DECLARATION OF LEONARD J. CHYALL, PH.D.

I, the undersigned, **Dr. Leonard J. Chyall**, U.S Passport No. 432624896, with a business address of 3065 Kent Avenue, West Lafayette, in the State of Indiana, USA, having been warned that I must state the truth and that I shall be liable to the penalties prescribed by law should I fail to do so, hereby declare in writing as follows:

I. INTRODUCTION

1. I have been retained by Teva Pharmaceutical Industries Ltd. ("Teva") to provide opinions and analyses related to sitagliptin phosphate. This report sets forth my analyses and opinions relating to this topic, based on the work carried out by me or under my supervision or instruction, as well as on the materials I reviewed for the purpose of that work. Based on this work and the materials I have reviewed, it is my conclusion, as explained below, that upon reaction of sitagliptin free base with phosphoric acid, only sitagliptin dihydrogen phosphate (1:1 adduct between sitagliptin base and phosphoric acid) is formed.

A. Background And Qualifications

2. I am a Ph.D. chemist specializing in the study of organic materials, including organic materials in the solid state and solution. I have specific qualifications in the preparation and analyses of pharmaceutical drug substances. As outlined below, I am an organic chemist by profession, with training and experience in these areas

3. I am currently employed by Aptuit, Inc. ("Aptuit"). Aptuit is a research and information company that provides problem solving and analytical research to a broad range of pharmaceutical companies. Aptuit provides a complete range of services covering all aspects of pharmaceutical development. Among other things, the scientists at Aptuit perform syntheses and chemical analyses of pharmaceuticals. Aptuit also offers a broad range of analytical testing services. Aptuit performs work for both innovator and generic companies. Aptuit also has expertise in the characterization of materials in the solid state and in solution. Aptuit also performs solid form and salt selection studies for active pharmaceutical ingredients ("API"). Aptuit also provides pharmacopoeia-based

analytical testing, stability testing of drug substances in the solid state and in solution, stabilization studies of drugs either as solids or solutions, and consulting on regulatory issues, among other services.

4. My laboratory at Aptuit, which is located in West Lafayette, Indiana, is a current Good Manufacturing Practices ("cGMP") laboratory. To maintain cGMP compliance, our employees constantly monitor and comply with United States Food and Drug Administration ("FDA") guidance documents. Our facility is the subject of routine compliance audits by FDA and our clients. Our work meets the highest standards of control and reliability set by FDA and the pharmaceutical industry. As a cGMP laboratory, our studies on drug substances and drug products (formulations) are routinely submitted to FDA.

5. I obtained my Bachelor of Arts degree in chemistry from Oberlin College in 1986 and my Ph.D. in chemistry from the University of Minnesota in 1991. My doctoral research involved the synthesis and characterization of novel organic molecules. My dissertation focused on understanding the reactivity of high-energy cyclopropane molecules.

6. I was a postdoctoral fellow from 1992-1996 in the chemistry department at Purdue University, where I furthered my understanding of the properties and reactivity of organic molecules. These studies involved both small molecular weight molecules as well as large biological molecules.

7. Following my postdoctoral fellowship, I worked as a research chemist at Great Lakes Chemical Corporation. My research involved the identification, characterization and development of new products for the company. In 2000, I became a research investigator at SSCI, Inc. and in 2003, I became a senior research investigator. In 2006, SSCI was acquired by Aptuit. SSCI now operates as an integrated division of Aptuit. I currently hold the title of Director in the SSCI division of Aptuit.

8. Through my education and work experiences, I have obtained extensive knowledge and training in chemistry with specific experience in the areas of organic

chemistry and pharmaceutical sciences. I have authored or co-authored 22 publications in peer reviewed scientific journals that are listed in the attached curriculum vitae (**Exhibit A**). The most recent of these publications have involved scientific research that is related to the properties of pharmaceuticals. I am the inventor or co-inventor of three patented inventions granted by the United States Patent and Trademark Office. I have given numerous scientific presentations at various technical meetings.

9. While at SSCI and Aptuit, I have worked on numerous projects providing research and consulting services related to the development of new pharmaceutical products. My scientific expertise has been applied to the characterization of drug substances in both the solid state and in solution. For example, I have managed research protocols involving the identification and selection of the appropriate crystalline forms of a drug substance that are suitable for further development and commercialization. These research protocols have included polymorph screening experiments, salt selection work, cocrystal screening and studies of amorphous pharmaceuticals, among other areas. I have worked with solid oral dosage forms and pharmaceuticals being developed for parenteral, topical and transdermal dosage forms.

10. Based upon my education and experience, I am qualified to conduct chemistry experiments and perform analytical tests involving organic chemicals and pharmaceuticals. In particular, I am qualified to perform the experiments and analyses set out herein. All of this work was conducted by either myself, by those under my direct supervision, or by qualified outside laboratories at my request and under my instruction.

11. My background and qualifications, and a complete list of my publications are more fully set out in my curriculum vitae, attached as **Exhibit A**.

B. Compensation

12. I have no financial interest in the outcome of this matter. I am not specially compensated for my work on this case, and receive my salary from my employer.

II. EXPERIMENTAL BACKGROUND

A. General Considerations

13. I have been asked by counsel for Teva to perform laboratory experiments and analyses of samples of sitagliptin free base and sitagliptin phosphate salt. More specifically, I have been asked to perform experiments designed to probe whether sitagliptin could combine with phosphoric acid in a manner to produce a salt other than sitagliptin dihydrogen phosphate.

14. I understand the chemical structure of sitagliptin base to be the structure depicted in Figure 1.

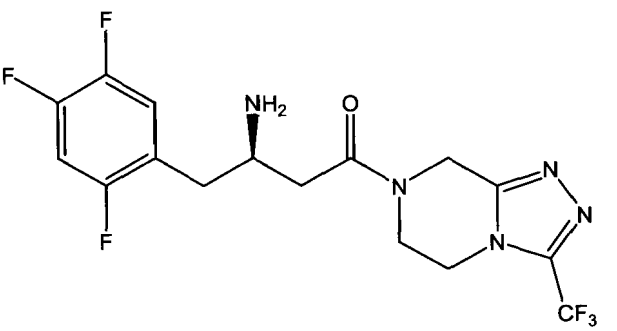

Figure 1: Structure of sitagliptin.

15. I understand phosphoric acid to have the molecular formula of H_3PO_4.

B. Materials Reviewed

16. I received from Teva two sample containers labeled Lot sal-069 and sal-008,087, which I understand to contain approximately 10 and 20 grams of sitagliptin base, respectively. Both samples were characterized by X-ray Powder Diffraction (XRPD). The XRPD patterns obtained for these samples (attached as **Exhibit B**) confirmed that the material is crystalline sitagliptin base, as disclosed in U.S. Patent Application Serial No. 12/740,693 (specification filed April 30, 2010) (attached as **Exhibit C**). The XRPD patterns for these materials were used as reference patterns in subsequent pH solubility experiments.

17. In addition, the two samples of sitagliptin base were analyzed by proton NMR spectroscopy and optical microscopy. These analyses are attached as **Exhibit D**. The chemical shifts and the integration of the proton resonances in the NMR spectrum indicate that the material is sitagliptin base. The NMR analysis also indicated that the sample was acceptably pure to conduct the experiments described herein. The optical microscopy images provide additional confirmation that the samples are crystalline due the presence of birefringence and extinction when viewed under magnification using polarized light.

18. I have also received from Teva two containers of material labeled lot no. D-1895NN-13067/3 and lot no. D1895MM14084/2. The sample container labeled lot no. D-1895NN-13067/3 contained approximately 10 grams of material that I understood to be sitagliptin dihydrogen phosphate. This sample was characterized by proton NMR spectroscopy, which confirmed the chemical identity of this material. In addition, it was characterized by XRPD, which demonstrated that the sample is a crystalline solid. The proton NMR spectra and XRPD patterns for lot no. D-1895NN-13067/3 are attached as **Exhibit E**. Comparison of the XRPD pattern so obtained with XRPD data of known solid forms of sitagliptin dihydrogen phosphate as disclosed in, inter alia, US 2010/0041885 A1 (**Exhibit F**) and WO 2005/020920 (**Exhibit G**), confirmed that the material in this vial is sitagliptin dihydrogen phosphate. The so obtained diffraction pattern was used as a reference pattern for subsequent pH solubility experiments described herein. In addition, this sample was analyzed by optical microscopy (images attached as **Exhibit H**). The optical microscopy images provide additional confirmation that the samples are crystalline due the presence of birefringence and extinction when viewed under magnification using polarized light.

19. The container labeled lot no. D1895MM14084/2 contains approximately 10 grams of material that I understand to be sitagliptin dihydrogen phosphate. I did not use this sample in any testing conducted at Aptuit.

20. Phosphoric acid and other chemical reagents were obtained from commercial suppliers and used as-received. As a cGMP-validated laboratory and in

order to be in compliance with our standard operating procedures (SOPs), my laboratory at Aptuit obtains Certificates of Analyses for all chemicals and reagents purchased from its suppliers, and no expired reagents were used in the testing conducted for the work described herein.

C. Materials Relied Upon

21. In reaching my opinions described herein, I relied on the documents referenced herein. I also relied on my general knowledge and experience, as well as my own scientific analyses. The opinions I express in this report are based on the information and evidence currently available to me.

III. SITAGLIPTIN SALT FORMATION EXPERIMENTS

22. I performed twelve salt formation experiments involving chemical reactions between sitagliptin base (Figure 1) and phosphoric acid and analyzed the solid products obtained from these reactions using a variety of techniques. The following summarizes these experiments.

23. The aforementioned twelve salt formation experiments were conducted by varying common parameters used in screening for potential salts of pharmaceutical APIs. These include the composition of the solvent, the temperature during the reaction and the molar ratio of acid to base. Many of these experiments were a deliberate attempt to obtain a phosphate salt other than a 1:1 adduct of sitagliptin and phosphoric acid.

24. A tabular summary of the salt formation experiments conducted between sitagliptin and phosphoric acid is given in Table 1.

Table 1: Salt Formation Experiments Conducted with Sitagliptin

Sample ID	Notebook No.	Ratio of API:H_3PO_4	Reaction Solvent	Temperature
233140	4063-02-01	1.00 : 1.05	methanol	ambient
233141	4063-03-01	1.00 : 2.10	methanol	ambient
234636	4063-18-01	3.00 : 1.00	methanol	ambient
233142	4063-04-01	2.04 : 1.00	methanol	ambient
234584	4063-19-01	1.00 : 5.01	methanol	ambient
234874	4063-35-01	2.04 : 1.00	12.5 % water in methanol	ambient
234872	4063-34-01	1.00 : 1.05	12.5 % water in methanol	ambient
234873	4063-32-01	1.00 : 2.10	12.5 % water in methanol	ambient
235805	4063-50-01	1.00 : 2.10	methanol	0 °C
235806	4063-51-01	2.04 : 1.00	methanol	0 °C
235848	4063-56-01	1.00 : 2.10	methanol	65 °C
235849	4063-57-01	2.04 : 1.00	methanol	65 °C

25. In some of these experiments an excess of sitagliptin base was used for the purpose of promoting the formation of a stable salt species containing either two or three molecules of sitagliptin for each molecule of phosphoric acid. As an example, for Sample ID 233142 I attempted to prepare a species corresponding to the salt with the formula $(SG\text{-}H^+)_2HPO_4^{2-}$ where "SG" corresponds to sitagliptin base (Figure 1). This stoichiometry represents a 2:1 adduct of sitagliptin with phosphoric acid.

26. In some of these experiments an excess of phosphoric acid was used for the purpose of attempting to promote formation of a stable salt species containing two or more molecules of phosphoric acid for each molecule of sitagliptin. As an example, for Sample ID 234873 I attempted to prepare a species corresponding to the salt with the formula $SG\text{-}H_2^{2+}(H_2PO_4^-)_2$. This stoichiometry represents a 1:2 adduct of sitagliptin with phosphoric acid.

27. For some experiments a slight excess of one of the reactants (either sitagliptin base or phosphoric acid) based on the stoichiometry of the intended chemical reaction was used. As an example, Sample ID 235805 was conducted with the intention

of providing a 1:2 adduct of sitagliptin with phosphoric acid, *e.g.*, SG-$H_2^{2+}(H_2PO_4^-)_2$. This experiment involved the use of 2.1 equivalents of phosphoric acid which is a 5% molar excess based on the intended stoichiometry. The motivation for using a slight excess of one of the reactants is to drive the chemical reaction to completion.

28. In the salt formation experiments, the salts were isolated from their solutions using common laboratory techniques as described below for each of the salt formation experiments performed at Aptuit.

29. Each of the twelve samples prepared at Aptuit during the salt formation experiments was extensively characterized by a variety of analytical techniques to determine the composition of the salt. The chemical identity and approximate purity of the samples was evaluated using proton NMR spectroscopy. XRPD was used to determine that crystalline products were obtained for each of the experiments. XRPD was also used to determine whether the product is sitagliptin dihydrogen phosphate, based on comparison with published XRPD data. Differential scanning calorimetry (DSC) was also used to confirm crystallinity by the presence of melt endotherms in the DSC plots for each of the samples, and to demonstrate that the samples are free of substantial amounts of solvent and other volatile material.

