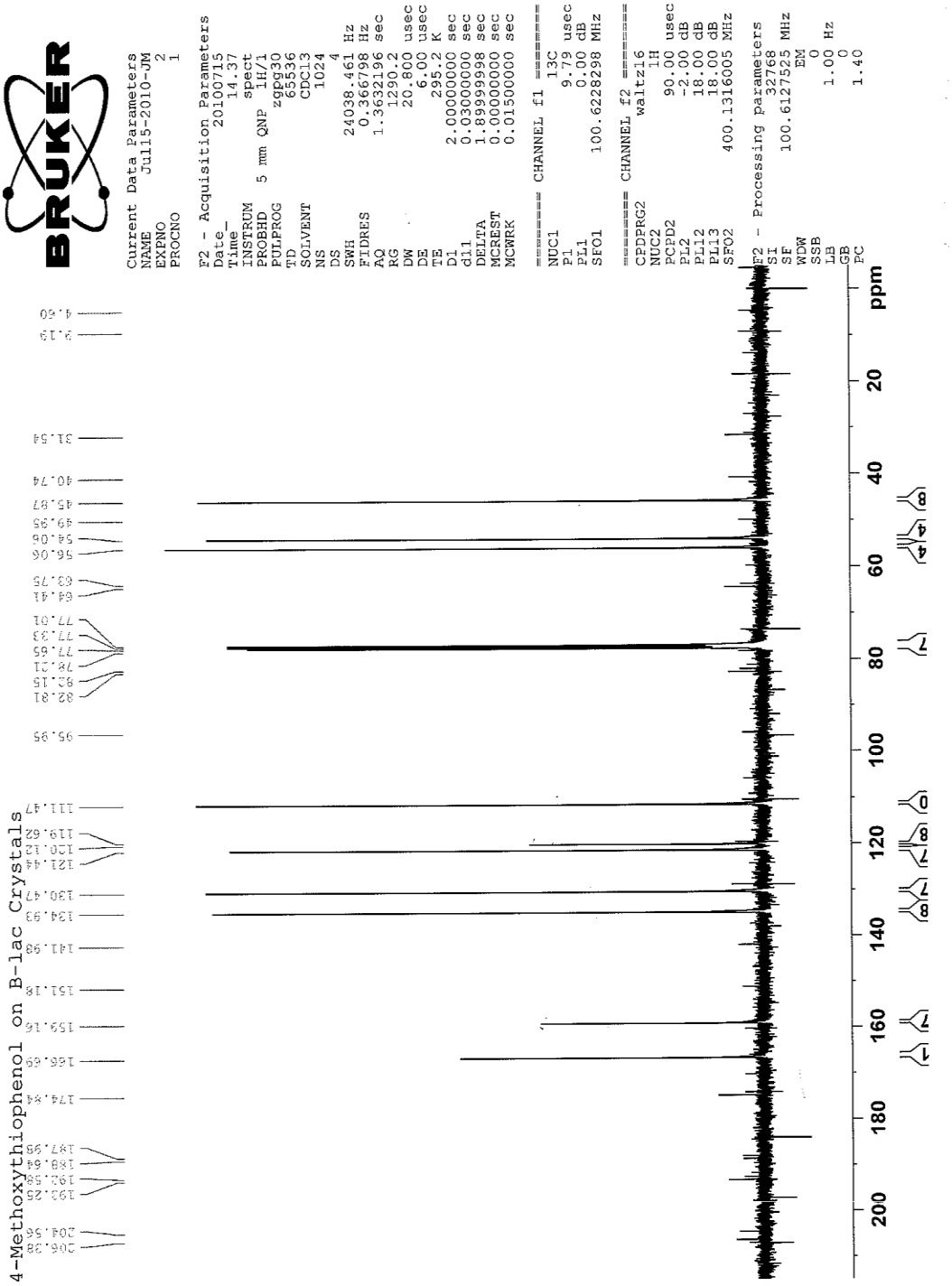


FIGURE 22: ^{13}C-NMR Spectrum of compound **5**.

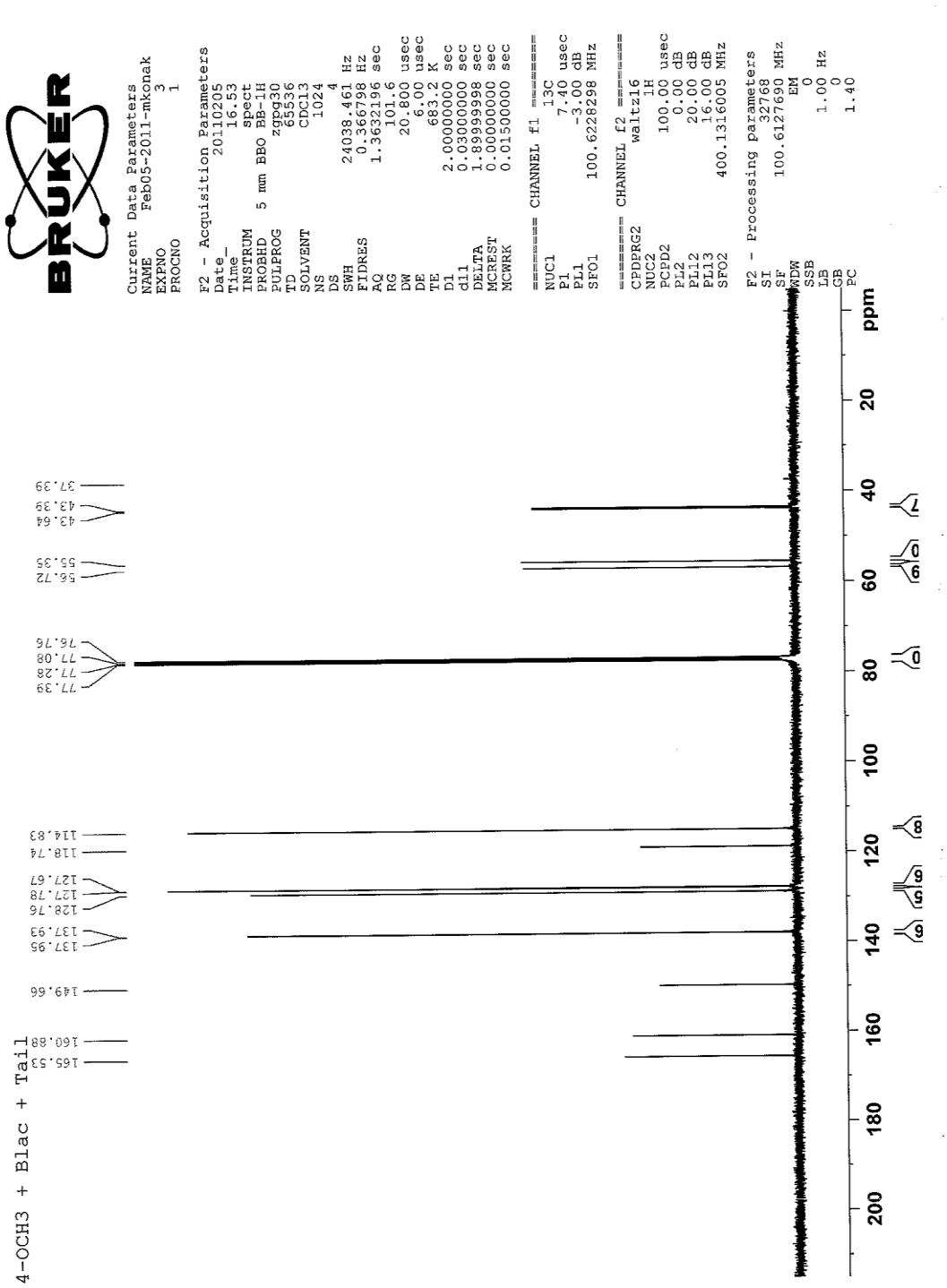


FIGURE 23: ^{13}C-NMR Spectrum of compound **6**.

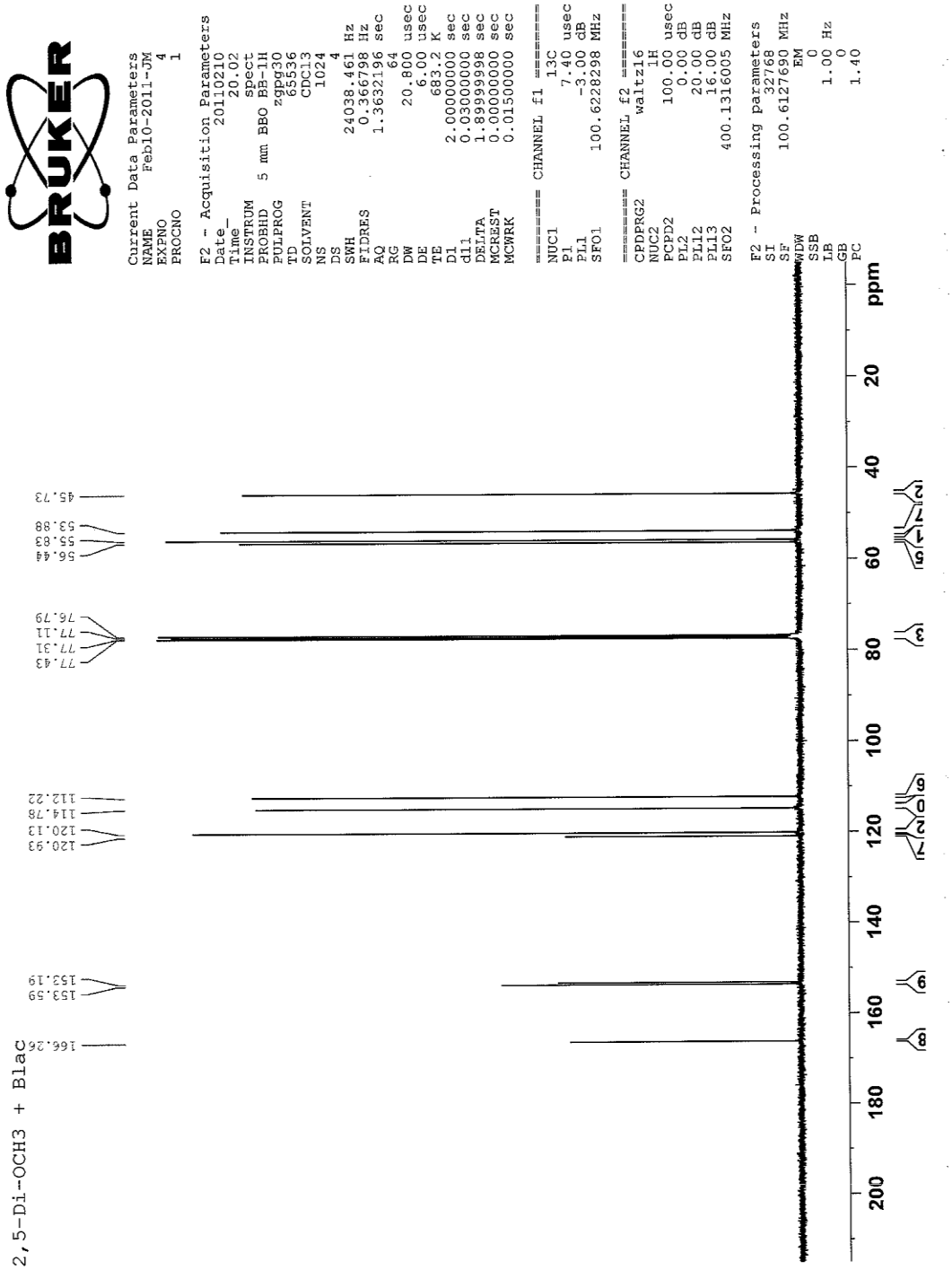


FIGURE 24: ^{13}C-NMR Spectrum of compound **7**.

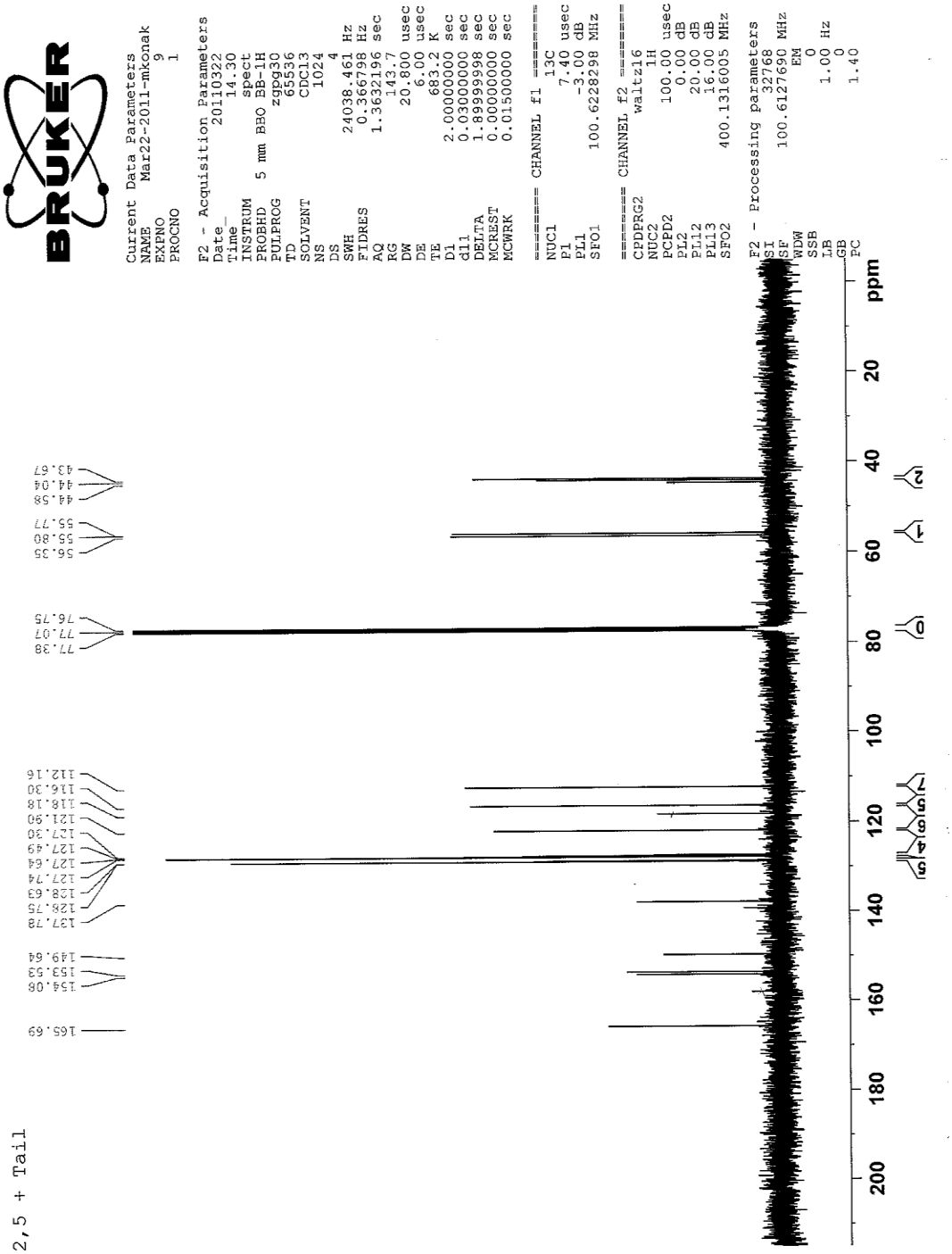


FIGURE 25: ^{13}C-NMR Spectrum of compound **8**.

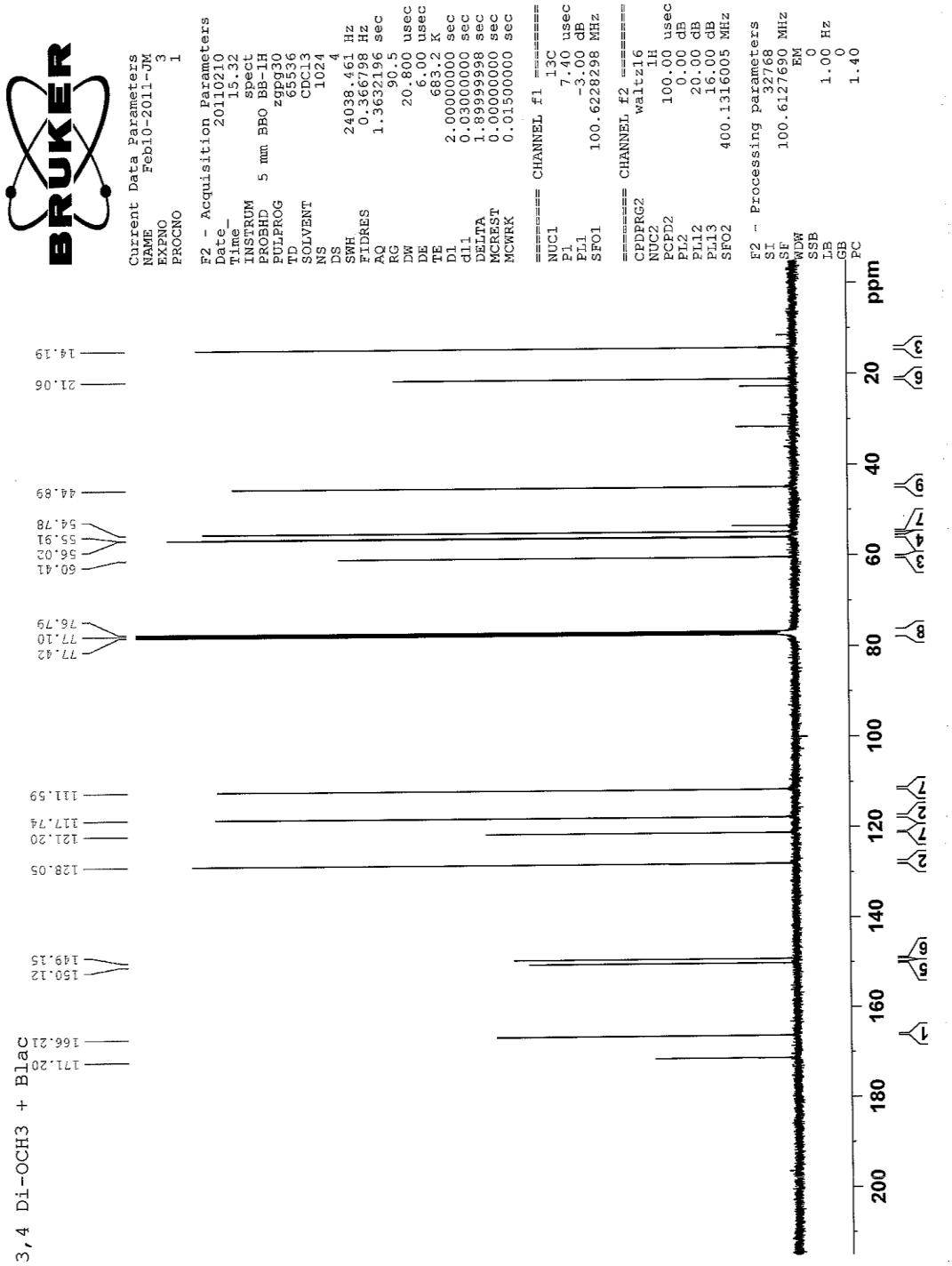


FIGURE 26: ^{13}C-NMR Spectrum of compound **9**.

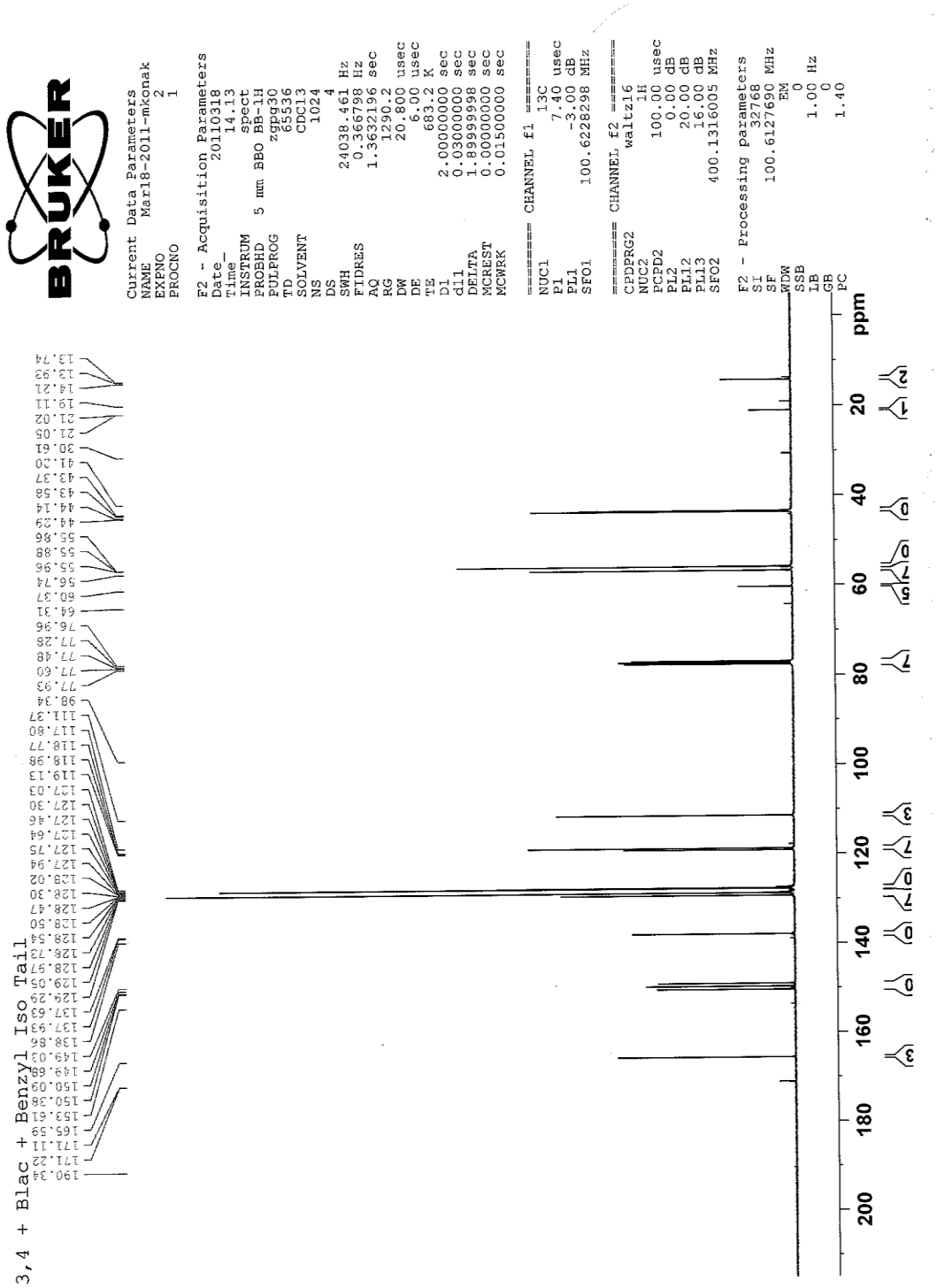


FIGURE 27: ^{13}C-NMR Spectrum of compound **10**.

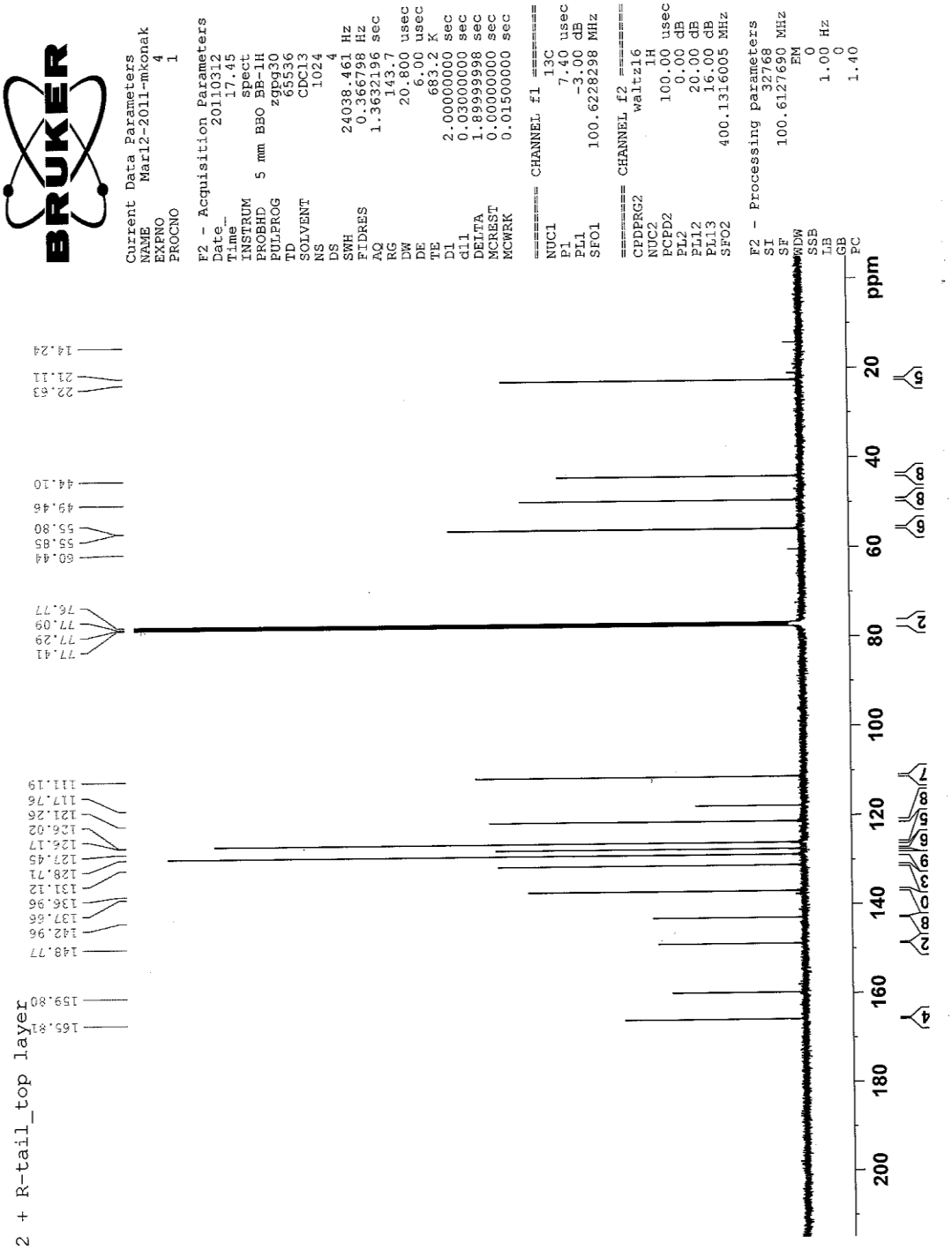


FIGURE 28: ^{13}C-NMR Spectrum of compound **11**.