30. Each of the twelve samples prepared at Aptuit during the salt formation experiments was also analyzed under my instruction and direction for carbon, hydrogen, phosphorus and nitrogen content. The purpose of this testing was to determine the ratio of sitagliptin to phosphoric acid in these samples and to obtain an indication of the overall purity of the samples. Galbraith Laboratories ("Galbraith") was hired by Aptuit as a subcontractor laboratory for this testing. Galbraith is a contract research and services laboratory which provides a variety of chemical testing services, including elemental analysis, on a fee for service basis. Its facilities are periodically audited by the FDA to ensure compliance with GMP regulations, and are also periodically audited by the Aptuit quality assurance department to ensure cGMP compliance. Galbraith Laboratories is a longstanding provider of testing services to SSCI and Aptuit. I have only had positive

experiences with Galbraith in over ten years of using them as sub-contractor laboratory, and consider the testing and data that Galbraith provides me as highly reliable.

31. A detailed description of the twelve salt formation experiments is provided below. Furthermore, as shall be explained below, the various analytical data obtained clearly show that despite the different conditions used in each experiment, all twelve of the salt formation experiments resulted in sitagliptin dihydrogen phosphate, which is a salt that results upon the combination of one molecule of sitagliptin for each molecule of phosphoric acid. This salt is generated in solution by transfer of one proton (H^+) from phosphoric acid to sitagliptin base.

A. Procedures For Salt Formation Experiments

32. Given below are the specific procedures used in each of the twelve salt formation experiments, observations made during the respective experiments, and identification of the analyses conducted on the solids resulting from the respective experiments.

33. **Sitagliptin base : phosphoric acid (1.00 : 1.05) in methanol (Sample 4063-02-01):** Sitagliptin base (503.4 mg) was weighed into a 100 mL round bottom flask. Methanol (5 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=22.0 °C). Phosphoric acid stock solution (1.298 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. White solids formed after approximately 15 minutes and were allowed to slurry for one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 398023), DSC (file 399011), TGA (file 398706), proton NMR (file 400883) and elemental analysis (LIMS 233140).

34. **Sitagliptin base : phosphoric acid (1.00 : 2.10) in methanol (Sample 4063-03-01):** Sitagliptin base (502.3 mg) was weighed into a 100 mL round bottom flask. Methanol (5 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=21.0 °C). Phosphoric acid stock solution (2.589 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. White solids

formed after approximately 15 minutes and were allowed to slurry for one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 398024), DSC (file 399012), proton NMR (file 400888), and elemental analysis (LIMS 233141).

35. **Sitagliptin base : phosphoric acid (3.00 : 1.00) in methanol (Sample 4063-18-01):** Sitagliptin base (1000.3 mg) was weighed into a 100 mL round bottom flask. Methanol (10 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=23.0 °C). Phosphoric acid stock solution (0.818 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. The solution remained clear and colorless for at least 4 hours. The sample was checked after approximately 15 hours and white solids were observed. The solids were allowed to slurry for approximately one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 401165), DSC (file 401166), proton NMR (file 401158), and elemental analysis (LIMS 234636).

36. **Sitagliptin base : phosphoric acid (2.04 : 1.00) in methanol (Sample 4063-04-01):** Sitagliptin base (501.0 mg) was weighed into a 100 mL round bottom flask. Methanol (5 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=21.0 °C). Phosphoric acid stock solution (0.603 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. White solids were observed after approximately 3 hours. The solids were allowed to slurry for approximately one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 398025), DSC (file 399013), proton NMR (file 400891), and elemental analysis (LIMS 233142).

37. **Sitagliptin base : phosphoric acid (1.00 : 5.01) in methanol (Sample 4063-19-01):** Sitagliptin base (499.9 mg) was weighed into a 100 mL round bottom flask. Methanol (5 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=22.0 °C). Phosphoric acid stock solution (1.230 mL of 5.0M phosphoric acid in methanol) was added drop-wise with stirring. Slight turbidity was noted after 3 hours 40 minutes. The solids were allowed to slurry for approximately

one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 401062), DSC (file 401063), proton NMR (file 401064), and elemental analysis (LIMS 234584).

38. **Sitagliptin base : phosphoric acid (2.04 : 1.00) in methanol and approximately 12.5% water (Sample 4063-35-01):** Sitagliptin base (750.4 mg) was weighed into a 100 mL round bottom flask. Methanol (7 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=21.0 °C). Phosphoric acid stock solution (1.002 mL of 0.9M phosphoric acid in water) was added drop-wise with stirring. White solids were noted after 35 minutes. The solids were allowed to slurry for approximately one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 401683), DSC (file 401686), proton NMR (file 401689), and elemental analysis (LIMS 2345874).

39. **Sitagliptin base : phosphoric acid (1.00 : 1.05) in methanol and approximately 12.5% water (Sample 4063-34-01):** Sitagliptin base (500.8 mg) was weighed into a 100 mL round bottom flask. Methanol (5 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=21.0 °C). Phosphoric acid stock solution (0.716 mL of 1.8M phosphoric acid in water) was added drop-wise with stirring. Slight turbidity was noted after 5 minutes. The solids were allowed to slurry for approximately one day. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 401681), DSC (file 401684), proton NMR (file 401687), and elemental analysis (LIMS 234872).

40. **Sitagliptin base : phosphoric acid (1.00 : 2.10) in methanol and approximately 12.5% water (Sample 4063-32-01):** Sitagliptin base (499.7 mg) was weighed into a 100 mL round bottom flask. Methanol (5 mL) was added resulting in a clear, colorless solution. The flask was placed in a water bath (T=21.0 °C). Phosphoric acid stock solution (0.716mL of 3.6M phosphoric acid in water) was added drop-wise with stirring. White solids were noted after approximately 2 hours. The solids were allowed to slurry for approximately one day. The solids were collected by vacuum

filtration and allowed to air-dry. The solids were analyzed by XRPD (file 401682), DSC (file 401685), proton NMR (file 401688), and elemental analysis (LIMS 234873).

41. **Sitagliptin base : phosphoric acid (1.00 : 2.10) in methanol at 0 °C (Sample 4063-50-01):** Sitagliptin base (1004.6 mg) was weighed into a 100 mL round bottom flask. The flask was placed in an ice-water bath (T=4.0 °C). Methanol (0 °C, 10 mL) was added resulting in a clear, colorless solution. Phosphoric acid stock solution (5.179 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. White solids were noted after approximately 45 minutes. The solids were slurried and the solution was allowed to come to ambient temperature over approximately 4 hours. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 403327), DSC (file 403329), proton NMR (file 403325), and elemental analysis (LIMS 235805).

42. **Sitagliptin base : phosphoric acid (2.04 : 1.00) in methanol at 0 °C (Sample 4063-51-01)):** Sitagliptin base (1004.4 mg) was weighed into a 100 mL round bottom flask. The flask was placed in an ice-water bath (T=0 °C). Methanol (0 °C, 10 mL) was added resulting in a clear, colorless solution. Pre-chilled phosphoric acid stock solution (1.208 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. Slight turbidity was noted after approximately 3.5 hours. The solids were slurried for an additional 10 hours. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 403328), DSC (file 403330), proton NMR (file 403326), elemental analysis (LIMS 235806).

43. **Sitagliptin base : phosphoric acid (1.00 : 2.10) in methanol under reflux (Sample 4063-56-01):** Sitagliptin base (1006.9 mg) was weighed into a 100 mL round bottom flask. Methanol (9 mL) was added resulting in a clear, colorless solution. The solution was heated to reflux (65 °C) in an oil bath with stirring. Phosphoric acid stock solution (5.1912 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. Immediate precipitation was observed. The solution was allowed to come to room temperature at a rate of approximately 5 °C/hour prior to harvesting solids. The solids were collected by vacuum filtration and allowed to air-dry. The solids were

analyzed by XRPD (file 403551), DSC (file 403548), proton NMR (file 403553), and elemental analysis (LIMS 235848).

44. **Sitagliptin base : phosphoric acid (2.04 : 1.00) in methanol under reflux (Sample 4063-57-01):** Sitagliptin base (1004.8 mg) was weighed into a 100 mL round bottom flask. Methanol (9 mL) was added resulting in a clear, colorless solution. The solution was heated to reflux (65-70 °C) in an oil bath with stirring. Phosphoric acid stock solution (1.2088 mL of 1.0M phosphoric acid in methanol) was added drop-wise with stirring. Turbidity was noted after 15 minutes; white solids after a total of 30 minutes. The solution was allowed to come to room temperature at a rate of approximately 5 °C/hour prior to harvesting solids. The solids were collected by vacuum filtration and allowed to air-dry. The solids were analyzed by XRPD (file 403552), DSC (file 403550), proton NMR (file 403554), and elemental analysis (LIMS 235849).

45. For each of the twelve salt formation experiments described above, the XRPD patterns are provided in **Exhibit I,** the NMR spectra are provided in **Exhibit J**, and the DSC plots are provided in **Exhibit K**.[1] A summary of the elemental analyses for the solids resulting from the salt formation experiments is provided in **Exhibit L**.

B. Results of the Salt Formation Experiments

46. Based on the analytical data obtained in the salt formation experiments (XRPD, proton NMR, DSC and elemental analysis), it is my conclusion that all twelve salt formation experiments resulted in sitagliptin dihydrogen phosphate. I note that elemental analysis and proton NMR provide information about the chemical content and structure of the compound. Furthermore, XRPD and DSC data provide information about the physical properties of the compound (crystalline form and melting temperature, respectively). Based on all of the analytical data I conclude that the samples are sitagliptin dihydrogen phosphate. The above conclusion is supported by the comparison of the observed physical properties to the characteristic physical properties of sitagliptin dihydrogen phosphate published in the literature.

[1] The TGA plot for Sample ID 233140 is also provided in **Exhibit K.**

47. Upon analysis of the XRPD patterns obtained on the twelve experimental samples, it is my conclusion that all salt formation experiments resulted in known crystalline forms or mixtures of known crystalline forms of sitagliptin dihydrogen phosphate disclosed in US 2010/0041885 A1 and WO 2005/020920. Additional support for the identification of these samples as crystalline solids was obtained from the DSC analyses of the materials, which display a melt endotherm for the materials. The chemical shift positions of the resonances and their corresponding integrated intensities in the proton NMR spectra demonstrate that the salt formation experiment samples prepared at Aptuit are sitagliptin dihydrogen phosphate. In addition, the NMR spectra for these twelve samples match the spectrum obtained for the sitagliptin dihydrogen phosphate sample obtained from Teva.

48. The elemental analyses of all twelve salt formation experiment samples are conclusive for the generation of sitagliptin dihydrogen phosphate in these samples. It is not possible for any of the samples to contain other than a 1:1 adduct of sitagliptin and phosphoric acid, which is sitagliptin dihydrogen phosphate. This is because the relative amounts of nitrogen and phosphorus in the ratio of 13.86/6.13 would be substantially different for other stoichiometries of sitagliptin and phosphoric acid. Table 2, below, provides theoretical values, the elemental analysis results, and the deviation between measured and theoretical values.

Table 2: Elemental Analyses of Salt Formation Experiment Samples

		Measured Values				**Deviation from Theory**			
Notebook No.	**API:acid ratio; solvent; temperature**	**C**	**H**	**N**	**P**	**C**	**H**	**N**	**P**
Theoretical	n/a	38.03	3.59	13.86	6.13	-	-	-	-
4063-19-01	1.00:5.01; methanol; ambient	37.72	3.67	13.64	6.03	-0.31	0.08	-0.22	-0.11
4063-18-01	3.00:1.00; methanol; ambient	37.89	3.64	13.71	6.12	-0.14	0.05	-0.15	-0.01
4063-02-01	1.00:1.05; methanol; ambient	37.92	3.43	13.54	6.15	-0.11	-0.16	-0.32	0.02
4063-03-01	1.00:2.10; methanol; ambient	37.76	3.47	13.60	5.97	-0.27	-0.12	-0.26	-0.16
4063-04-01	2.04:1.00; methanol; ambient	37.78	3.41	13.62	5.91	-0.25	-0.18	-0.24	-0.22
4063-50-01	1.00:2.10; methanol; 0 °C	36.51	3.62	13.56	6.31	-1.53	0.03	-0.30	0.18
4063-51-01	2.04:1.00; methanol; 0 °C	37.96	3.60	14.08	5.78	-0.08	0.00	0.22	-0.35
4063-56-01	1.00:2.10; methanol; 65 °C	37.64	3.56	13.98	5.92	-0.39	-0.03	0.12	-0.22
4063-57-01	2.04:1.00; methanol; 65 °C	37.87	3.58	13.98	5.85	-0.16	-0.01	0.12	-0.29
4063-34-01	1.00:1.05; methanol-water; ambient	37.82	3.63	13.96	6.22	-0.21	0.04	0.10	0.09
4063-32-01	1.00:2.10; methanol-water; ambient	37.75	3.58	13.97	6.29	-0.28	-0.01	0.11	0.16
4063-35-01	2.04:1.00; methanol-water; ambient	37.82	3.62	13.91	6.12	-0.21	0.03	0.05	-0.01

49. As can be readily seen from Table 2, above, all but one of the elemental analysis values fall within ±0.4% of the theoretical values for anhydrous sitagliptin dihydrogen phosphate. A deviation within ±0.4% from the theoretical value is generally considered to be an acceptable demonstration of compound purity by peer-reviewed

scientific journals.[2] The agreement between the values obtained for the twelve samples is remarkable when one considers that the products were crystallized from the reaction mixture and air-dried. No additional purification or drying of the samples was performed.

50. The carbon value for sample notebook no. 4063-50-01 deviated from theory by -1.53%, and was the only elemental analysis value that deviated from theory by more than ±0.4%. The reason for this observed deviation is likely to be that the sample contains minor amounts of inorganic and/or organic impurities. For example, the DSC plot for this sample shows a lower onset of melting relative to the other eleven DSC plots, which is consistent with the presence of minor amounts of inorganic and/or organic impurities. However the agreement between the nitrogen and phosphorus experimental values with the theoretical values for these elements along with the other analytical data collected for this compound allows me to conclude that the sample notebook no. 4063-50-01 is sitagliptin dihydrogen phosphate. In particular, the agreement between theory and experiment for the nitrogen and phosphorus content for this sample is conclusive evidence that there is a 1:1 molecular ratio of sitagliptin and phosphoric acid in this sample.