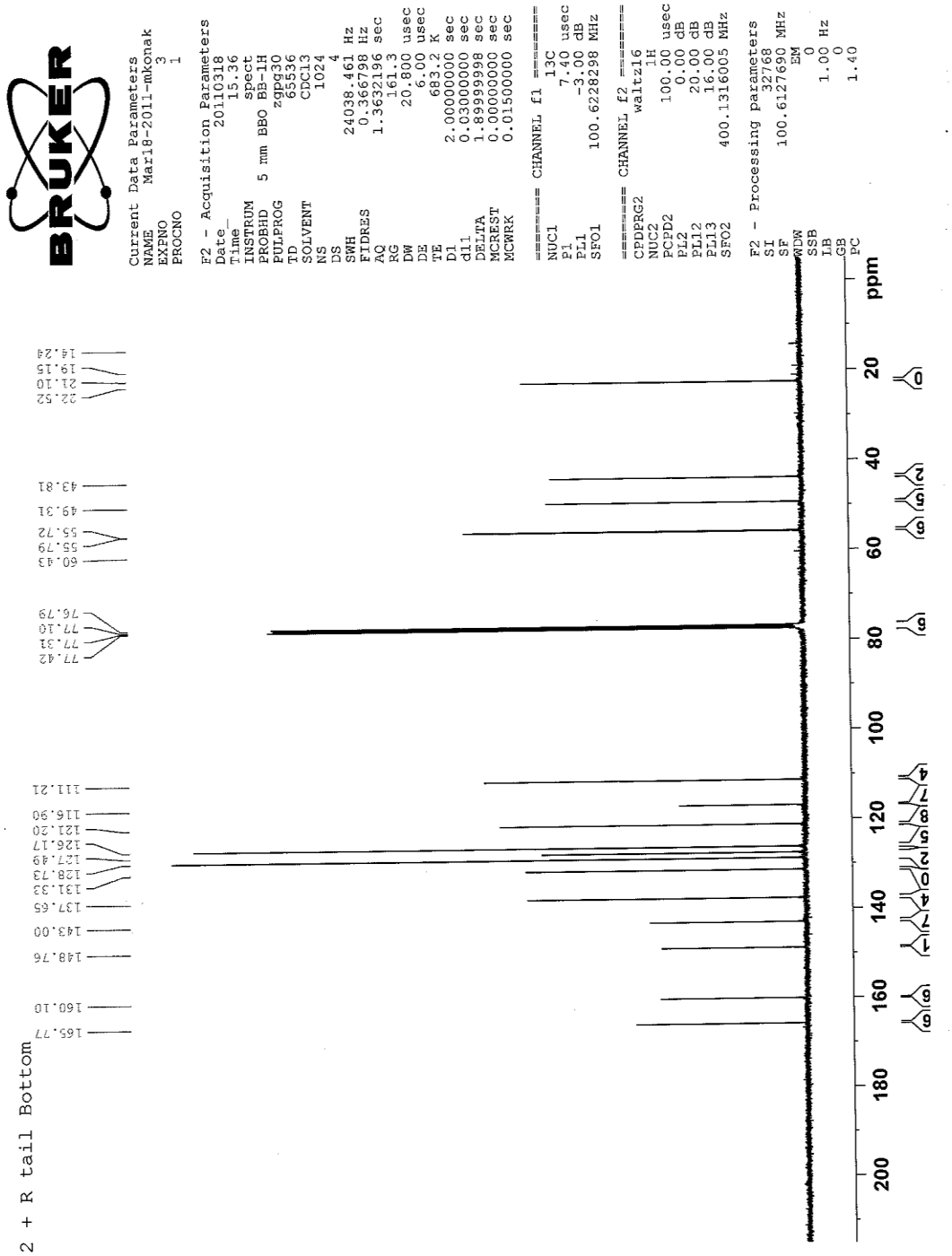


FIGURE 29: ^{13}C-NMR Spectrum of compound **12**.

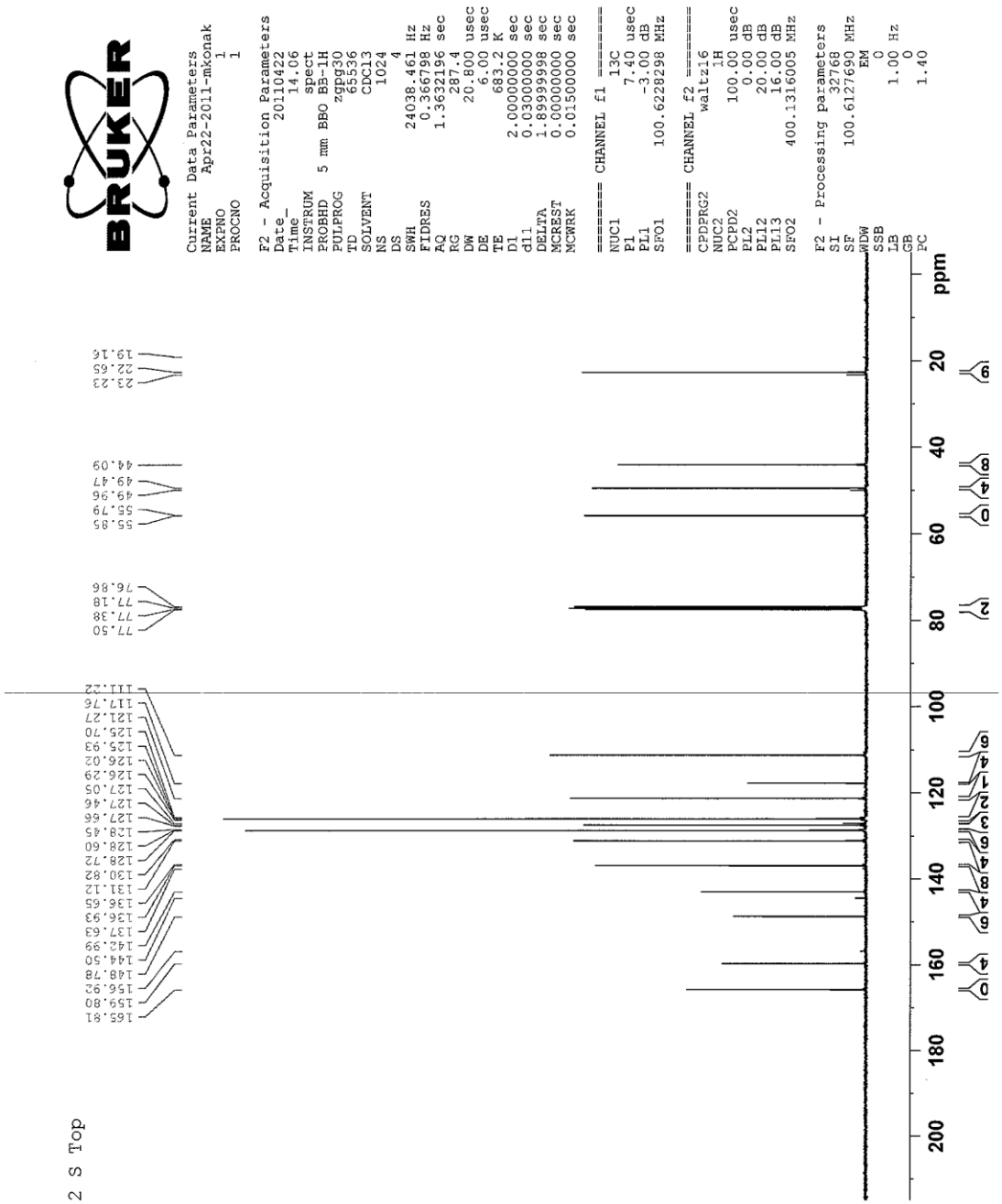


FIGURE 30: ^{13}C-NMR Spectrum of compound **13**.

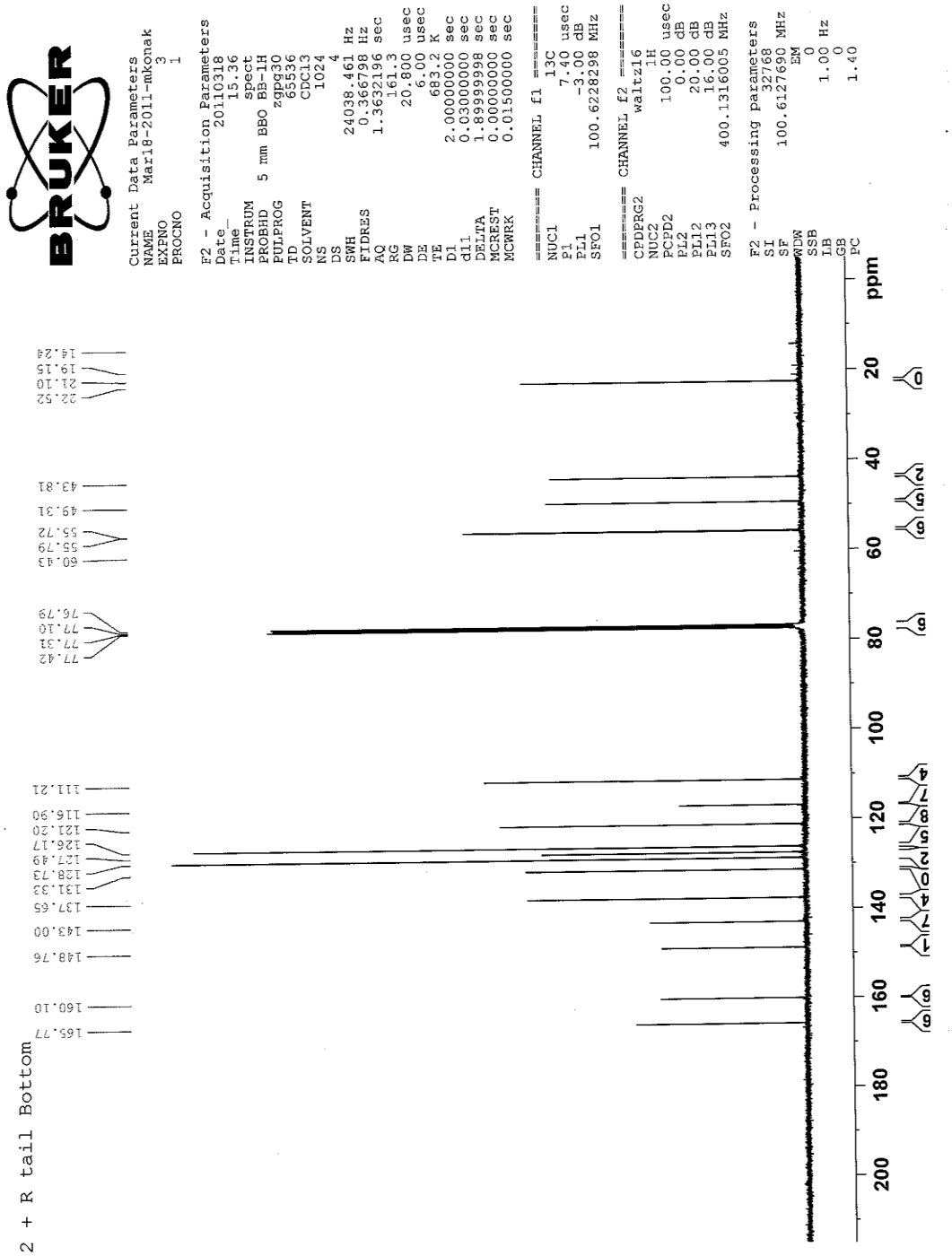


FIGURE 31: ^{13}C-NMR Spectrum of compound **14**.

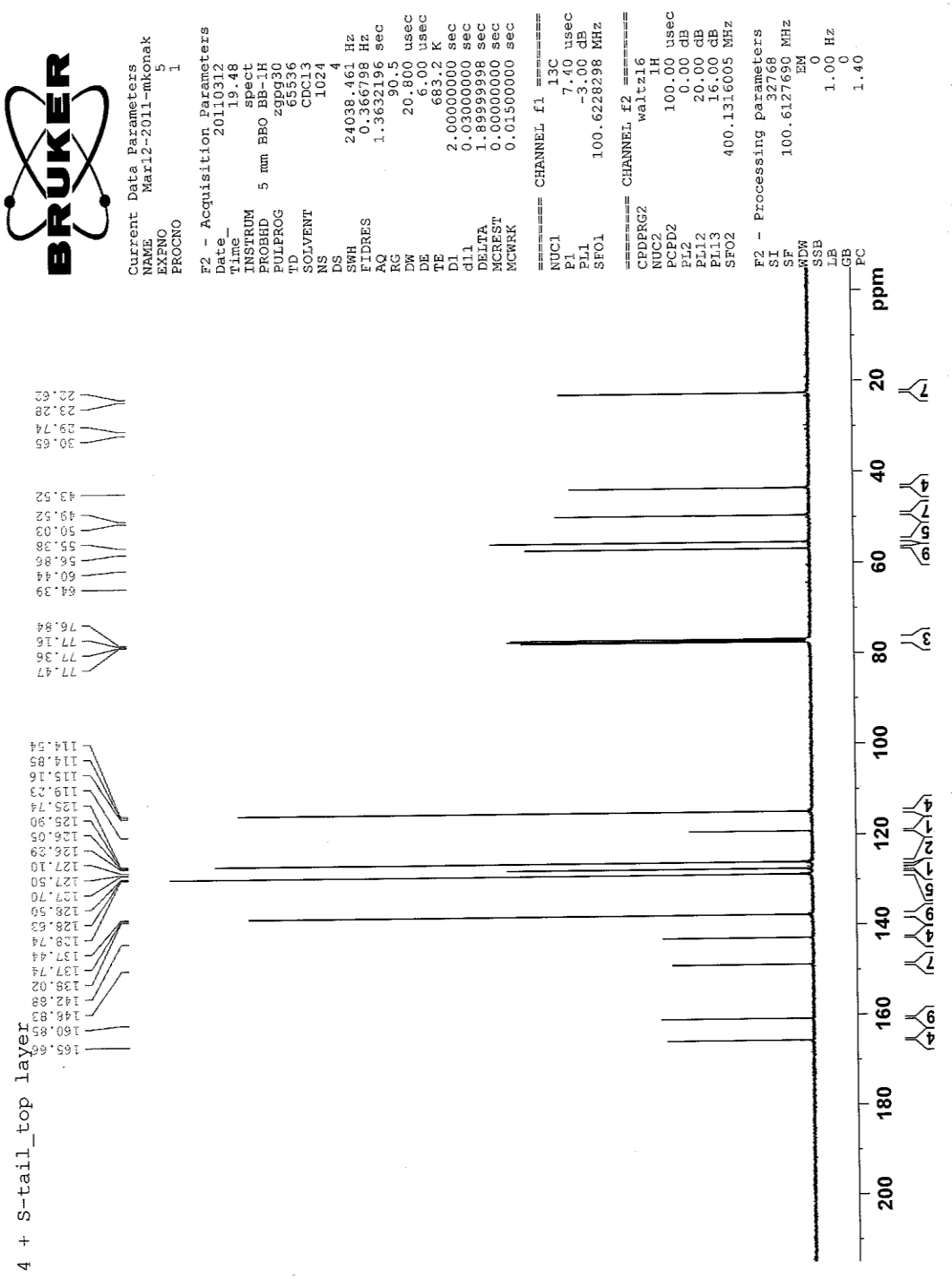


FIGURE 32: ^{13}C-NMR Spectrum of compound **15**.

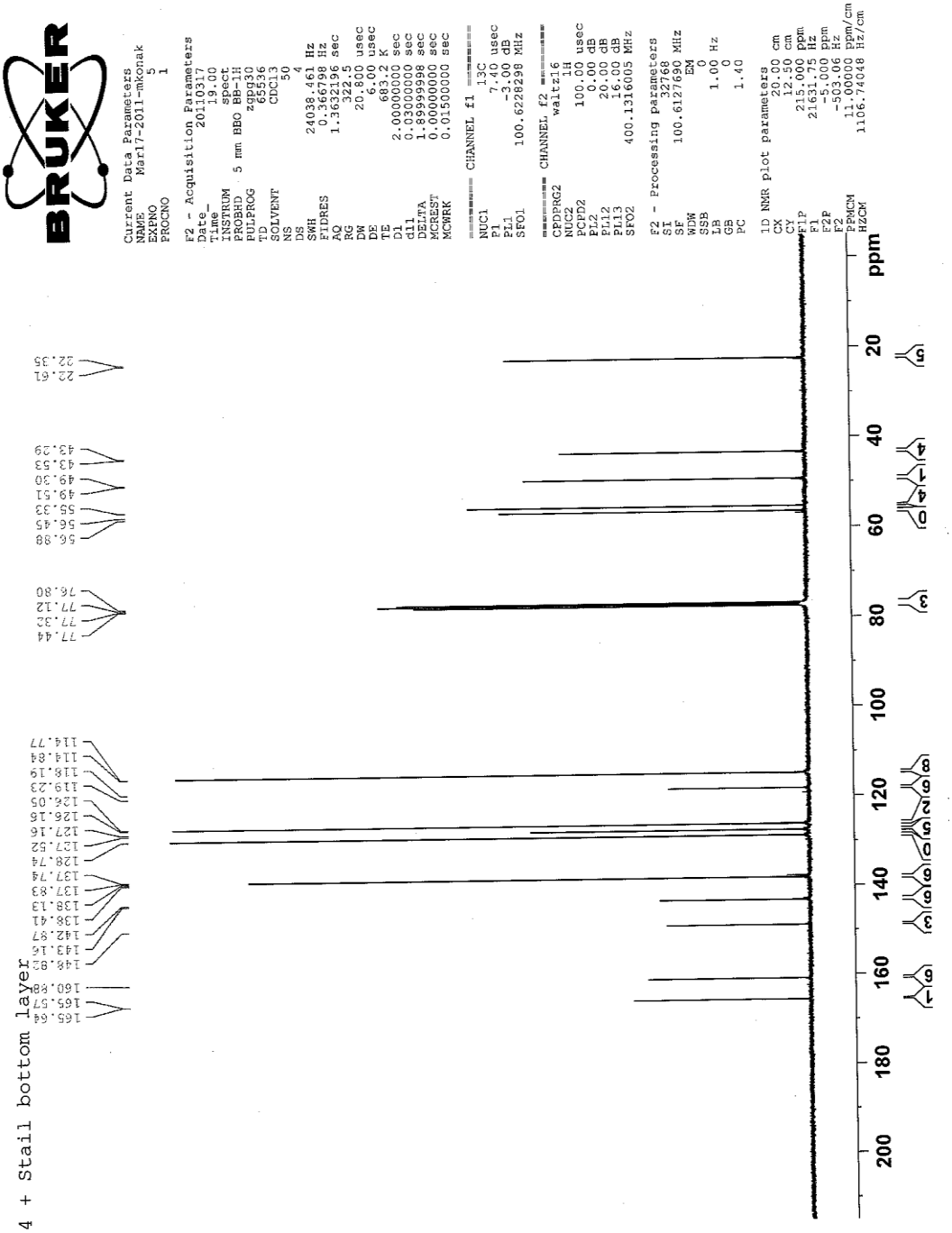


FIGURE 33: ^{13}C-NMR Spectrum of compound **16**.

APPENDIX C

FT-IR SPECTRUM OF COMPOUNDS

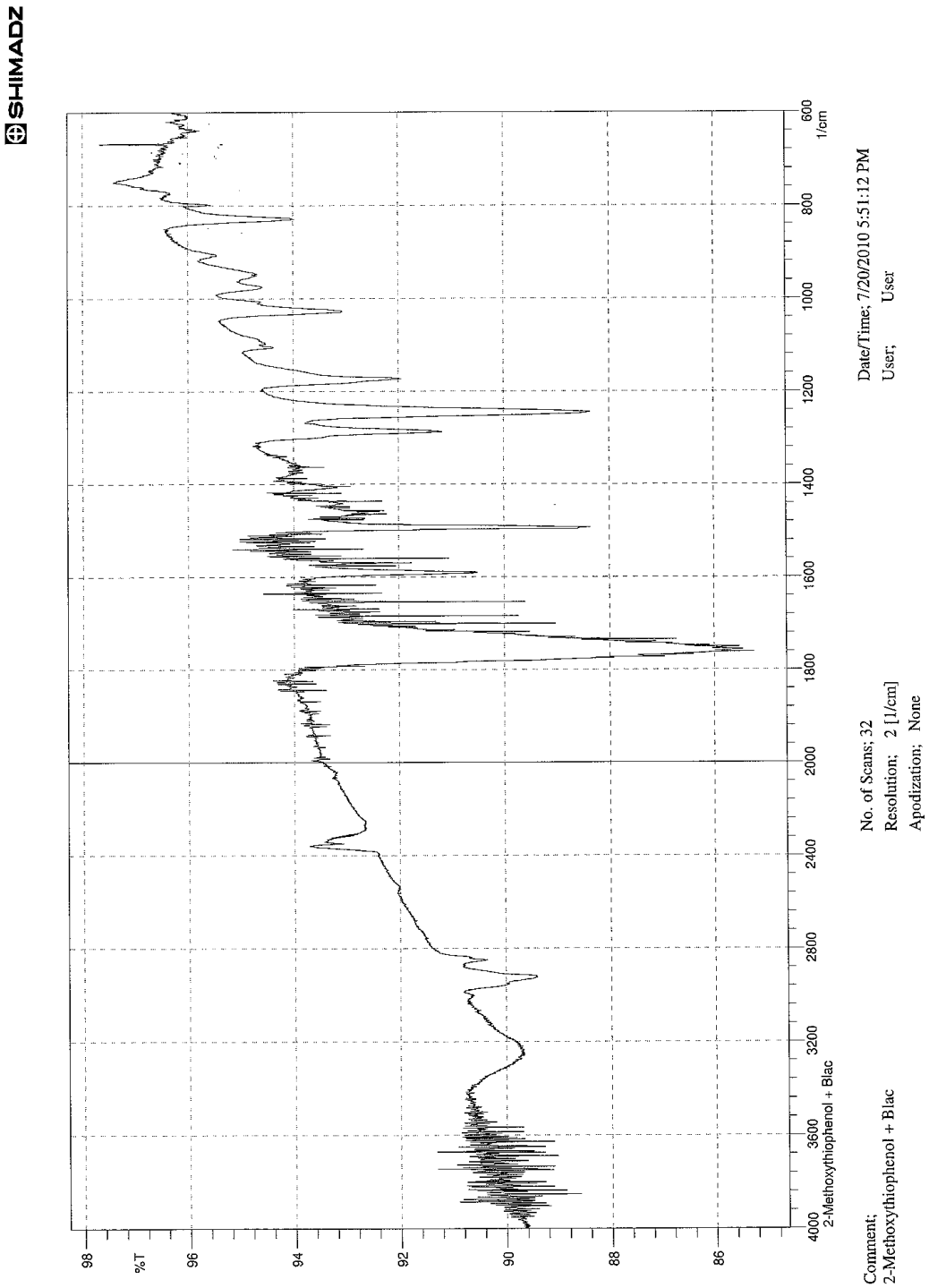


FIGURE 34: FT-IR Spectrum of compound **1**.

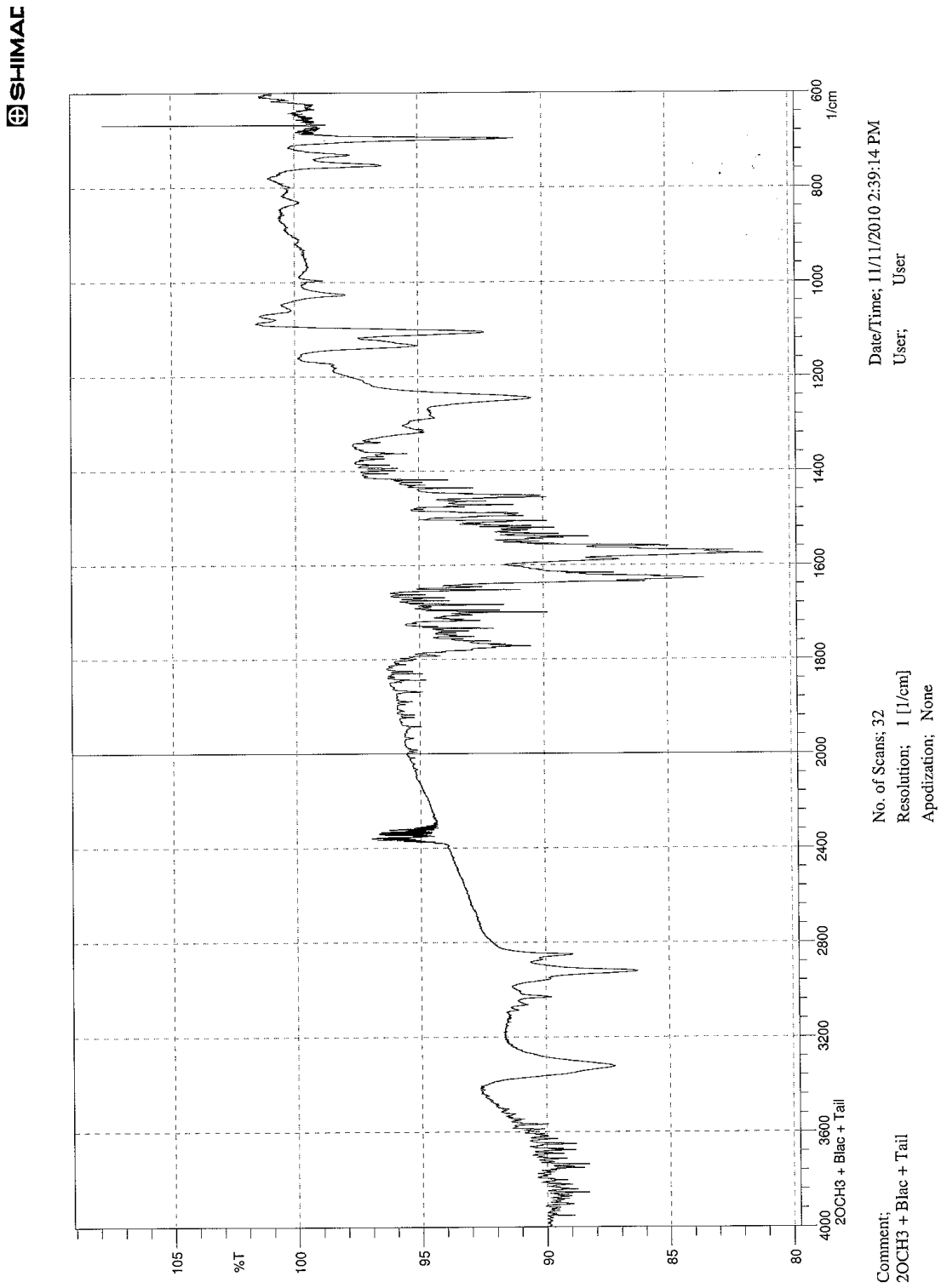


FIGURE 35: FT-IR Spectrum of compound **2**.