51. The elemental analysis results detailed in Table 2 above, which clearly indicate a 1:1 ratio of sitagliptin and phosphoric acid, rule out the possibility that either a 1:2 ($\mathbf{SG\text{-}H_2^{2+}(H_2PO_4^-)_2}$) or a 2:1 ($\mathbf{(SG\text{-}H^+)_2HPO_4^{2-}}$) adduct of sitagliptin with phosphoric acid has been formed in any of the twelve salt formation experiments. Formation of species $\mathbf{SG\text{-}H_2^{2+}(HPO_4^{2-})}$ is also readily excluded, *inter alia,* in view of the XRPD patterns for the products of the twelve salt formation experiments, all of which correspond to those of known crystalline forms, or mixtures of known crystalline forms, of $\mathbf{SG\text{-}H^+(H_2PO_4^-)}$, namely sitagliptin dihydrogen phosphate.

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[2] For example, the journal *Chemical Communications* published by the Royal Society of Chemistry (RSC) requires the following for characterization of organic substances: "Elemental analysis (within ±0.4% of the calculated value) is required to confirm 95% sample purity and corroborate isomeric purity." www.rsc.org/Publishing/ReSourCe/AuthorGuidelines/JournalPolicy/CC/sect3.asp

C. Conclusion Based On Salt Formation Experiments

52. The experiments described above represent common and reasonable attempts to deliberately obtain different possible molecular combinations of a basic API, such as sitagliptin, and phosphoric acid. Even when either sitagliptin or phosphoric acid was used in great excess, only sitagliptin dihydrogen phosphate resulted. Furthermore, experiments conducted at sub-ambient and elevated temperature, and experiments conducted in the presence of water, also only resulted in sitagliptin dihydrogen phosphate. These experiments indicate that there is only one possible molecular ratio, a 1:1 ratio, that will be present as a pharmaceutically suitable salt of sitagliptin and phosphoric acid, namely sitagliptin dihydrogen phosphate.

IV. pH SOLUBILITY EXPERIMENTS

53. The equilibrium solubility of a drug is defined as the concentration of the drug in solution where the dissolution rate is in equilibrium with the rate of recrystallization (of the same solid form). It is an important property to understand and characterize in pharmaceutical development due to its impact on bioavailability (aqueous solubility) and critical development issues such as chemical and physical stability of a pharmaceutical salt.

54. Aqueous solubility is dependent on many factors. For ionizable drugs, compounds with molecular sites that can become ionized when exposed to acids or bases of a given concentration, the intrinsic solubility in an aqueous solution is defined by the solubility of the unionized form at a given temperature. It is also dependant on factors such as the pH of the aqueous media (the acidity or basicity of the solution) in relation to the pKa(s) of the ionization site(s) on the drug, the solid form of the material in suspension (*e.g.*, base, salt, hydrate etc.), and the ionic strength of the media.

55. The pH solubility profile of an ionizable drug at equilibrium can be calculated with knowledge of the intrinsic solubility and the pK_a of the drug.[3] For

[3] For a detailed discussion of pH solubility theory see Pudipeddi *et al.* In Handbook of Pharmaceutical Salts Properties, Selection and Use; Stahl, C. D.; Wermuth, C. G. Eds.; Wiley-VCH, Zurich Switzerland, 2002, Chapter 2 pp. 19-29. (**Exhibit M**).

example, for a slightly soluble weak acidic electrolyte with a single pK_a, the pH can be expressed as follows:

$$HA_{(solid)} \rightarrow HA_{(sol)}$$

$$HA_{(solid)} \rightarrow A^- + H^+$$

$$K_a = \frac{[A^-][H^+]}{[HA]_{(sol)}}$$

$$pK_a = \log\frac{[A^-][H^+]}{[HA]_{(sol)}} = \log[H^+] + \log\frac{[A^-]}{[HA]_{(sol)}}$$

$$pH = pK_a + \log\frac{[A^-]}{[HA]_{(sol)}} \quad \text{(Henderson-Hasselbalch equation)}$$

56. An important parameter in the pH solubility relationship of a given compound is the pH_{max}. At this pH value, the solution is saturated with the free base and the salt form.[4] The equations for expressing the solubility of a weak base are given below. *See* **Exhibit M**

$$S = S_o\left(1 + \frac{[H^+]}{K_a}\right)$$

$$S = \left(1 + \frac{K_a}{[H^+]}\right)\sqrt{K_{sp}}$$

where S = total solubility of the species
S_o = intrinsic solubility of the base
K_a = acid dissociation constant
K_{sp} = solubility product of the salt

[4] *Cf* **Exhibit M** at p. 23.

57. At pH_{max} both of the above solubility equations are equal to one another, which can be used to derive an expression for pH_{max} based on the pK_a, S_o, and K_{sp}:

$$pH_{max} = pK_a + \log \frac{S_o}{\sqrt{K_{sp}}}$$

58. From the equations presented above, it can be seen that knowledge of the solubilities of the base (S_o) and salt (K_{sp}) can be used along with the pK_a to construct a theoretical pH solubility profile for the compound. However, experimentally determined pH solubility curves may differ from the curve predicted by theory. Factors such as lack of equilibrium due to slow dissolution, chemical degradation, or solid form change may cause the experimental data to depart from the theoretically predicted curve. In addition, factors such as ionic strength of the solution or a common ion effect may also cause a measured curve to differ from theory. Nonetheless, knowledge K_{sp}, pK_a and S_0 for a given system will allow one to calculate a pH solubility curve based on the equations given above.

59. According to a Merck submission during an opposition proceeding on European Patent No. 1 654 263, the pK_a value of sitagliptin free base is 7.7. *See* Opposition of European Patent No. 1 654 263, February 19, 2007 submission by Merck & Co., Inc., page 3 (**Exhibit N**). As mentioned above, this pK_a value may be used in combination with the solubility of sitagliptin phosphate (*Ksp*) and the free base (S_0) to construct a theoretical pH solubility curve for sitagliptin.

60. According to Merck, sitagliptin is chemically unstable in aqueous solutions: *See* **Exhibit N** at p. 3; *see also* Declaration of Robert M. Wenslow, Jr., Ph.D., December 21, 2009, at paragraph 11 (**Exhibit O**). Therefore, for experimental solubility values measured in water to be valid, it is important to consider the time for equilibration versus degradation when conducting equilibrium solubility experiments.

61. The pH solubility experiments performed at Aptuit, and their results, are described in detail below.

A. pKa Analyses

62. As mentioned above, the pK_a for sitagliptin has been reported by Merck to be 7.7. Given in Table 3 are the known pK_a values for phosphoric acid.[5]

Table 3: pKa(s) and Species for Phosphoric Acid (H_3PO_4)

Species	pKa
H_3PO_4	2.12
$H_2PO_4^-$	7.21
HPO_4^{2-}	12.67

63. The pK_a values for phosphoric acid indicate that phosphoric acid is a strong enough acid to protonate sitagliptin to generate a dihydrogen phosphate salt. The second pK_a of phosphoric acid of 7.2 is slightly lower than the pK_a of sitagliptin base. However, a difference of approximately 0.5 units is not believed to be large enough for the species $H_2PO_4^-$ to protonate sitagliptin base to generate a stable salt. Typically differences in pK_a on the order of 2 to 3 units between the acidic and basic species are required to generate a stable salt. *See e.g.*, Bastin *et al.*, Salt Selection and Optimisation Procedures for Pharmaceutical New Chemical Entities, *Organic Process Research & Development*, 4(5), 427-35 (2000) (**Exhibit Q**) (stating that a pK_a difference of 3.0 is necessary to generate a stable salt); Stahl and Wermuth, Wiley-VCH, Zurich Switzerland, 2002, Chapter 6, p. 138 (**Exhibit R**) ("*Tong* and *Whitesell* recommended that, for the preparation of salt forms of a basic drug, the pK_a of the acid used should be at least 2 pH units lower than the pK_a of the drug."). Therefore, neither a 2:1 salt between sitagliptin and phosphoric acid nor a 1:1 monohydrogen phosphate salt are expected to occur.

B. Experimental pH Solubility Values

64. The solubilities of sitagliptin base and sitagliptin dihydrogen phosphate were determined by slurrying these materials in water for approximately 24 hours. A stability study was conducted which demonstrated that this time period is sufficient to permit the solutions to become saturated with the material, but not too long to cause

[5] CRC Handbook of Chemistry and Physics, 82nd Ed. David R. Lide Ed, 2001-2002. CRC Press Boca Raton, FL (**Exhibit P**).

degradation of the sitagliptin species. All samples were equilibrated at 25 °C, which is the temperature corresponding to equilibrium solubility measurements.

65. To provide confidence that equilibrium was achieved for these measurements, two samples corresponding to sitagliptin base and sitagliptin phosphate were initially equilibrated at 40 °C. This temperature would result in the solutions being more concentrated than at their equilibrium solubility values at 25 °C. The samples were then cooled to 25 °C and allowed to equilibrate for 3 hours. The solubility values obtained for the two experiments conducted in this manner provide added confidence that equilibrium was achieved for all experiments given in Table 4 as equilibrium was approached from experiments involving both undersaturated and supersaturated solutions.

66. The average solubility of sitagliptin base (S_0) was found to be approximately 6.6 mg/mL (n = 3) with the solutions achieving a pH of approximately 9.3 at equilibrium. For the equilibrium solubility of sitagliptin dihydrogen phosphate an average value of ~ 93 mg/mL (n = 4) at an approximate pH of 4.1 was obtained. The solubility of 93 mg/mL is expressed in terms of free base equivalents so that the solubilities of the free base and salt may be compared directly. These experimental values are presented in Table 4.

Table 4: Aqueous Solubility Values at 25 °C for Sitagliptin Base and Sitagliptin Dihydrogen Phosphate

Sample No.	pH	Solubility (mg/ml) per base	Solubility (mg/ml) per salt	Slurry Time (hr)	Starting solids	Solids recovered after slurry
4031-11-05	4.10	93.0	115	22	sitagliptin phosphate	sitagliptin phosphate
4031-24-01	4.13	88.6	110	24	sitagliptin phosphate	sitagliptin phosphate
4031-11-07	4.16	94.5	117	22[a]	sitagliptin phosphate	sitagliptin phosphate
4031-27-05	4.18	94.4	117	24	sitagliptin phosphate	sitagliptin phosphate
4031-11-03	9.22	7.07	-	22[a]	sitagliptin base	sitagliptin base
4031-24-03	9.33	6.42	-	24	sitagliptin base	sitagliptin base
4031-11-01	9.42	6.39	-	22	sitagliptin base	sitagliptin base

[a] 40 °C slurry for 19 hours then 25 °C for 3 hours

67. The measured solubility values for the free base and phosphate salt of sitagliptin were used with the pK_a value of 7.7 to derive the solubility curve shown in Figure 2 using the solubility theory and equations presented above

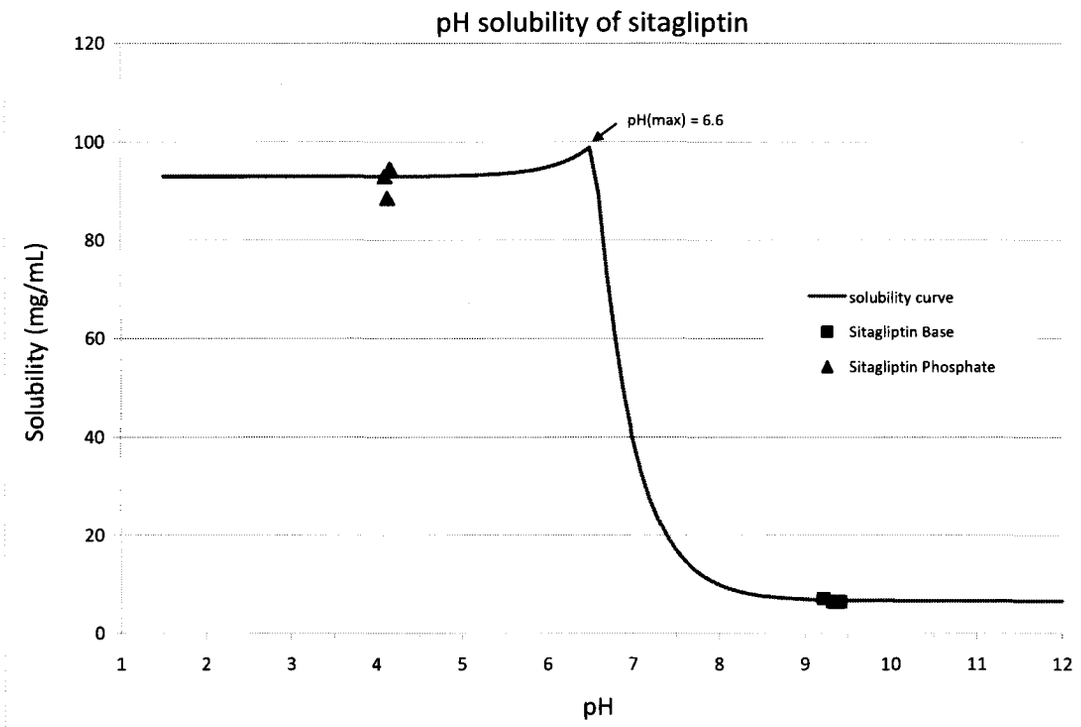

Figure 2: pH solubility curve for sitagliptin

68. The calculated pH_{max} for sitagliptin from this analysis is 6.6. This value along with the other features of the solubility profile indicate that sitagliptin will not form a stable and isolable salt with phosphoric acid in aqueous media at pH values above 6.6. This is indicated by rapid decrease in solubility of the species above this value.