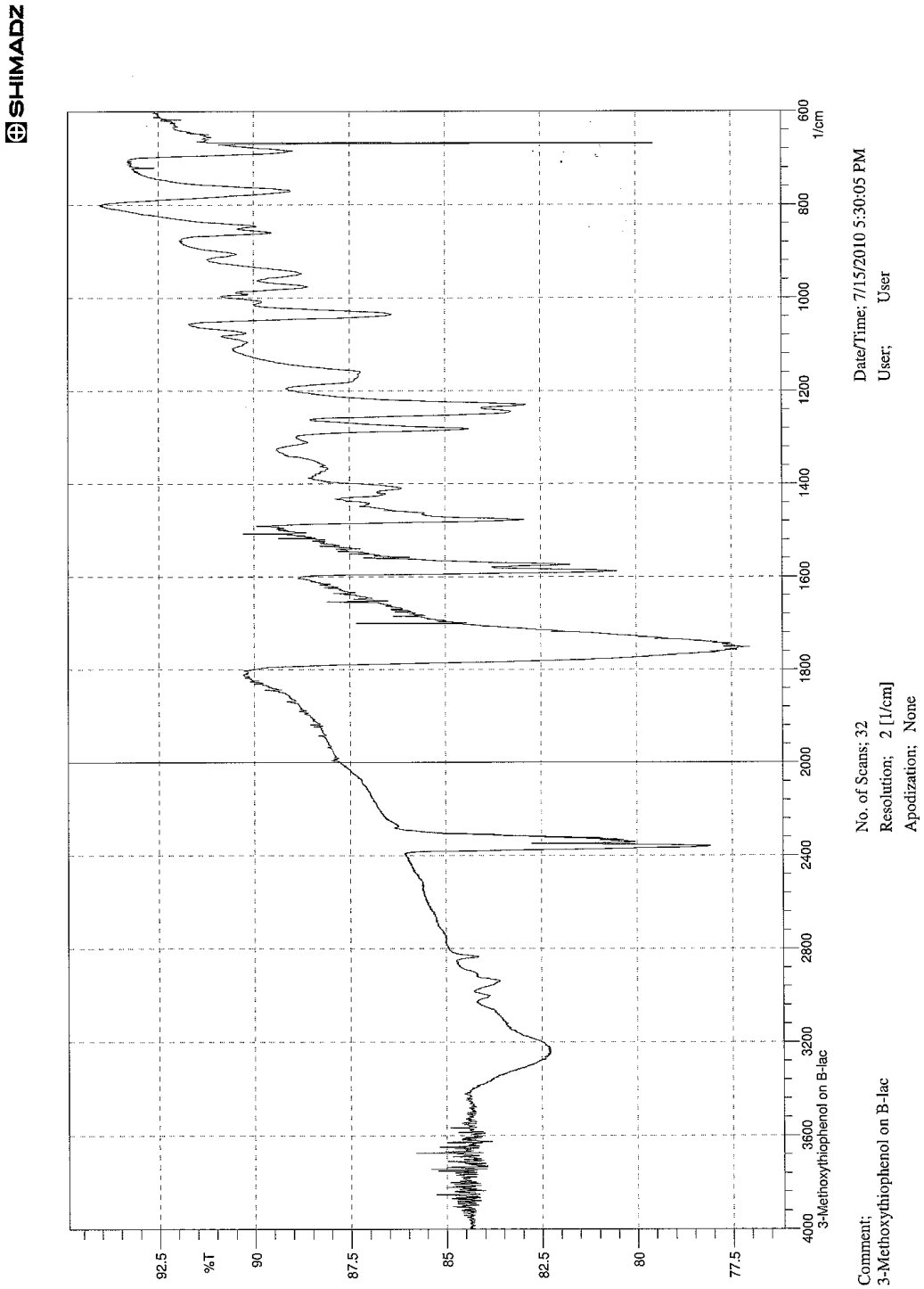


FIGURE 36: FT-IR Spectrum of compound **3**.

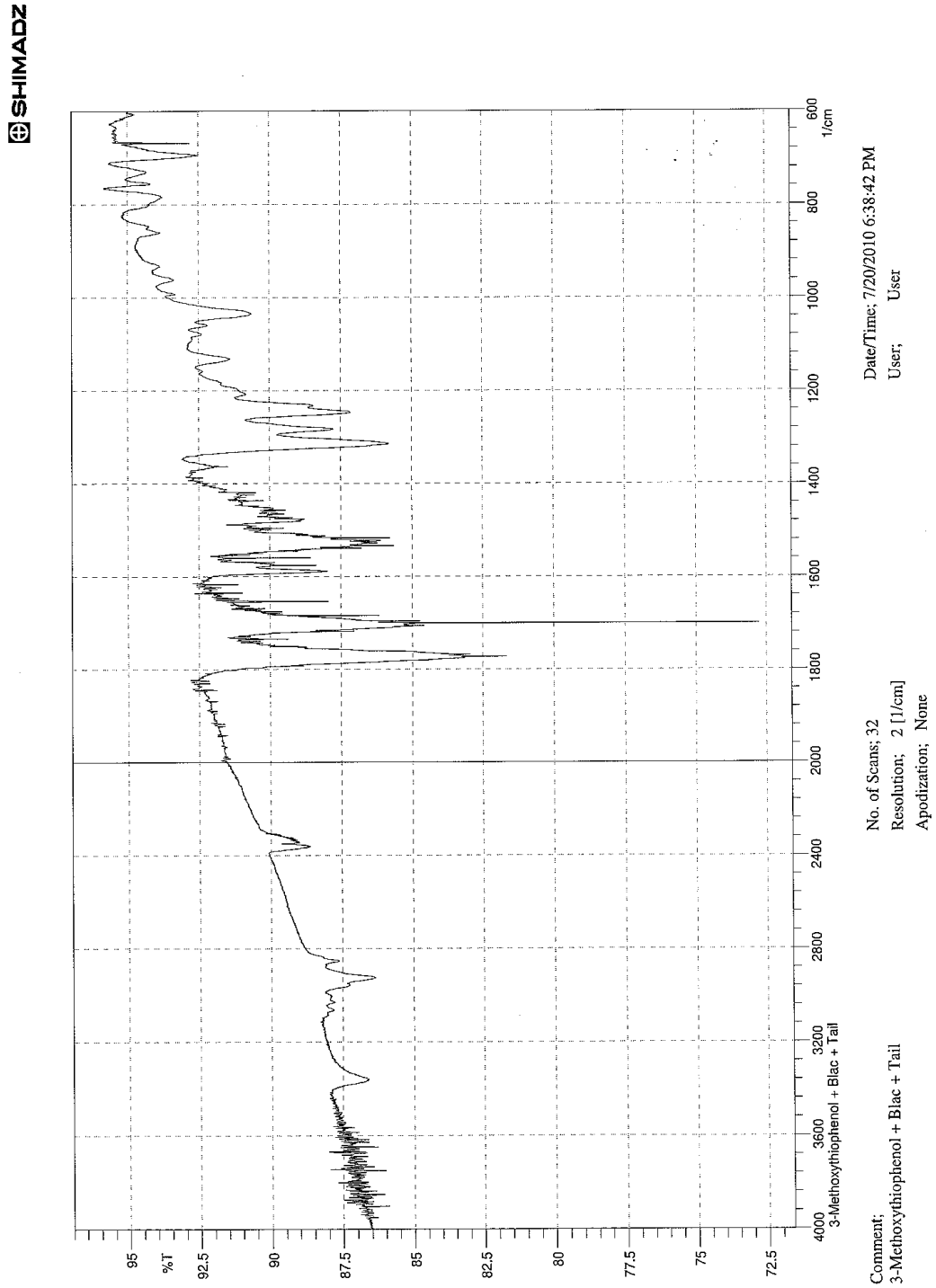


FIGURE 37: FT-IR Spectrum of compound **4**.

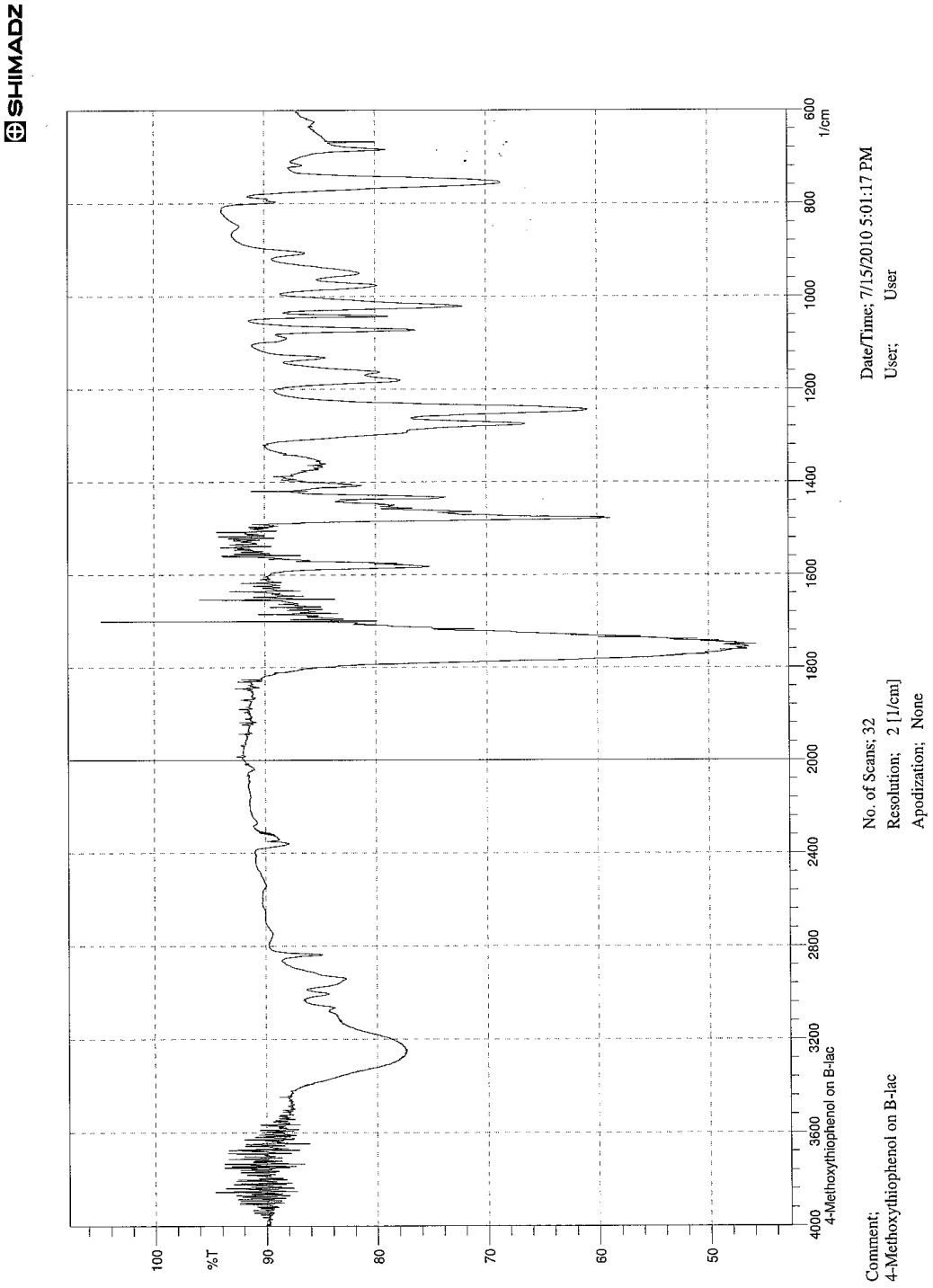


FIGURE 38: FT-IR Spectrum of compound **5**.

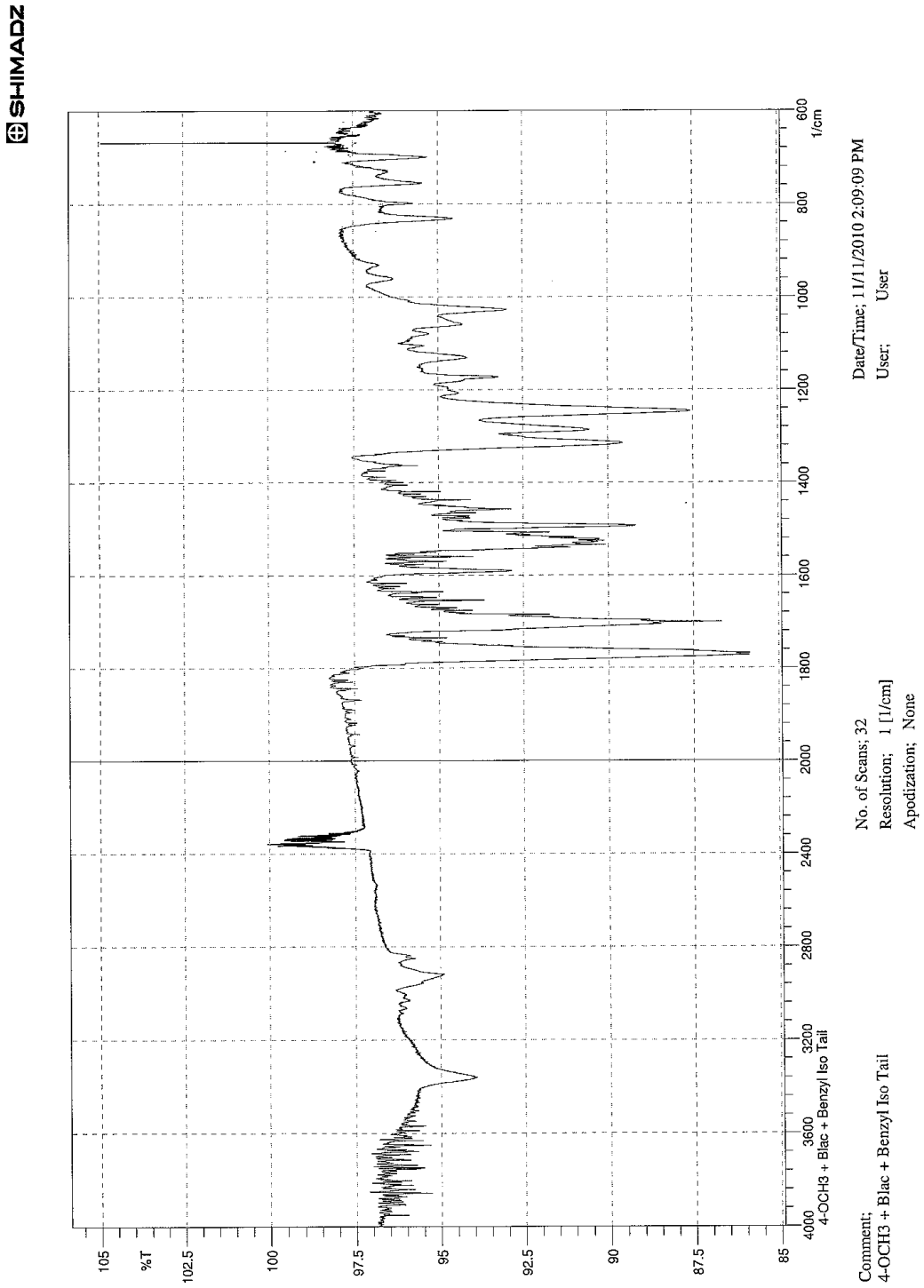


FIGURE 39: FT-IR Spectrum of compound **6.**

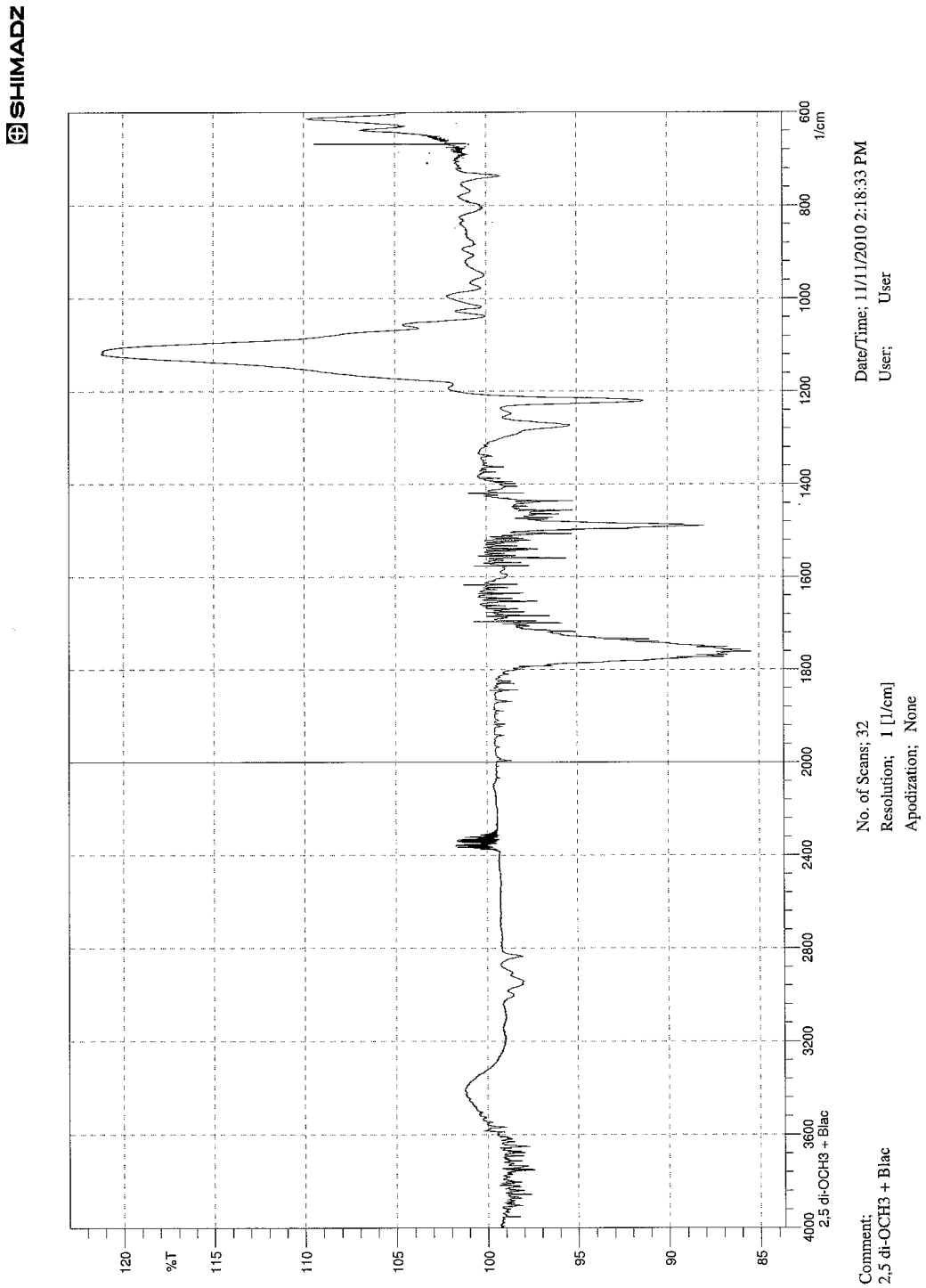


FIGURE 40: FT-IR Spectrum of compound **7**.

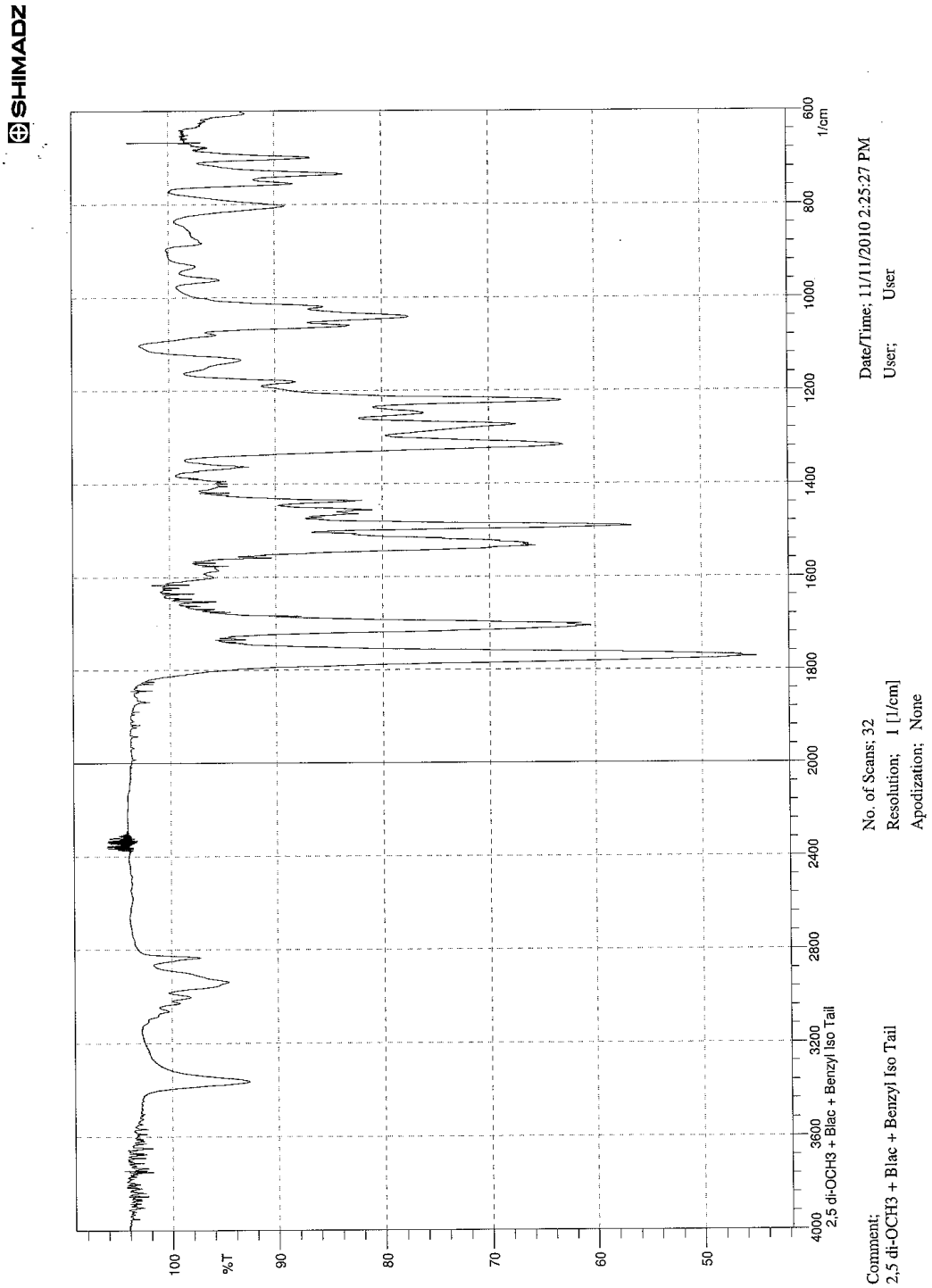


FIGURE 41: FT-IR Spectrum of compound **8**.

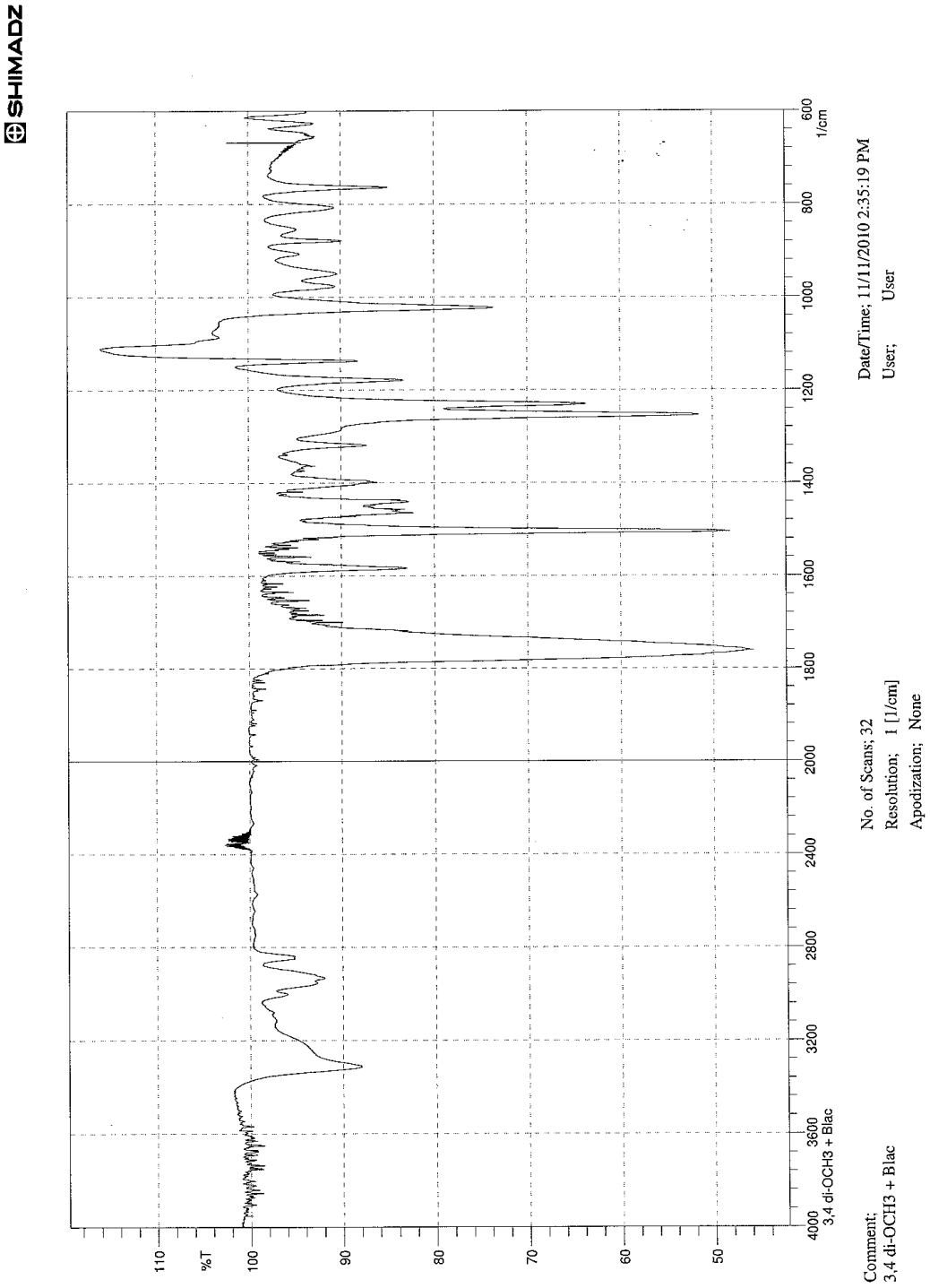


FIGURE 42: FT-IR Spectrum of compound **9**.