69. Additional solubility data points for sitagliptin phosphate were collected in aqueous media at pH values other than those listed in Table 4. To do this, solutions of either sitagliptin base or sitagliptin phosphate in water were pH-adjusted by the addition of either sodium hydroxide or phosphoric acid. The results of these experiments are listed in Table 5.

Table 5: Aqueous Solubility Values for Sitagliptin Base and Sitagliptin Dihydrogen Phosphate pH-Adjusted Experiments

Sample No.	pH	Solubility (mg/ml) per base	Solubility (mg/ml) per salt	Slurry Time (hr)	Starting solids	Solids recovered after slurry
4031-24-02	1.51	157	195	24	sitagliptin phosphate	sitagliptin phosphate
4031-27-04	1.57	178	221	24	sitagliptin phosphate	sitagliptin phosphate
4031-27-03	2.32	116	144	24	sitagliptin phosphate	sitagliptin phosphate
4031-27-02	3.01	107	133	24	sitagliptin phosphate	sitagliptin phosphate
4031-30-02	3.41	103	127	24	sitagliptin phosphate	sitagliptin phosphate
4031-24-08	7.04	29.9	-	24	sitagliptin base	sitagliptin base
4031-24-07	7.96	10.3	-	24	sitagliptin base	sitagliptin base
4031-24-06	11.0	5.74	-	24	sitagliptin base	sitagliptin base
4031-24-05	12.0	5.90	-	24	sitagliptin base	sitagliptin base
4031-24-04	12.8	2.41	-	24	sitagliptin base	sitagliptin base

70. A plot of the data points given in Table 4 and Table 5 along with the theoretical pH solubility profile (Figure 3) shows that these additional solubility values generally agree with the theoretical profile. However, there is disagreement at strongly acidic pH values for the experimental data and predicted values. This is likely because the highly concentrated solutions with extremely high ionic strengths no longer behave as ideal solutions.

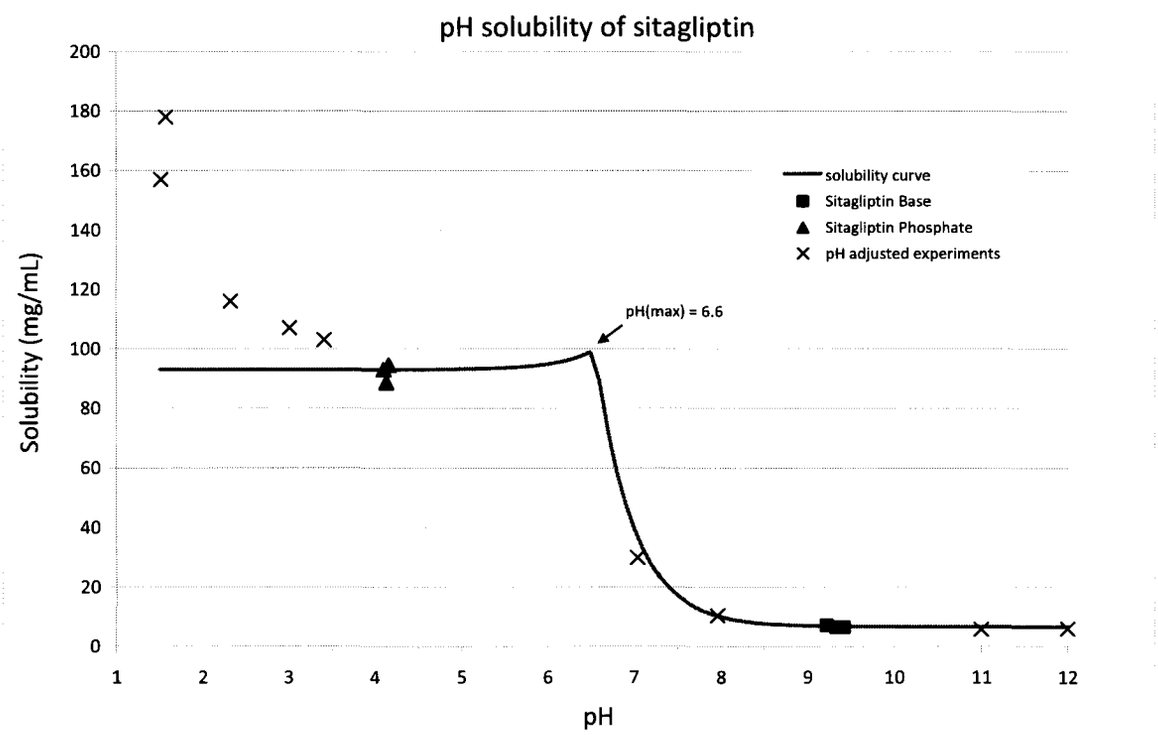

Figure 3: Calculated pH solubility profile for sitagliptin and pH-adjusted solubility values.

C. Conclusion of pH Solubility Experiments

71. An experimental pH solubility profile for sitagliptin was collected and compared to the theoretical profile based on experimental and literature data. It was found to be in good agreement between pH 4 and 12. The pH max value obtained from this profile of 6.6 indicates that a salt of sitagliptin phosphate will not be stable in a media with a pH above 6.6. As a result, the second ionization of phosphoric acid is not energetically favored to protonate sitagliptin base such that a stable and pharmaceutically

acceptable salt could be isolated. Therefore, neither 2:1 adducts of sitagliptin and phosphoric acid nor 1:1 monohydrogen phosphate salts are expected to be stable and isolable species.

V. CONCLUSION

72. The salt formation experiments performed on sitagliptin phosphate encompassed a wide range of experimental conditions in deliberate attempts to prepare a phosphate salt other than a 1:1 adduct of sitagliptin and phosphoric acid. None of these experiments were successful in this regard, providing only crystalline sitagliptin dihydrogen phosphate (1:1 adduct) as an isolable product. From these experiments alone, I believe it is not possible to prepare any pharmaceutically suitable salt of sitagliptin with phosphoric acid other than sitagliptin dihydrogen phosphate using common and standard laboratory procedures.

73. Support for my conclusion that only a 1:1 salt of sitagliptin and phosphoric acid is isolable can be found in the pH solubility studies that were conducted in my laboratory. I found that the pH max for sitagliptin phosphate is about 6.6, which is too low to facilitate the formation of a stable 2:1 salt between sitagliptin and phosphoric acid.

74. My pH solubility studies demonstrated that even at low pH values near 1.0, only sitagliptin dihydrogen phosphate can be isolated from solution. Therefore, it is not possible for two molecules of phosphoric acid to bond to one molecule of sitagliptin base to produce a 1:2 salt.

75. In summary, it is my scientific opinion that the only possible outcome of an experiment to obtain a stable and isolable salt (*i.e.*, a pharmaceutically suitable salt) involving sitagliptin and phosphoric acid is the generation of sitagliptin dihydrogen phosphate.

I hereby state and attest that all statements herein are based on my own information and belief, and are accurate to the best of my knowledge.

Date: 3-August-2010

Leonard J. Chyall

Leonard J. Chyall, Ph.D.
Director
Aptuit, Inc.

EXHIBIT A

Leonard J. Chyall, Ph.D.

3105 Cedar Lane, Lafayette, Indiana 47905 USA
Tel 765.463.0112 ext 3361 (work); Tel 765.448.1165 (residence)

Education

B.A. (Chemistry), 1986, Oberlin College, Oberlin, OH
Ph.D. (Chemistry), 1991, University of Minnesota, Minneapolis, MN
Postdoctoral Fellow, 1992-1996, Purdue University, West Lafayette, IN
Pharmaceutical Solids and Regulatory Affairs Short Course, Purdue University, 2001

Employment

2000- **Aptuit, Inc. (formerly SSCI, Inc.), West Lafayette, Indiana**
Director (August 2010 – present)
Principal (January 2007 – July 2010)
Senior Research Investigator (2003 - 2006)
Research Investigator (2000 - 2003)
Project manager and group leader for external client projects involving various aspects of organic and analytical chemistry. These projects involve the development of new products (primarily pharmaceuticals) or providing scientific consulting to support patent litigation, counterfeit analysis or tampering analysis projects.

1996-2000 **Great Lakes Chemical Corp., West Lafayette, Indiana**
Research Chemist
Lead Scientist for a new technology development program in the GLCC Corporate R&D division. Technology Coordinator for contract research programs. Project Leader for a new product development project in GLCC Polymer Additives R&D.

Representative Technical Skills

Analytical Chemistry
- X-ray powder diffraction (XRPD)
- Thermogravimetric analyses (TGA)
- Differential scanning calorimetry (DSC)
- Optical microscopy
- Infrared (IR) and Raman spectroscopy
- Moisture sorption/desorption analyses
- NMR spectroscopy
- Spectrophotometry
- Solubility and dissolution testing
- pH measurements and potentiometric titrations

Organic Chemistry
- Synthetic chemistry
  - mg to kg scale laboratory reactions
  - high pressure reactions
  - air-sensitive procedures
- Crystallization methods
- Enantiomer Resolutions
- Polymorph, salt and cocrystal screening and characterization
- Analytical chromatography (HPLC and TLC)
- Preparative chromatography

Publications

1. Park, A.; Chyall, L.; Dunlap, J.; Schertz, C.; Jonaitis, D.; Stahly, B.; Bates, S.; Shipplett, R.; Childs S. New solid-state chemistry technologies to bring better drugs to market: knowledge-based decision making. *Exp. Opin. Drug Disc.*, **2007**, *2(1)*, 145-154.
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Patents

1. Chyall, L. J.; Hodgen, H. A.; Vyverberg, F. J.; Chapman, R. W. Intumescent Polymer Compositions. US 6,905,693 (June 14, 2005).

2. Chyall, L. J.; Hodgen, H. A.; Vyverberg, F. J.; Chapman, R. W.; Chou, P. K. Intumescent Polymer Compositions. US 6,632,442 B1 (October 14, 2003).

3. Robin, M. L.; Mazac, C. J.; Chyall, L. J.; Kleindl, P. Bromine-containing 1,2-bis(phenyl)difluoromethanes and method of imparting flame retardancy to flammable materials. US 6,348,633 (February 19, 2002).

Papers Presented

1. "Crystallization Studies of Nabumetone: Preparation and Characterization of a Novel, High-Energy Polymorph." Chyall, L. J.; Tower, J. M.; Coates, D. A.; Houston, T. L., 223rd National Meeting of the American Chemical Society, Orlando, FL, April 7-11, 2002: Abstract IEC 268

2. "The Synthesis and Properties of 7,7-Dichloro-*trans*-bicyclo-[4.1.0]-hept-3-ene." P. G. Gassman and L. J. Chyall, 22nd Great Lakes Regional Meeting of the American Chemical Society, Duluth, MN, May 31-June 2, 1989: Abstract 98.

3. "The Synthesis and Thermal Isomerization of 7,7-Dihalo-*trans*-bicyclo-[4.1.0]-hept-3-enes." P. G. Gassman and L. J. Chyall, 201st National Meeting of the American Chemical Society, Atlanta, GA, April 14-19, 1991: Abstract ORGN 228.
4. "Free Radical Rearrangements of Dihalo-*trans*-bicyclo-[4.1.0]-hept-3-enes." P. G. Gassman and L. J. Chyall, 32nd National Organic Chemistry Symposium, University of Minnesota, Minneapolis, MN, June 16-20, 1991: Abstract B-65.
5. "Kinetic Versus Equilibrium Control in the Deprotonation of Unsymmetrical Ketones in the Gas Phase." L. J. Chyall, M. D. Brickhouse, M. E. Schnute, and R. R. Squires, 205th National Meeting of the American Chemical Society, Denver, CO, March 28-April 2, 1993: Abstract ORGN 29.
6. "Ion-Molecule Chemistry of the Methylene Dimethylsulfonium Ion: A Novel Alpha-Distonic Ion." L. J. Chyall, R. L. Smith, K. M Stirk, and H. I Kenttämaa, 41st ASMS Conference on Mass Spectrometry, San Francisco, CA, May 30-June 4, 1993: Abstract MOD 12:10
7. "Radical-Type Reactivity of Distonic Ions: The 4-Dehydroanilinium Ion." L. J. Chyall and H. I Kenttämaa, 208th National Meeting of the American Chemical Society, Washington, DC, August 21-25, 1994: Abstract ORGN 404.
8. "Astrophysical Thermochemistry: The Heats of Formation of C_3H_2 Isomers." L. J. Chyall and R. R. Squires, 43rd ASMS Conference on Mass Spectrometry, Atlanta, GA, May 21-26, 1995: Abstract WOE 11:50.