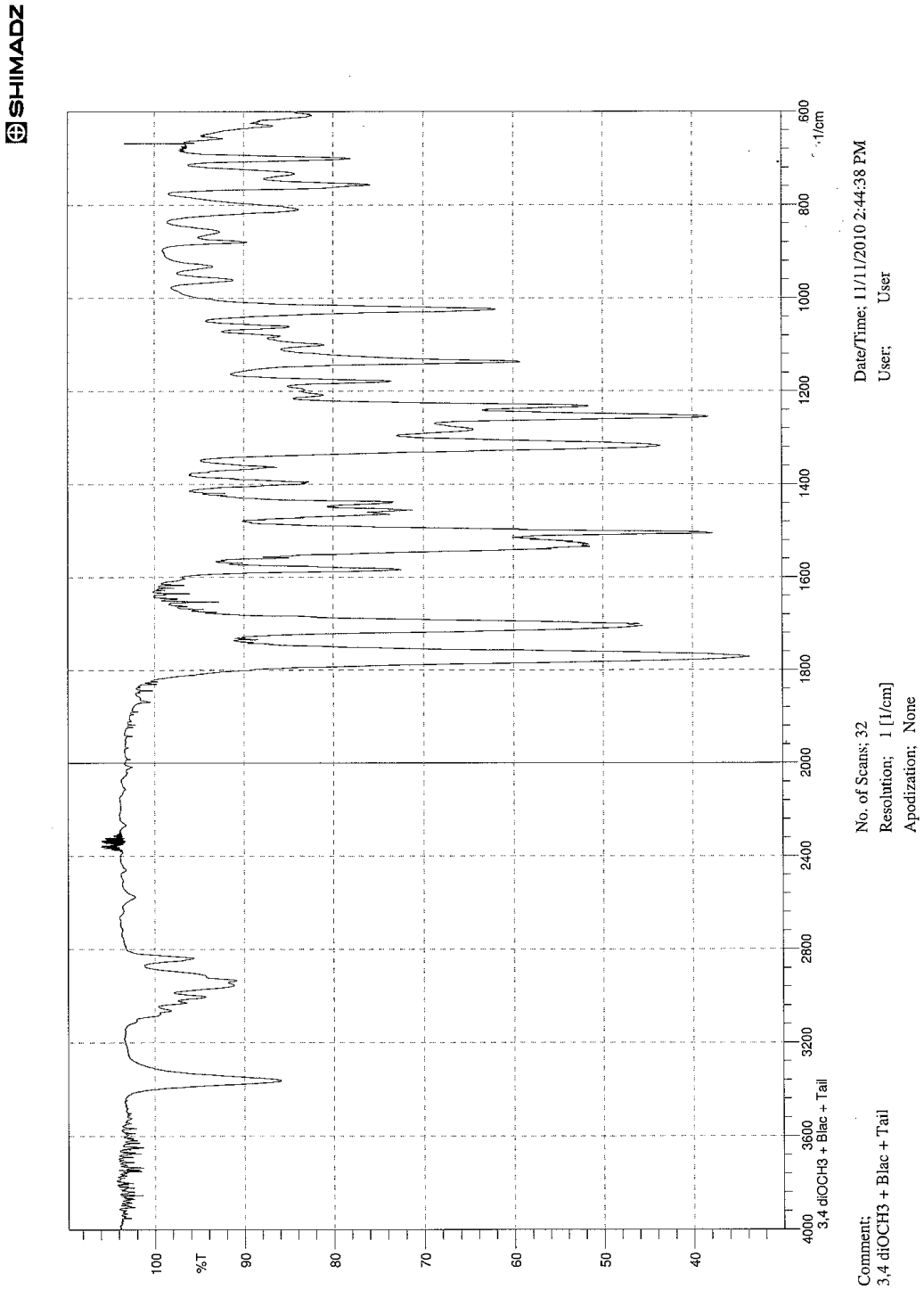


FIGURE 43: FT-IR Spectrum of compound **10**.

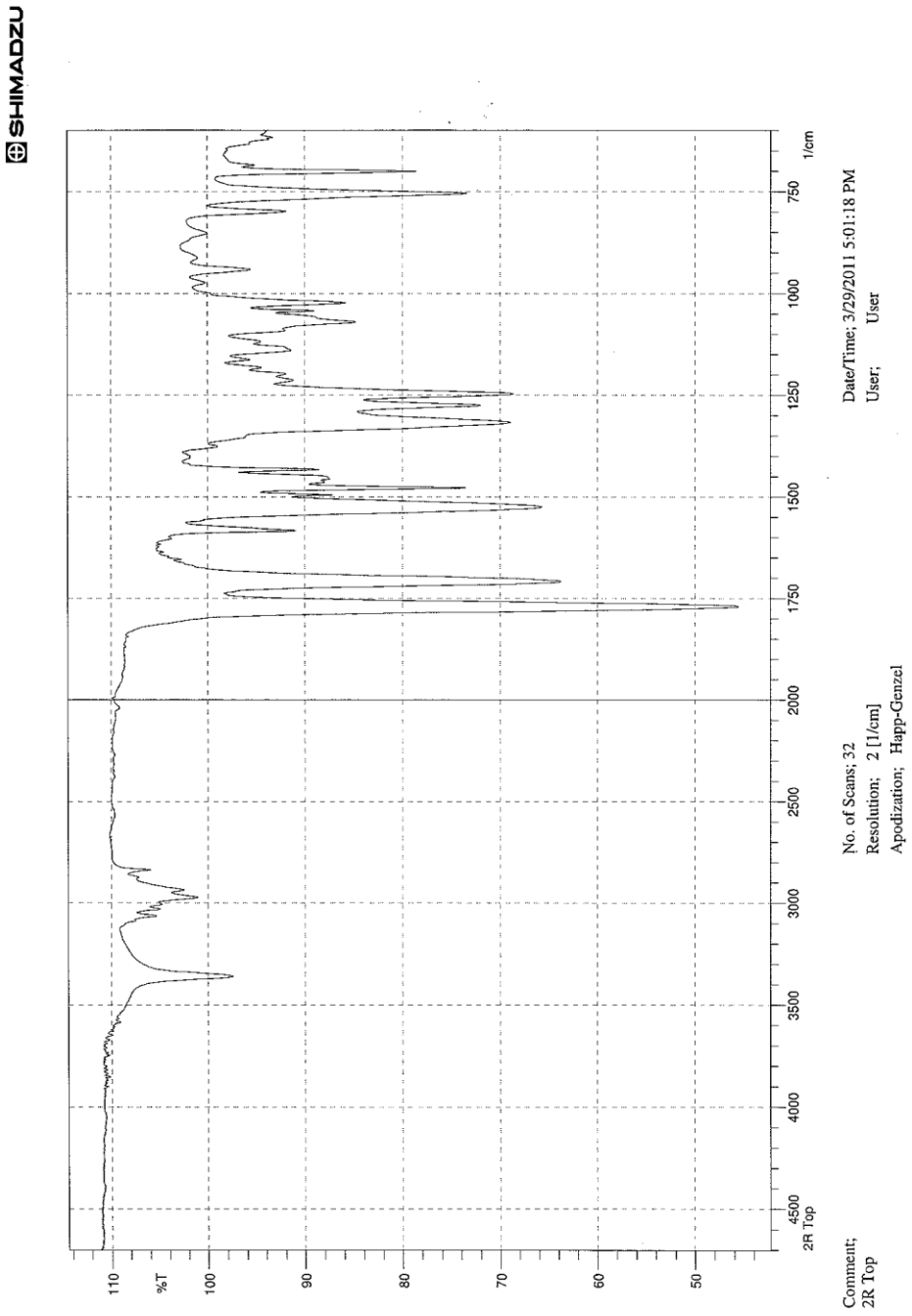


FIGURE 44: FT-IR Spectrum of compound **11**.

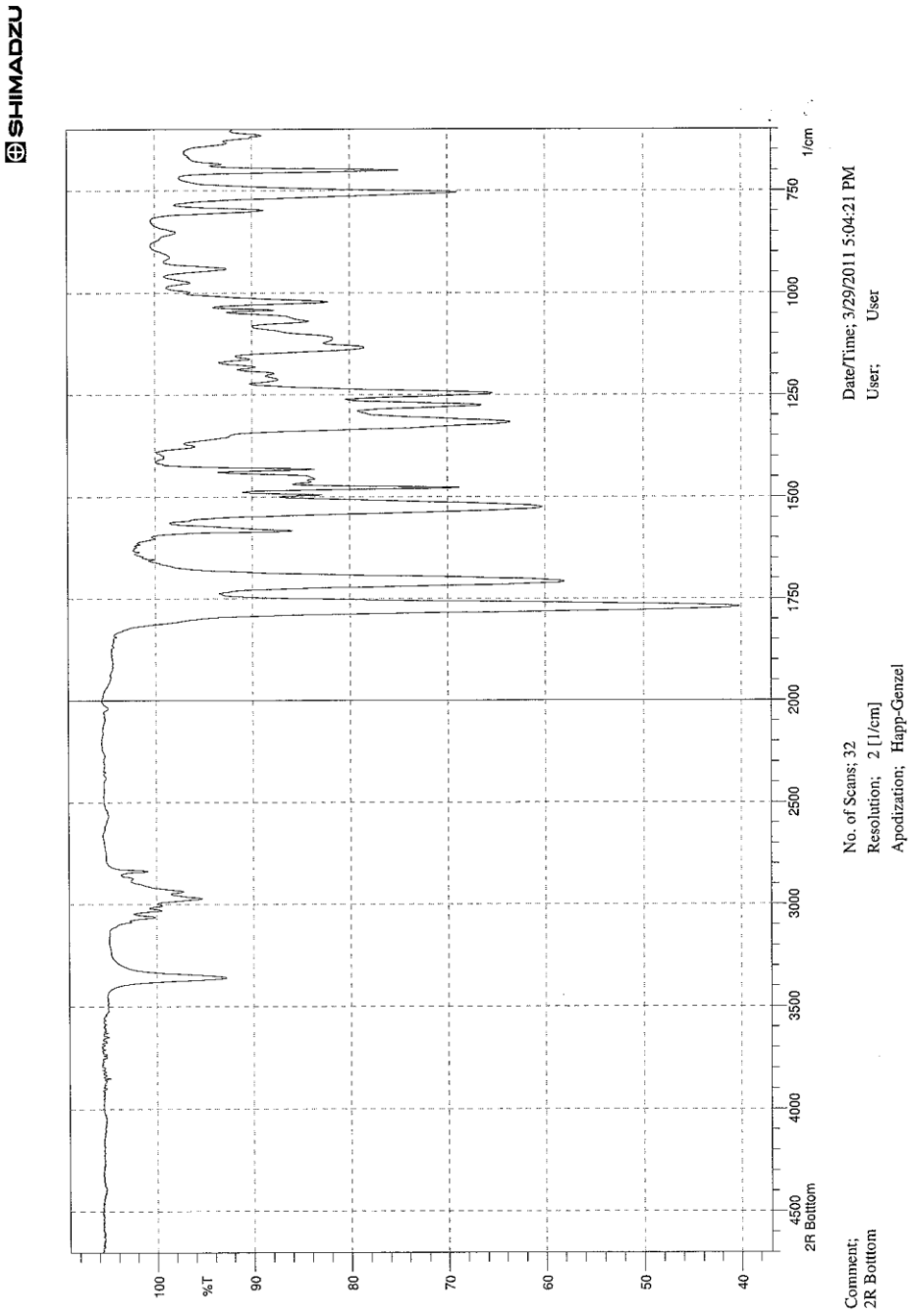


FIGURE 45: FT-IR Spectrum of compound **12**.