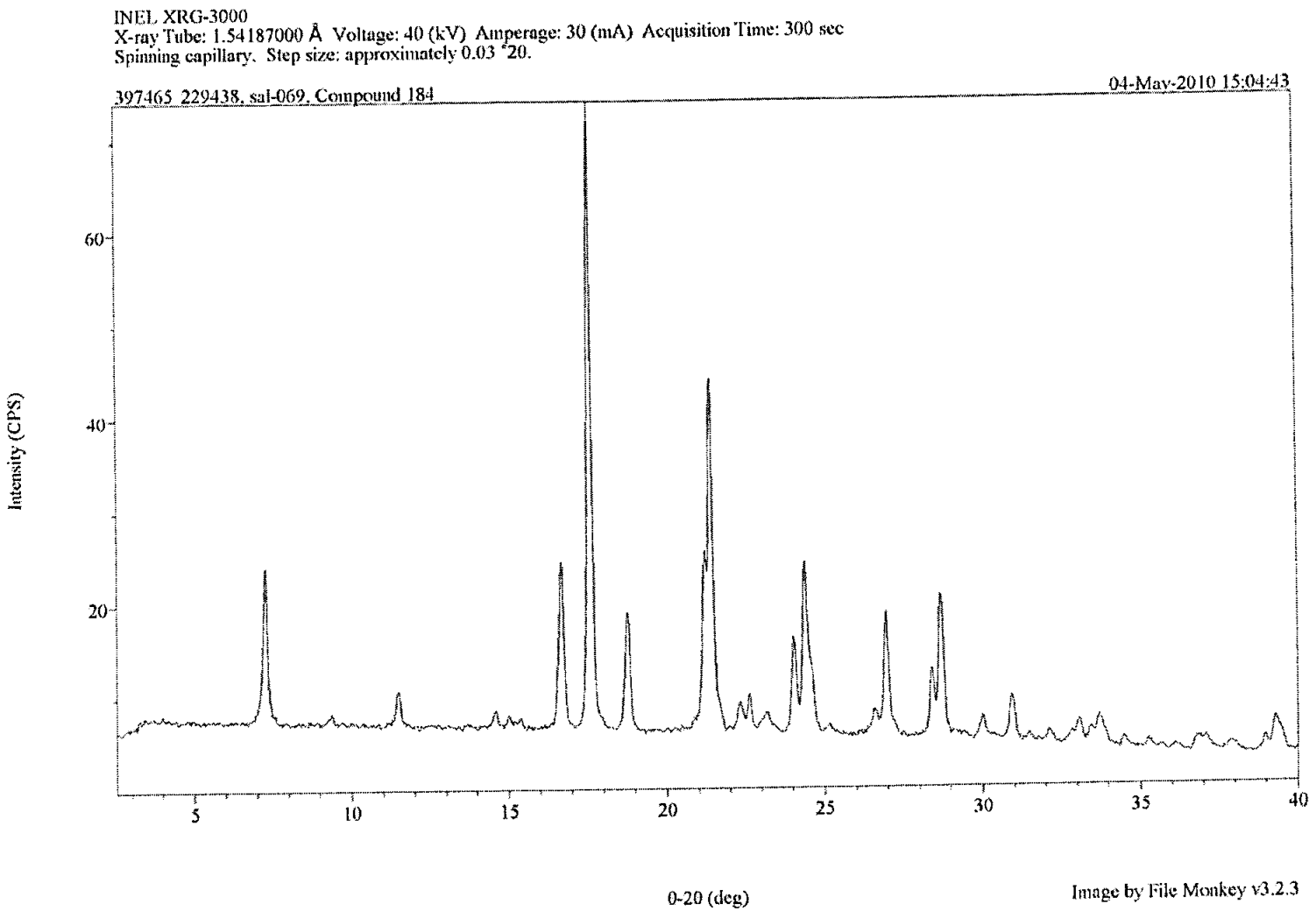
Invited Lectures

1. "Environmentally Friendly Fire Suppression Technology." Department of Chemistry, Purdue University, West Lafayette, IN. April 8, 1997.
2. "The Proton Affinity and Absolute Heat of Formation of Trifluoromethanol." Joint Institute Laboratory for Astrophysics, University of Colorado, Boulder, CO. March 1, 1996.
3. "Understanding the Atmospheric Fate of Hydrofluorocarbons: Thermochemisty of Trifluoromethanol." Aeronomy Laboratory, National Oceanic and Atmospheric Administration, Boulder, CO. February 29, 1996.

Dissertation

Chyall, L. J. The synthesis and thermal rearrangements of 7,7-dibromo-*trans*-bicyclo[4.1.0]hept-3-ene and 7,7-dichloro-*trans*-bicyclo[4.1.0]hept-3-ene. (1991) 192 pp. Avail.: Univ. Microfilms Int., Order No. DA9209417 From: Diss. Abstr. Int. B 1992, 52(10), 5264.

EXHIBIT B



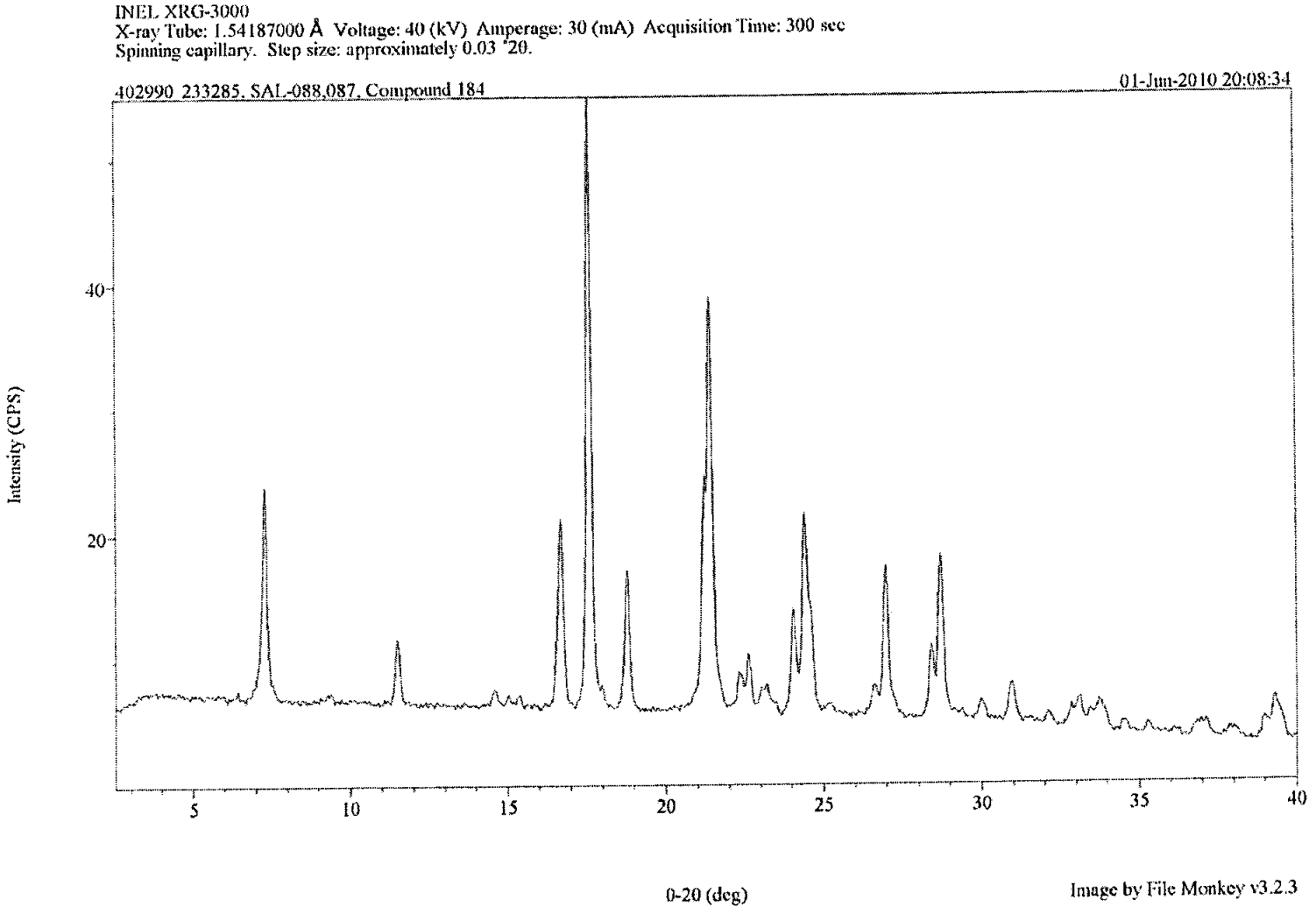


EXHIBIT C

Electronic Acknowledgement Receipt

EFS ID:	7473897
Application Number:	12740693
International Application Number:	PCT/IN08/00707
Confirmation Number:	1353
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	WO2009084024A2-Glenmark.pdf	1538690 258b04c553fc57708c49d50a3a40536fafc29f51	no	39
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Information:					
2	Information Disclosure Statement (IDS) Filed (SB/08)	API-0028-SITA-PCT-US_IDS_SB-08.pdf	38123 a8709d3ac21de51571624793a3a3f216256eee29	no	4
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3	Authorization to access Appl. by Trilateral Office	API-0028-SITA-PCT-US_SB039.pdf	79902 b1a2edfe55cfd2e4fe2265c5535ac0d1d537d7ce0	no	2
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4	Specification	API-0028-SITA-PCT-US_PCT_Appl_Asls_SPEC.pdf	419963 23456da271c94acd78f4c2dd4cba8c63844584a9	no	39
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5	Drawings-only black and white line drawings	API-0028-SITA-PCT-US_Figures.pdf	30406 7273b990f0f72e2b4e6a11064e82795795119450	no	4
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6	Oath or Declaration filed	API-0028-SITA-PCT-US_OathDec_SB01SB02.pdf	875950 583c6cb343946fcd9685e3efb03f124223994e25	no	4
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7	Documents submitted with 371 Applications	RO-01.pdf	190583 95ac8ef5e0fded0369b12edfb3b19670cdbf5a6a	no	6
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8	Documents submitted with 371 Applications	PrioDoc.pdf	1047292 4887b8bc85e56e5ce9461388f0c75a85e3d650a2	no	33
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9	Applicant Arguments/Remarks Made in an Amendment	API-0028-SITA-PCT-US_CoverLetter.pdf	41155 59b91fea89062f6cf8966a5eabe3f8dabf68bb99	no	3
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11	Application Data Sheet	API-0028-SITA-PCT-US_SB-014_ADS.pdf	39374 8c78bf779a228e59c85a18753eda07da4b3fdf6f	no	5
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12	Claims	API-0028-SITA-PCT-US_CLMS-PrelimAmndt.pdf	11109 ab2f2f8ad4966cff08a6bc15c80ba9db8f747fbb	no	1
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A PROCESS FOR THE PREPARATION OF R-SITAGLIPTIN AND ITS PHARMACEUTICALLY ACCEPTABLE SALTS THEREOF

PRIORITY

[0001] This application is a 35 U.S.C. 371 National Stage Filing of International Application No. PCT/IN2008/000707, filed October 27, 2008, which claims priority under 35 U.S.C. 119 (a-d) to IN 2190/MUM/2007 filed on November 2, 2007, the contents of which are incorporated by reference herein.

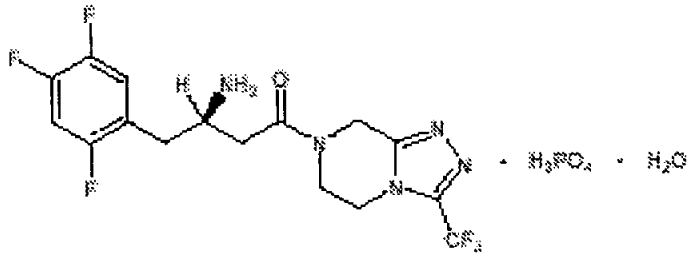
BACKGROUND OF THE INVENTION

1. Technical field

[0002] The present invention relates to a novel process for the preparation of R-sitagliptin and its pharmaceutical acceptable salts thereof. The present invention also provides structurally novel intermediates useful in the disclosed process, a pharmaceutical composition and a method of treating Type-2-diabetes.

2. Description of the Related Art

[0003] R-sitagliptin is commonly available as sitagliptin phosphate, 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate, and has the following structural formula:



[0004] Sitagliptin phosphate is an orally administered dipeptidyl peptidase-4 (DPP-4) inhibitor. Sitagliptin has been developed for the treatment of Type-2-diabetes and is available in the market under the brand name JANUVIA® as tablets in the dosage strengths of 25, 50, or 100 mg equivalent base.

[0005] International Patent Publication WO2004087650 describes a process for the preparation of sitagliptin via benzyloxy protected tetrazolylpyrazine intermediate.

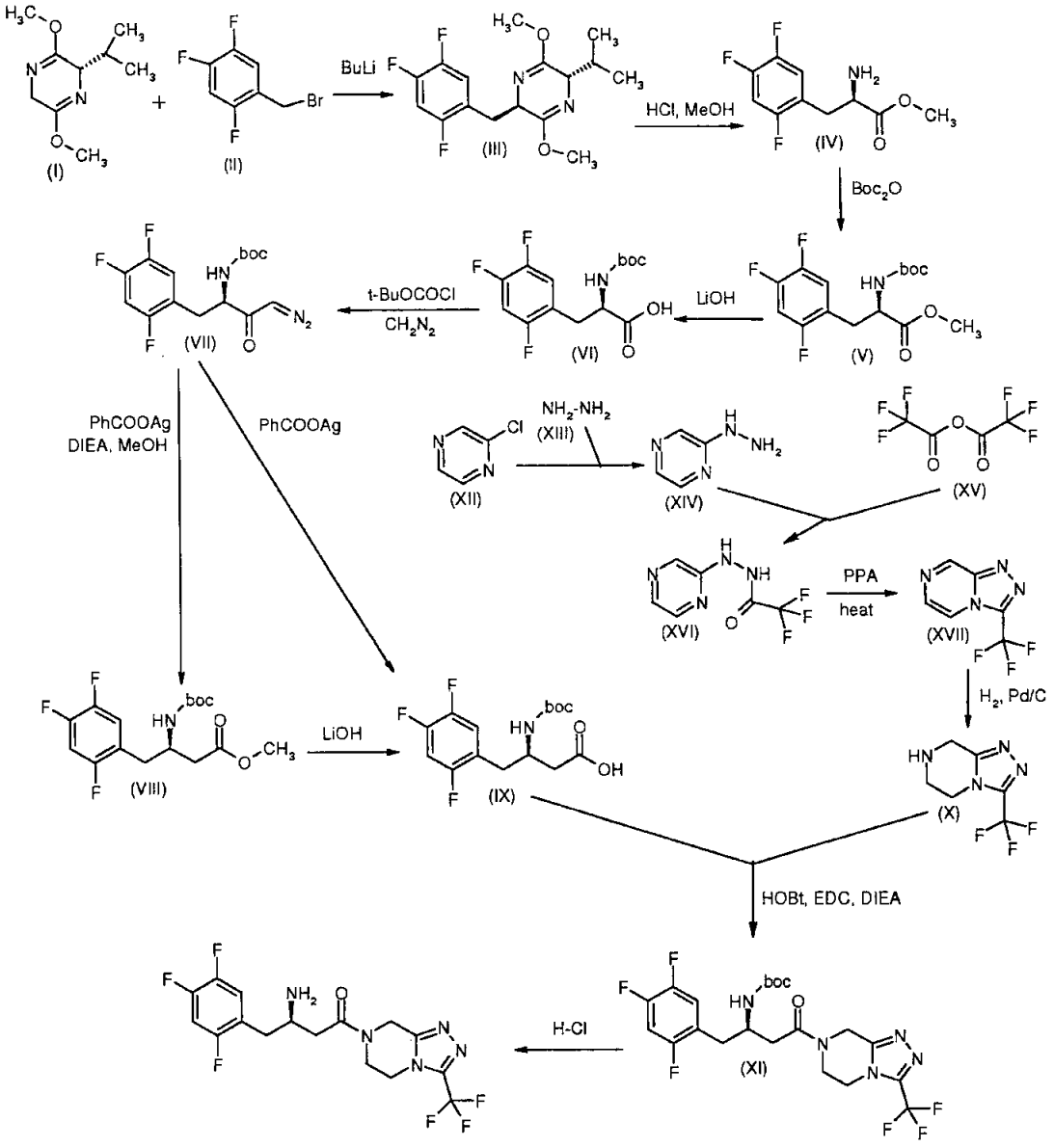
[0006] International Patent Publication WO2004085661 describes a process for the preparation of enantiomerically enriched sitagliptin via (S)-phenylglycine amide protected tetrazolyl pyrazine intermediate.

[0007] US PG Publication US20080058522 describes a process generically for the preparation of sitagliptin and its pharmaceutically acceptable salts using specific chiral bisphosphine ligands.

[0008] International Patent Publication WO2006081151 describes a process generically for the preparation of sitagliptin and its pharmaceutically acceptable salts using rhodium metal precursor complexed to a ferrocenyl diphosphine ligand.

[0009] US PG Publication US20060194977 describes a process for the preparation of Enantiomerically enriched sitagliptin using specific chiral ferrocenyl diphosphine ligands.

[0010] US Patent No. 6,699,871 describes various DPP-4 inhibitors including sitagliptin and their pharmaceutically acceptable salts, a pharmaceutical composition and method of treatment and a process for the preparation of sitagliptin hydrochloride as follows:



[0011] The aforementioned processes involve reactions that use specific chiral ligands or a stereo specific/stereoselective reduction process with specific stereoselective reducing agents, which are expensive and may not be commercially available, which may subsequently render the processes unsuitable on commercial scale.

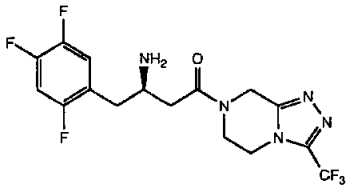
[0012] Hence, there is a need for an improved process for the preparation of R-sitagliptin or its pharmaceutically acceptable salts which alleviates the problems associated with aforementioned processes as referred above.

[0013] The process of the present invention provides a process which is simple, ecofriendly, inexpensive, reproducible, robust and well suited on commercial scale.

SUMMARY OF THE INVENTION

[0014] The present invention relates to a process for the preparation of R-sitagliptin and its pharmaceutically acceptable salts thereof.

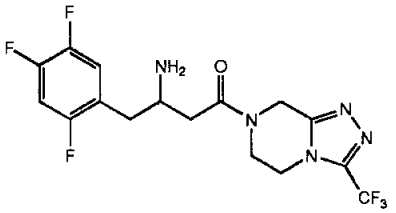
[0015] In one aspect, the present invention relates to a process for preparing R-sitagliptin of formula [Ia]



[Ia]

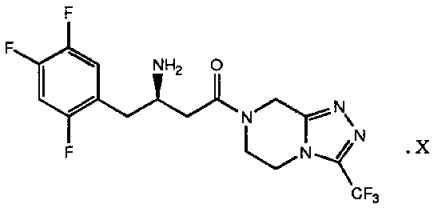
or a pharmaceutically acceptable salt thereof , comprising:

(a) resolving racemic sitagliptin of formula III



(III)

with a chiral acid to obtain a salt of the chiral acid and R-sitagliptin of formula II



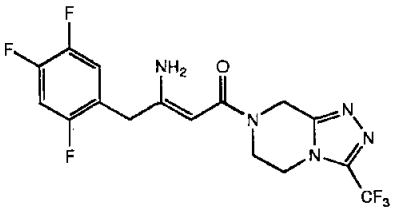
(II)

where X is the chiral acid; and

b) converting the salt of the chiral acid and R-sitagliptin to R-sitagliptin of Formula [Ia] or a pharmaceutically acceptable salt thereof.

[0016] In a second aspect, the present invention relates to a process for the preparation of racemic sitagliptin of formula [III] comprising:

a) reduction of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)but-2-en-2-amine compound of Formula [IV]

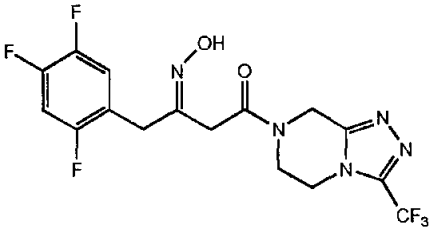


[IV]

with a reducing agent in the presence of organic solvent to give the racemic sitagliptin compound of Formula III.

[0017] In a third aspect, the present invention relates to an alternate process for the preparation of racemic sitagliptin of formula [III] comprising:

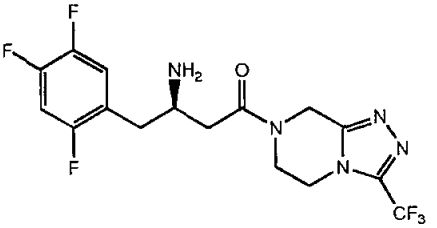
a) reaction of 3-oxime-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo [4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one compound of Formula [IVa]



[IVa]

with a reducing agent in an organic solvent.

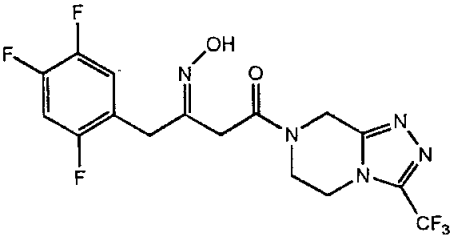
[0018] In a fourth aspect, the present invention relates to a to a process for preparing R-sitagliptin of formula [Ia]



[Ia]

comprising :

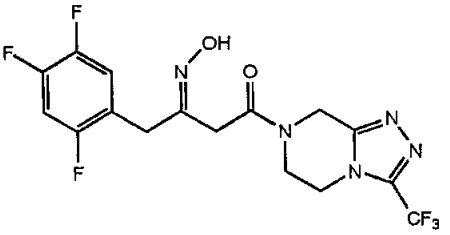
a) chiral reduction of compound 3-oxime-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one of Formula [IVa]



[IVa]

with a chiral reducing agent in the presence of an organic solvent.

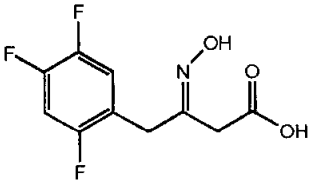
[0019] In a fifth aspect, the present invention relates to a process for the preparation of 3-oxime-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one of Formula [IVa]



[IVa]

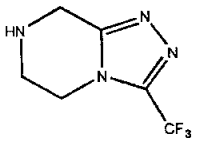
comprising:

a) reaction of 4-(2,4,5-trifluorophenyl)-3-oximebutanoic acid compound of Formula [VIIa]



[VIIa]

with 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-α]pyrazine compound of formula [VI] or salt thereof,

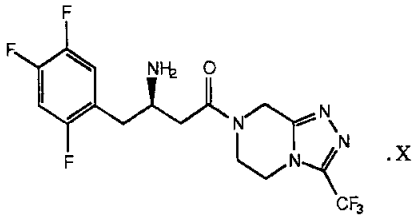


[VI]

in the presence of a coupling reagent and an organic solvent.

[0020] In a sixth aspect, the present invention provides R-sitagliptin or its pharmaceutically acceptable salt thereof having less than 0.15% by weight of the corresponding (S)-enantiomer by chiral HPLC.

[0021] In a seventh aspect, the present invention provides R-sitagliptin dibenzyl-L-tartrate of formula II



[II]

where X is dibenzyl-L-tartaric acid.

[0022] In an eighth aspect, the present invention provides R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of the present invention having an X-ray powder diffraction (XRPD) pattern with reflections at about: 6.5, 7.4, 10.9, 12.8, 14.9, 17.4, 17.9, 19.2, 21.5, 22.4, and 23.7 $\pm$ 0.2 degrees 2 theta.

[0023] In a ninth aspect, the present invention provides R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of present invention having an XRPD pattern which is substantially in accordance with Fig. 1.

[0024] In a tenth aspect, the present invention provides R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of present invention having a differential scanning calorimetry (DSC) thermogram with sharp endotherm at about 176.73°C with onset at about 171.49°C and endset at about 176.73°C.

[0025] In an eleventh aspect, the present invention provides R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of the present invention having a DSC thermogram which is substantially in accordance with Fig. 2.

[0026] In a twelfth aspect, the present invention provides R-sitagliptin of formula Ia

obtained by the process of the present invention having an X-ray powder diffraction (XRPD) pattern with reflections at about: 7.3, 17.6, 18.8, 21.2, 21.5, 22.4, 24.1, 24.4, 24.7, 27.0, and 28.7 $\pm$ 0.2 degrees 2 theta.

[0027] In a thirteenth aspect, the present invention provides R-sitagliptin of formula Ia obtained by the process of the present invention having an XRPD which is substantially in accordance with Fig. 3.

[0028] In a fourteenth aspect, the present invention provides R-sitagliptin of formula Ia obtained by the process of the present invention is having a differential scanning calorimetry (DSC) thermogram with sharp endotherm at about 117.66 °C with onset at about 116.37°C and endset at about 119.58°C.

[0029] In a fifteenth aspect, the present invention provides R-sitagliptin of formula Ia obtained by the process of the present invention having a DSC thermogram which is substantially in accordance with Fig. 4.

[0030] In a sixteenth aspect, the present invention provides a pharmaceutical composition comprising sitagliptin or its pharmaceutically acceptable salts obtained by the processes of the present invention and at least a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Fig. 1: Shows a powder X-ray diffraction pattern of R-sitagliptin dibenzyl-L-tartrate of Formula II as prepared by example 5c.

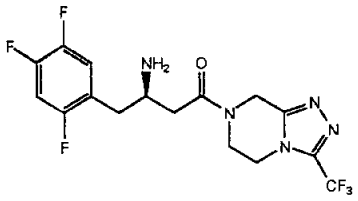
[0032] Fig. 2: Shows a Differential scanning calorimetry (DSC) of R-sitagliptin dibenzyl-L- tartrate of Formula II as prepared by example 5c.

[0033] Fig. 3: Shows a powder X-ray diffraction pattern of R-sitagliptin of Formula Ia as prepared by example 6.

[0034] Fig. 4: Shows a Differential scanning calorimetry (DSC) of R-sitagliptin of Formula Ia as prepared by example 6.

DETAILED DESCRIPTION OF THE INVENTION

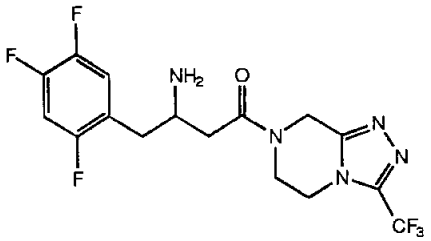
[0035] The present invention provides a process for preparing R-sitagliptin of formula [Ia]



[Ia]

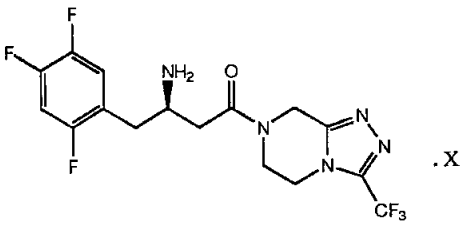
or a pharmaceutically acceptable salt thereof , comprising:

(a) resolving racemic sitagliptin of formula III



(III)

with a chiral acid to obtain a salt of the chiral acid and R-sitagliptin of formula II,



(II)

where X is chiral acid, and

b) converting the salt of the chiral acid and R-sitagliptin to R-sitagliptin of Formula [Ia] or a pharmaceutically acceptable salt thereof.