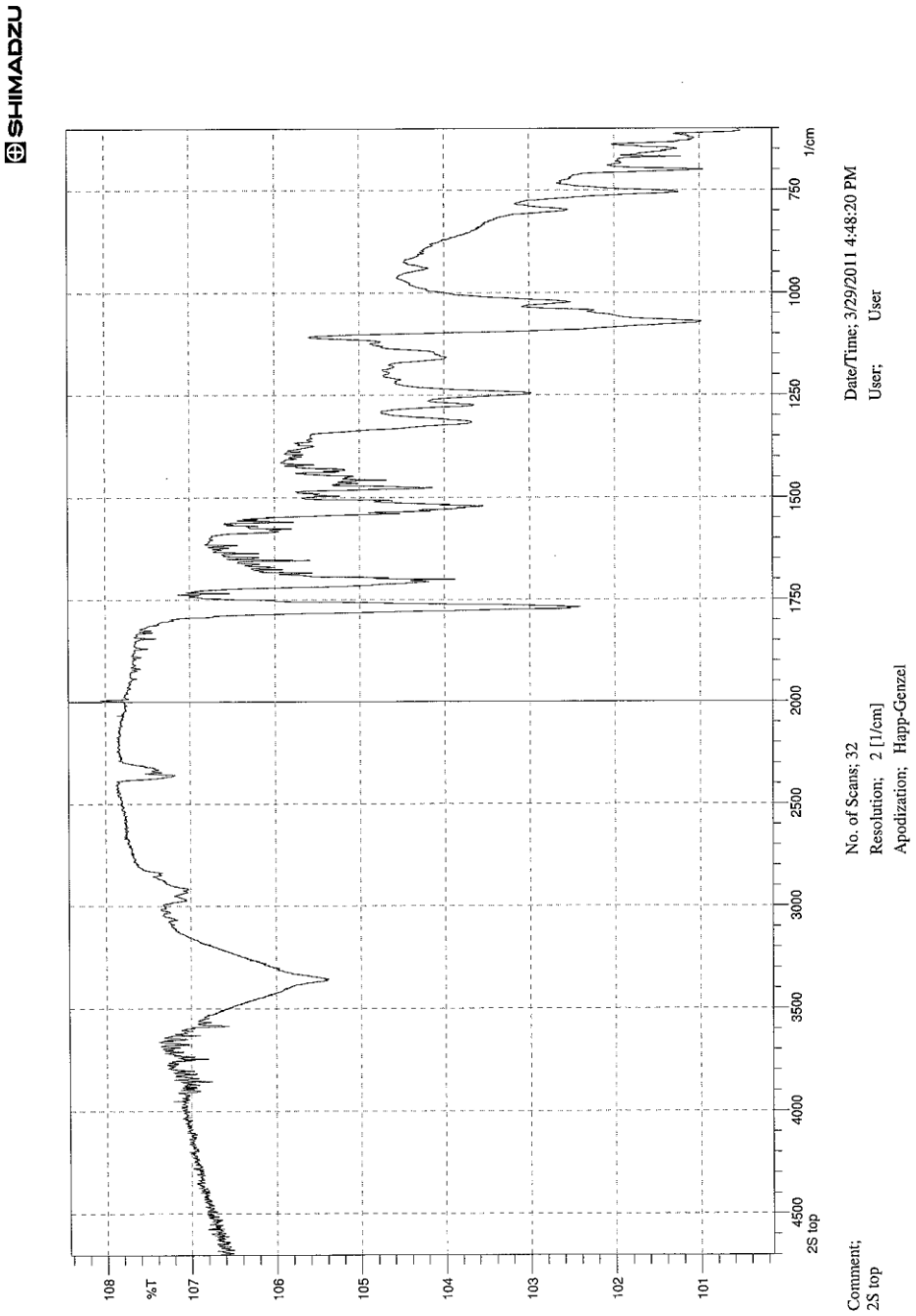


FIGURE 46: FT-IR Spectrum of compound **13**.

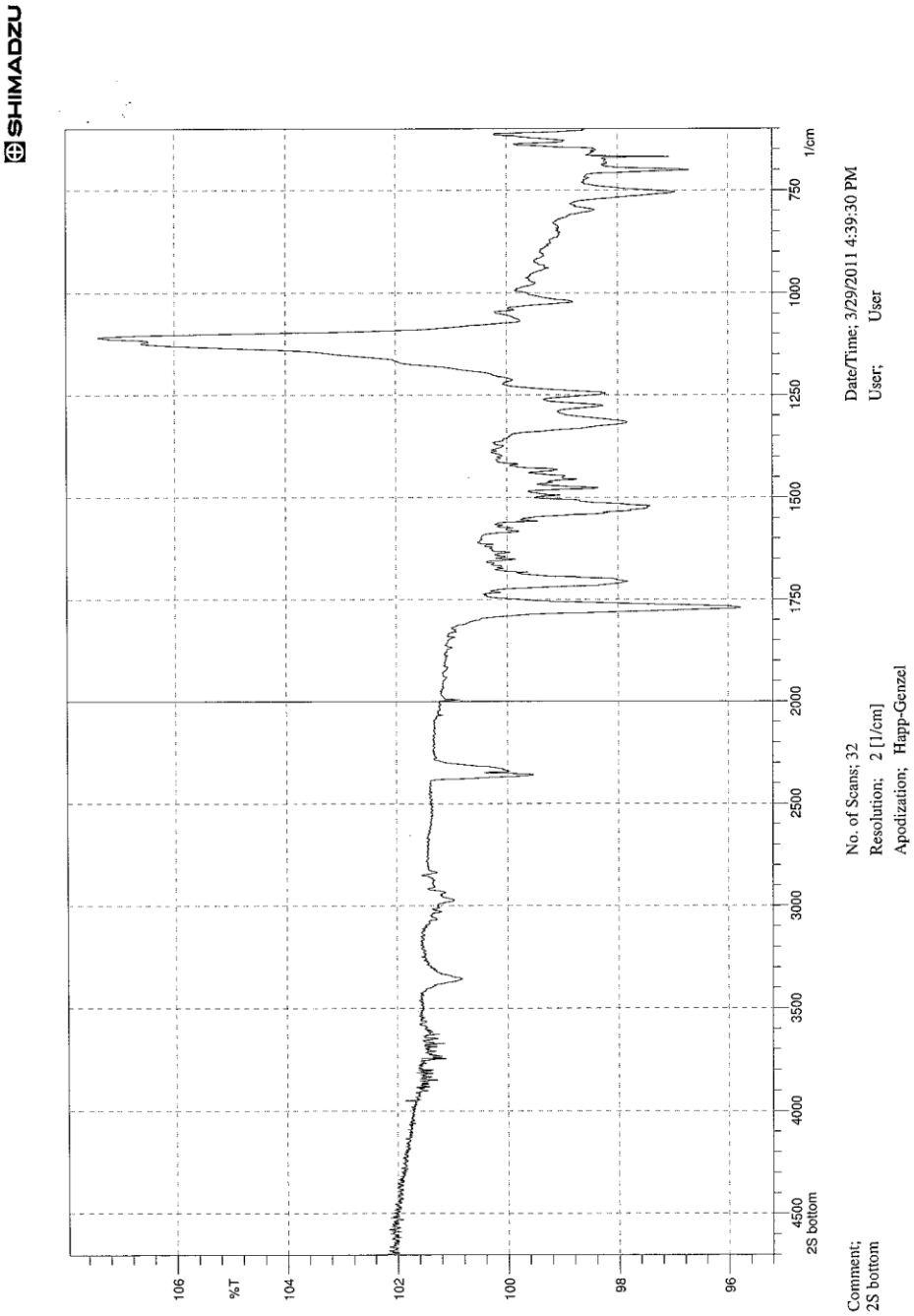


FIGURE 47: FT-IR Spectrum of compound **14**.

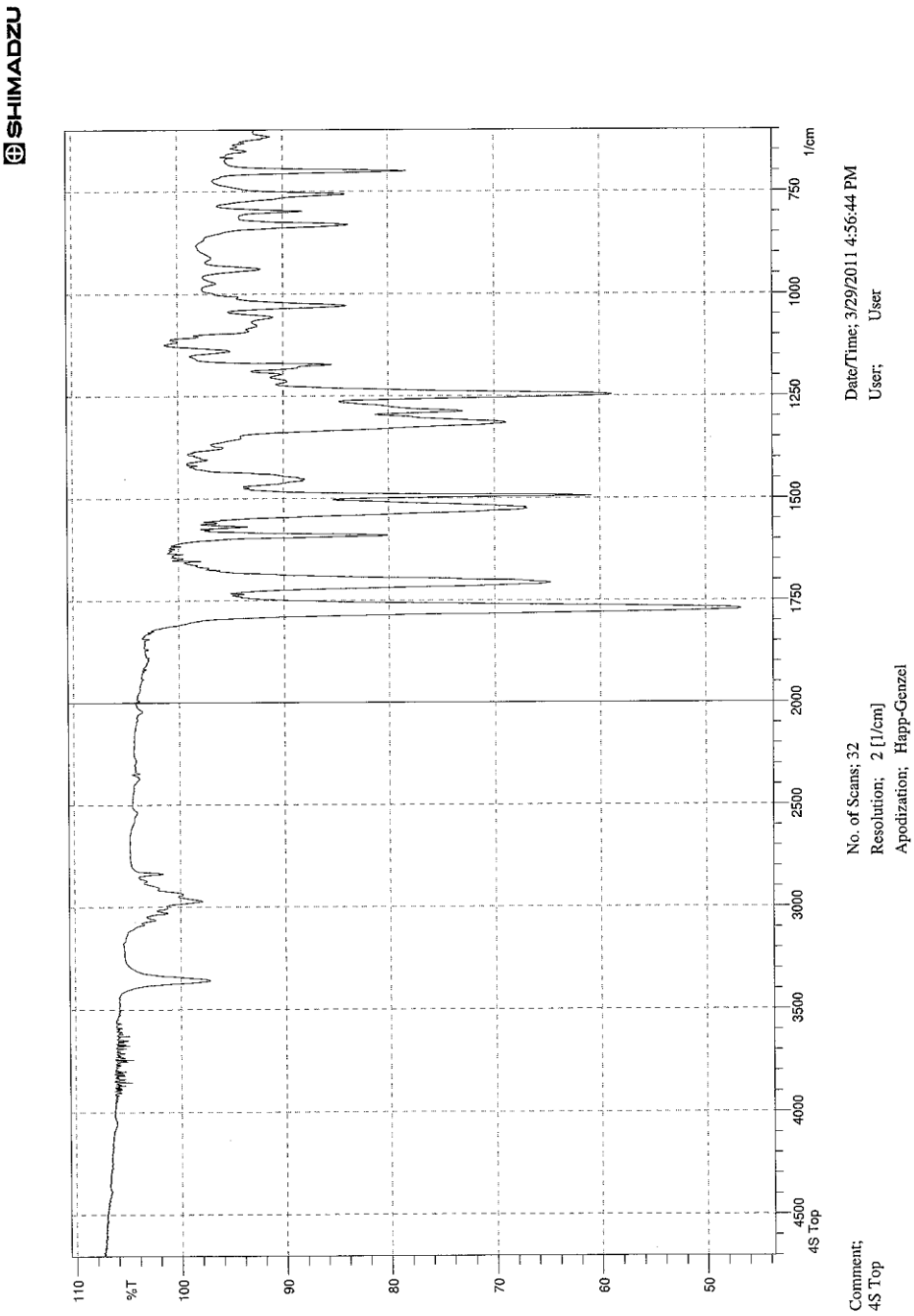


FIGURE 48: FT-IR Spectrum of compound **15**.

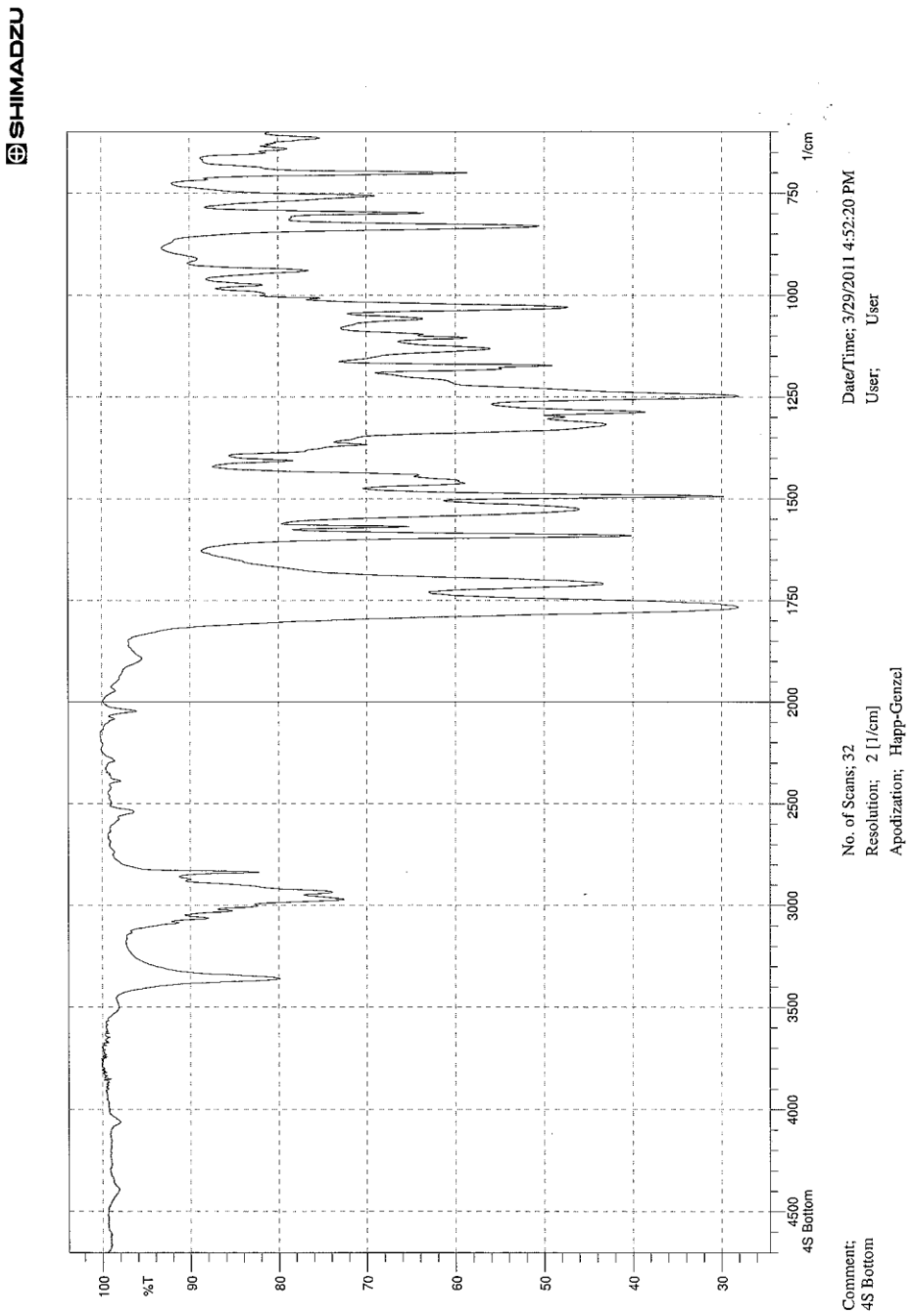


FIGURE 49: FT-IR Spectrum of compound **16.**

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