[0036] The chiral acid that can be used for resolution of racemic sitagliptin is selected from the group of S-(+) mandelic acid, R-(-) mandelic acid, L-(+)tartaric acid, D-(-) tartaric acid, (-)-dibenzoyl-L-tartaric acid, (-)-dibenzoyl-L-tartaric acid monohydrate, (+)-dibenzoyl-D -tartaric acid, (+)-dibenzoyl-D -tartaric acid monohydrate, (+)-dipara-toluoyl–D-tartaric acid, (+)-dipara-toluoyl–D-tartaric acid monohydrate, (-)-dipara-toluoyl–D-tartaric acid, (-)-dipara-toluoyl–D-tartaric acid monohydrate, (1R)-(-)-10-camphorsulfonic acid, and (1S)-(+)-10-camphorsulfonic acid. Preferably the chiral acid used is (-)-dibenzoyl-L-tartaric acid.

[0037] The organic solvent that can be used is selected from the group of alcohols such as methanol, ethanol, isopropyl alcohol and the like; ketones such as acetone, ethyl methyl ketone, methyl isobutyl ketone and the like; nitriles such as acetonitrile, propionitrile and mixtures thereof or their aqueous mixtures. Preferably the solvent used is methanol.

[0038] The resolution process can be carried out at temperature range of about 0°C to about 100°C or reflux temperatures of the solvents used. Preferably from about 20°C to about 70°C.

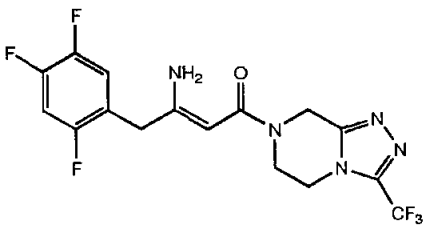
[0039] The molar equivalents of chiral acid used can be from about 0.5 to about 10 moles per moles of the racemic sitagliptin of formula III. Preferably 1:1 ratio of chiral acid and racemic sitagliptin is used.

[0040] The base that can be used is selected from the group of inorganic bases such as sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate, sodium bicarbonate, potassium bicarbonate, sodium methoxide, potassium methoxide and the like; organic bases such as liquid ammonia, triethylamine, diisopropylethylamine, pyridine and the like; aqueous or alcoholic mixtures thereof. Preferably aqueous sodium hydroxide.

[0041] The molar equivalents of base used can be from about 0.5 to about 10 moles per mole of the racemic sitagliptin of formula III. Preferably 1:1 ratio of base and racemic sitagliptin is used.

[0042] The present invention provides a process for the preparation of racemic sitagliptin of formula [III] comprising:

a) reducing 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*] pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)but-2-en-2-amine compound of Formula [IV]



[IV]

with a reducing agent in the presence of organic solvent to give the racemic sitagliptin compound of Formula III.

[0043] The reducing agents that can be used is selected from the group Raney nickel, palladium carbon, platinum, platinum dioxide, sodium borohydride, sodium triacetoxy borohydride, sodium cyanoborohydride, lithium aluminium hydride(LAH), diisobutylaluminium hydride (DIBAL-H), sodium bis(2-methoxyethoxy)aluminium hydride, tributyltin hydride, triethylsilane and the like. Preferably the reducing agent used is sodium cyanoborohydride.

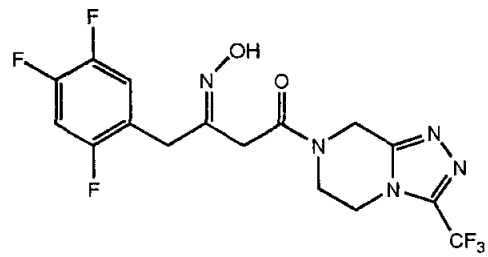
[0044] The organic solvent that can be used is selected from the group of alcohols such as methanol, ethanol, isopropyl alcohol and the like; ketones such as acetone, ethyl methyl ketone, methyl isobutyl ketone and the like; nitriles such as acetonitrile, propionitrile and mixtures thereof or their aqueous mixtures. Preferably the solvent used is methanol.

[0045] The resolution process can be carried out at temperature range of about 0°C to about 100°C or reflux temperatures of the solvents used, preferably from about 20°C to about 70°C.

[0046] The molar equivalents of reducing agent used can be from about 0.25 to about 10 moles per mole of racemic sitagliptin of formula III, preferably 1:1 ratio of reducing agent and racemic sitagliptin is being used.

[0047] The present invention further provides an alternate process for the preparation of racemic sitagliptin of formula [III] comprising:

a) reaction of 3-oxime-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one compound of Formula [IVa]



[IVa]

with a reducing agent in an organic solvent.

[0048] The reducing agents that can be used is selected from the group of Raney nickel, palladium carbon, platinum, platinum dioxide, sodium borohydride, sodium triacetoxy

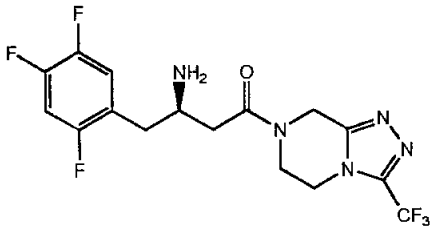
borohydride, sodium cyanoborohydride, lithium aluminium hydride (LAH), diisobutylaluminium hydride (DIBAL-H), sodium bis(2-methoxyethoxy)aluminium hydride, tributyltin hydride, triethylsilane and the like. Preferably the reducing agent used is palladium carbon.

[0049] The organic solvent that can be used is selected from the group of alcohols such as methanol, ethanol, isopropyl alcohol and the like; ketones such as acetone, ethyl methyl ketone, methyl isobutyl ketone and the like; nitriles such as acetonitrile, propionitrile and mixtures thereof or their aqueous mixtures. Preferably the solvent used is methanol.

[0050] The resolution process can be carried out at temperature range of about 20°C to about 100°C or reflux temperatures of the solvents used, preferably from about 20°C to about 70°C.

[0051] The molar equivalents of reducing agent used can be from about 0.25 to about 10 moles per mole of the racemic sitagliptin of formula III, preferably 1:1 ratio of reducing agent and racemic sitagliptin is being used.

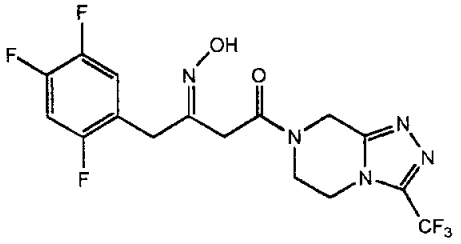
[0052] The present invention also provides an alternate process for preparing R-sitagliptin of formula [Ia]



[Ia]

comprising:

a) chiral reduction of compound 3-oxime-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one of Formula [IVa]



[IVa]

with a chiral reducing agent in the presence of an organic solvent.

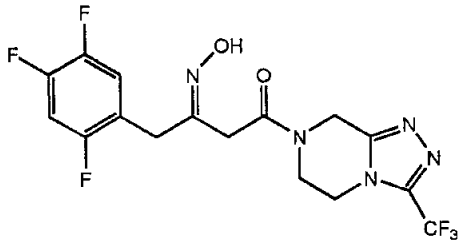
[0053] The chiral reducing agents that can be used is selected from the group of Borne-THF complex with chiral Auxiliaries of ephedrine derivatives such as L-(−)-norephedrine , (1*R*,2*R*)-(−)-pseudoephedrinepropionamide (*S*)-1- methyl-3,3-diphenyl-tetrahydro-pyrrolo[1,2*c*][1,3,2]oxazaborole, and (*S*)-(−)-4- isopropyl-5,5-diphenyl-2-oxazolidinone and the like, preferably L-(−)-norephedrine.

[0054] The organic solvent that can be used is selected from the group of alcohols such as methanol, ethanol, isopropyl alcohol and the like; ketones such as acetone, ethyl methyl ketone, methyl isobutyl ketone and the like; nitriles such as acetonitrile, propionitrile and mixtures thereof or their aqueous mixtures. Preferably the solvent used is methanol.

[0055] The reduction process can be carried out at temperature range of about 30°C to about 100°C or reflux temperatures of the solvents used, preferably from about 60°C to about 70°C.

[0056] The molar equivalents of chiral reducing agent used can be from about 0.25 to about 10 moles per mole of the compound of formula IVa, preferably 1:1 ratio of reducing agent and compound of formula IVa is being used.

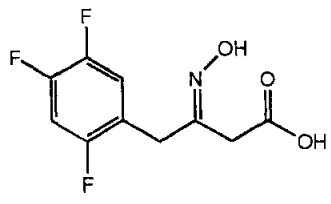
[0057] In embodiment of the present invention, there is provided a process for the preparation of 3-oxime-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one of Formula [IVa]



[IVa]

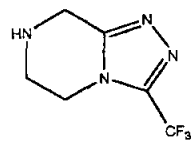
comprising:

a) reaction of 4-(2,4,5-trifluorophenyl)-3-oximebutanoic acid compound of Formula [VIIa]



[VIIa]

with 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-α]pyrazine compound of formula [VI] or salt thereof,



[VI]

in the presence of a coupling reagent and an organic solvent.

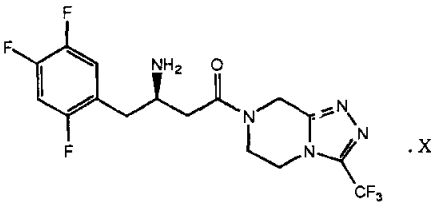
[0058] The coupling agent that can be used is selected from the group of benzotriazole-1-yl-oxy-tris(dimethylamino)phosphoniumhexafluoro-phosphate (BOP),N,N'-dicyclohexylcarbodiimide (DCC), 1-hydroxibenzotriazol anhydrous(HOBt), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) and the like. Preferably the coupling agent used is 1-hydroxibenzotriazol anhydrous (HOBt).

[0059] The organic solvent that can be used is selected from the group of ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran and the like; halogenated solvents such as methylene chloride, ethylene dichloride, chloroform and the like; alcohols such as methanol, ethanol, isopropyl alcohol and the like; hydrocarbon solvents such as n - hexane, n-heptane, cyclohexane, toluene and the like; or mixtures thereof. Preferably the solvent used is toluene.

[0060] The process can be carried out at temperature range of about 30°C to about 150°C or reflux temperatures of the solvents used, preferably from about 100°C to about 110°C.

[0061] The R-sitagliptin or its pharmaceutically acceptable salt thereof of the present invention may have less than 0.15% by weight of the corresponding (S)-enantiomer by chiral HPLC.

[0062] The present invention further provides R-sitagliptin dibenzyl-L-tartrate of formula II



[II]

where X is dibenzyl-L-tartaric acid.

[0063] In yet another embodiment, the present invention provides a process for preparing sitagliptin phosphate of formula I, as shown in Scheme 1.

c) allowing the obtained R-sitagliptin racemate which is enriched in (R) enantiomer to mix with methanol,

d) removing the precipitate;

e) recovering from the mother liquid the optically substantially pure R-sitagliptin by crystallization.

[0067] The mother liquor from resolution step or the mother liquor from each recrystallisation, is enriched with (S)-sitagliptin. (S)-sitagliptin present in one or more of these liquors, or the pooled liquors, may be converted into racemic sitagliptin for reuse in a process according to the present invention substantially as hereinbefore described.

[0068] A further preferred aspect of a process according to the present invention comprises:

(a) resolving racemic sitagliptin with a chiral acid and obtaining a mother liquor enriched in (S)-sitagliptin;

(b) converting (S)-sitagliptin obtained from (a) to racemic sitagliptin; and

(c) if desired, employing racemic sitagliptin obtained from (b) in a process according to the present invention substantially as hereinbefore described.

[0069] Suitably, one or more mother liquors obtained from a process as described above, or pooled such mother liquors, may be treated with a base to remove any residual chiral acid and to thereby afford the free base enriched in (S)-sitagliptin. The free base can then be converted to the racemate, typically by reflux in a suitable solvent for several hours, optionally in the presence of a suitable acid for example HCl or a base for examples NaOH, which racemate can then be recycled for use in a process according to the present invention substantially as hereinbefore described.

[0070] The term "optically substantially pure" means here optical purity over about 90%, preferably over 95%, and more preferably over 99%, expressed as the percent enantiomeric excess. The terms "resolve" and "resolution" are intended to compass the complete or partial separation of the two optical enantiomers.

[0071] The crystalline diastereomeric salt can be filtered and the free base liberated by basifying the salt with e.g. potassium carbonate solution or ammonia. The mother liquid can be recovered after filtering and be further treated in order to recover the enantiomer

which was not previously removed by precipitation. The treatment may comprise cooling the mother liquid and recovering the resulting crystalline diastereomeric salt.

[0072] R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of present invention having an X-ray powder diffraction (XRPD) pattern with reflections at about: 6.5, 7.4, 10.9, 12.8, 14.9, 17.4, 17.9, 19.2, 21.5, 22.4, and 23.7 ± 0.2 degrees 2 theta.

[0073] The R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of present invention may have an XRPD pattern which is substantially in accordance with Fig. 1.

[0074] R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of the present invention is further characterized by differential scanning calorimetry (DSC) having thermogram with sharp endotherm at about 176.73°C with onset at about 171.49°C and endset at about 176.73°C.

[0075] The R-sitagliptin dibenzyl-L-tartrate of formula II obtained by the process of present invention may have a DSC thermogram which is substantially in accordance with Fig. 2.

[0076] R-sitagliptin of formula Ia obtained by the process of present invention having an X-ray powder diffraction (XRPD) pattern with reflections at about: 7.3, 17.6, 18.8, 21.2, 21.5, 22.4, 24.1, 24.4, 24.7, 27.0, and 28.7 ± 0.2 degrees 2 theta. X-ray powder diffraction measurements were performed on a Philips X'pert PRO Diffractometer using Cu Kα radiation (Cu Kα1=1.54060Å). The X-ray source is operated at 45 kV and 40mA. Spectra are recorded at start angle from 2° to 50° 2θ, a step size 0.0167° with a time per steps of 50 seconds.

[0077] The R-sitagliptin of formula Ia obtained by the process of present invention may have an XRPD which is substantially in accordance with Fig. 3.

[0078] R-sitagliptin of formula Ia obtained by the process of present invention is further characterized by differential scanning calorimetry (DSC) having thermogram with sharp endotherm at about 117.66 °C with onset at about 116.37°C and endset at about 119.58°C.

[0079] The R-sitagliptin of formula Ia obtained by the process of present invention may have a DSC thermogram which is substantially in accordance with Fig. 4.

[0080] The DSC thermogram is recorded by following the procedure: Take the empty aluminum standard 40µL pan and put it in the microbalance. Take and weigh approximately below 2.0mg of sample. Slightly pierce the cover of the pan and seal it. Place the sample pan in the left position on mark "S" and empty reference pan in the right position on mark "R" of the blue DSC sensor. Place the furnace lid, and maintain the nitrogen gas as purge gas. Select the method temperature range is 30°C to 350°C and heating rate is 10°C/Minute .Under these conditions run the sample.

[0081] Typically the product obtained by the above described method contains about 90 wt% of the desired enantiomer of (Ia). The purity of the product can be increased to about 96 wt% by recrystallization. Methanol is the preferred recrystallization solvent. For example, the product which is enriched in (-) enantiomer is recrystallized by adding the product to methanol solvent, refluxing the mixture and filtering precipitate. The filtrate is concentrated, if necessary, and cooled in order to crystallize the (R)-enantiomer of (Ia). This allows recovering the substantially pure (-) enantiomer of (I) from the mother solution by crystallization.

[0082] The precipitation is carried out with cooling, decreasing the amount of the solvent and/or by adding a contrasolvent

[0083] The free base obtained may be optionally purified by recrystallization or slurrying in suitable solvents.

[0084] Recrystallization involves providing a solution of crude R-sitagliptin in a suitable solvent and then crystallizing the solid from the solution.

[0085] Suitable solvents in which R-sitagliptin can be dissolved for purification include but are not limited to: C_1-C_5 ketones such as acetone, ethyl methyl ketone, butanone and the like; alcohols such as ethanol, methanol, and isopropanol; ethers such as such as tetrahydrofuran, 1,4-dioxane, ethyl acetate and the like; water; and mixtures thereof.

[0086] The concentration of the R-sitagliptin in a solvent or mixture of solvents can range from about 40% to about 80% or more. The solution can be prepared at an elevated temperature if desired to achieve a higher solute concentration. Any temperature is acceptable for the dissolution as long as a clear solution of the R-sitagliptin is obtained and is not detrimental to the drug substance chemically or physically. The solution may be brought down to a lower temperature for further processing if required or an elevated

temperature may be used. A higher temperature for dissolution will allow the precipitation from solutions with higher concentrations of R-sitagliptin, resulting in better economies of manufacture.

[0087] The product may optionally be further dried. Drying can be suitably carried out in a tray dryer, vacuum oven, air oven, fluidized bed drier, spin flash dryer, flash dryer and the like. The drying can be carried out at temperatures of about 35°C to about 70°C. The drying can be carried out for any desired time periods to achieve the desired product purity, times from about 1 to 20 hours frequently being adequate.

[0088] R-sitagliptin prepared by above methods can also be converted into its pharmaceutically acceptable salts such as phosphate, hydrochloride, and the like; preferably phosphate.

[0089] The process briefly involves the reacting a pharmaceutically acceptable acid with R-sitagliptin in solution.

[0090] Suitable pharmaceutically acceptable acids which can be used include, but are not limited to: inorganic acids such as phosphoric acid, hydrochloric acid, hydrobromic acid, hydroiodic acid; and organic acids such as acetic acid, tartaric acid, oxalic acid, and the like. Preferably phosphoric acid.

[0091] Optionally, the acid is dissolved in a solvent before adding it to the solution of R-sitagliptin free base.

[0092] The solvent used for the dissolution of R-sitagliptin and the acid may be the same, or different solvents may be used.

[0093] Optionally, the acid addition salt obtained can be purified further by recrystallization or slurrying in suitable solvents.

[0094] Suitable solvents in which the acid addition salt of R-sitagliptin can be dissolved for purification include but are not limited to: C_1-C_5 ketones such as acetone, ethyl methyl ketone, butanone and the like; alcohols such as ethanol, methanol, and isopropanol; ethers such as tetrahydrofuran, 1, 4-dioxane, ethyl acetate and the like; water; and mixtures thereof.

[0095] The product may optionally be further dried. Drying can be suitably carried out in a tray dryer, vacuum oven, air oven, fluidized bed drier, spin flash dryer, flash dryer and the like. The drying can be carried out at temperatures of about 35°C to about 90°C. The

drying can be carried out for any desired time until the required product purity is achieved, time periods from about 1 to 20 hours frequently being sufficient.

[0096] R-sitagliptin or any of the pharmaceutically acceptable salts of R-sitagliptin prepared in accordance with the present invention contains less than about 0.5%, of the corresponding impurities as characterized by a chiral HPLC (high performance liquid chromatography) chromatogram obtained from a mixture comprising the desired compound and one or more of the said impurities, preferably less than about 0.1%. The percentage here refers to weight percent obtained from the area-% of the peaks representing the impurities. R-sitagliptin and salts thereof also are substantially free of other process-related impurities

[0097] The process of the present invention advantageously provides R-sitagliptin or its pharmaceutically acceptable salts in relatively high purity, e.g., greater than about 98% ee and preferably greater than about 99%.

[0098] The R-sitagliptin or its pharmaceutically acceptable salts obtained by the processes of the present invention has residual organic solvent less than the amount recommended for pharmaceutical products, as set forth for example in ICH guidelines and U.S. Pharmacopoeia; the recommended amount is less than 5000 ppm for methanol, ethyl acetate and acetone; less than 800ppm for toluene, dichloromethane, dimethyl formamide and diisopropyl ether. Preferably, the amount is less than about 5000 ppm residual organic solvent, preferably, more preferably less than about 2000 ppm residual organic solvent, most preferably, less than about 700 ppm.

[0099] The pharmaceutical composition comprising R-sitagliptin or its pharmaceutically acceptable salts prepared by the processes of present invention may be formulated for oral administration. Accordingly, D_{90} particle size of the unformulated sitagliptin or pharmaceutically acceptable salts thereof used as starting material in preparing a pharmaceutical composition generally is less than 300 microns, preferably less than about 200 microns, more preferably less than 100 microns, still more preferably less than about 50 microns and still more preferably less than about 20 microns.

[00100] Any milling, grinding micronizing or other particle size reduction method known in the art can be used to bring the solid state sitagliptin or its pharmaceutically acceptable salt thereof into any desired particle size range as set forth above.

[00101] Another aspect of the present invention is directed to a pharmaceutical dosage form containing sitagliptin or its pharmaceutically acceptable salts thereof. The pharmaceutical dosage may be in any form, for example, compacted tablets, powder suspensions, capsules, and the like. The compositions of the present invention can be administered to humans and animals in such dosage forms as oral, rectal, parenteral (intravenous, intramuscular, or subcutaneous), intracistemal, intravaginal, intraperitoneal, local (powders, ointments or drops), ophthalmic, transdermal, or sublingual forms or as a buccal or nasal spray. Oral dosage forms include, but are not limited to, pills, capsules, troches, sachets, suspensions, powders, lozenges, elixirs, tablets, capsules (including soft gel capsules), ovules, solutions, and the like which may contain flavoring or coloring agents, for immediate-, delayed-, modified-, or controlled-release such as sustained-, dual-, or pulsatile delivery applications. R-sitagliptin or its pharmaceutically acceptable salt thereof prepared by the process as described herein also may be administered as suppositories, ophthalmic ointments and suspensions, and parenteral suspensions, which are administered by other routes. The most preferred route of administration of the sitagliptin or its pharmaceutically acceptable salts thereof of the present invention is oral.

[00102] The active ingredient of the invention may also be administered via fast dispersing or fast dissolving dosage forms or in the form of high energy dispersion or as coated particles. Suitable pharmaceutical composition of the invention may be in coated or uncoated form as desired.

[00103] Tableting compositions may have few or many components depending upon the tableting method used, the release rate desired and other factors. For example, the compositions of the present invention may contain diluents such as cellulose-derived materials like powdered cellulose, microcrystalline cellulose, microfine cellulose, methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, carboxymethyl cellulose salts and other substituted and unsubstituted celluloses; starch; pregelatinized starch; inorganic diluents such calcium carbonate and calcium diphosphate and other diluents known to one of ordinary skill in the art. Yet other suitable diluents include waxes, sugars (e.g. lactose) and sugar alcohols like mannitol and sorbitol, acrylate polymers and copolymers, as well as pectin, dextrin and gelatin.

[00104] Other excipients contemplated by the present invention include binders, such as acacia gum, pregelatinized starch, sodium alginate, glucose and other binders used in wet and dry granulation and direct compression tableting processes; disintegrants such as sodium starch glycolate, crospovidone, low-substituted hydroxypropyl cellulose and others; lubricants like magnesium and calcium stearate and sodium stearyl fumarate; flavorings; sweeteners; preservatives; pharmaceutically acceptable dyes and glidants such as silicon dioxide.

[00105] Capsule dosages will contain the solid composition within a capsule which may be coated with gelatin. Tablets and powders may also be coated with an enteric coating. The enteric-coated powder forms may have coatings comprising phthalic acid cellulose acetate, hydroxypropylmethyl cellulose phthalate, polyvinyl alcohol phthalate, carboxymethylethylcellulose, a copolymer of styrene and maleic acid, a copolymer of methacrylic acid and methyl methacrylate, and like materials, and if desired, they may be employed with suitable plasticizers and/or extending agents. A coated tablet may have a coating on the surface of the tablet or may be a tablet comprising a powder or granules with an enteric coating.

[00106] Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification. The invention is further defined by reference to the following examples describing in detail the preparation of the composition and methods of use of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

EXAMPLES

[00107] Example 1: PREPARATION OF 5-[1-HYDROXY-2-(2,4,5 TRIFLUOROPHENYL) ETHYLIDENE]-2,2-DIMETHYL-1,3-DIOXANE-4,6-DIONE

200 gm of 2,4,5-triflurophenyl acetic acid, 108 ml of oxalic acid,10 ml of dimethyl formamide and 2000 ml of methylenedichloride were charged into a clean and dry round bottom flask followed by stirring at about 25-30°C for about 2-3 hours., the progress of the reaction was monitored by thin layer chromatography (TLC) including high performance liquid chromatography (HPLC), after the completion of the reaction, the reaction mass was cooled to about -5°C. Pre-reacted solution of 280 gm of 4-dimethylamino pyridine and 226 gm of 2,2-dimethyl-1,3-dioxane-4,6-dione in 1000 ml of methylenedichloride was added to the reaction mass between the temperature of about -5~0°C and maintained the reaction mixture at the same temperature until the completion of reaction, which was monitored by TLC or HPLC. 300 gm of dried product of 5-[1-hydroxy-2-(2,4,5-trifluorophenyl)ethylidene]-2,2-dimethyl-1,3-dioxane-4,6-dione (Formula VII) was obtained by the acidic aqueous workup followed by solid precipitation with diisopropyl ether . The isolated compound has been characterized by Melting points, Mass ^{1}H NMR and HPLC purity.

Mass	: 315.31 [M-H]$^{-}$
^{1}H NMR(300 MHz,CDCl3)	: 7.1,6.9 (2 H,m), 4.43 (2 H,s),1.7(6 H,s)
Melting point	: [86.44 – 109.5] °C
HPLC purity	: NLT = 98 %.

[00108] Example 2: PREPARATION OF 4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-a]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUOROPHENYL)BUTAN-2-ONE

547 gm of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one (Formula V) was obtained by adding 500 gm of 5-[1-hydroxy-2-(2,4,5-trifluorophenyl)ethylidene]-2,2-dimethyl-1,3-dioxane-4,6-dione in to the solution 5000 ml toluene containing 440 gm of 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazine .hydrochloride (Formula VI) and 1100 ml of diisopropyl ethylamine at about 25-30°C followed by heating the reaction mass to toluene reflux temperature until the completion of the reaction, as monitored by TLC or HPLC. The isolation of the above said product was done by ethylacetate and aqueous–acidic workup followed by distillation.

The isolated compound has been characterized by Mass and HPLC purity.

Mass : 407.18 $[M+H]^+$

HPLC purity : NLT = 85 %.

[00109] Example 3 : PREPARATION OF 4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-a]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUORO PHENYL) BUT-2-EN-2-AMINE

540 gm of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one dissolved in 5400 ml of methanol and cooled to 0-5°C. 540 gm of ammonium acetate and 1080 ml of aqueous ammonia (25w/w) solution was added and maintained for about 15-30 minutes at about 40-45°C until the completion of the reaction, as monitored by TLC or HPLC. 387 gm of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl) but-2-en-2-amine (Formula IV) was obtained by filtration and drying . The isolated compound has been characterized by Melting Points, Mass ^{1}H NMR and HPLC purity.

Mass : 406.74 $[M+H]^+$

^{1}H-NMR (400MHz,DMSO) : 7.5(2H,m),4.86(1H,s),4.82(1H,s),4.1(2 H,m),3.8(2H,m), 3.4(1 H,s),3.32(1 H,s).

Melting point : 193.77 °C

HPLC purity : NLT = 95 %.

[00110] Example 4:4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO [1,2,4]TRIAZOLO[4,3-*A*]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUOROPHENYL) BUTAN-2-AMINE

50 gm of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)but-2-en-2-amine with 150 ml of methanol and 350 ml of methylenedichloride with 19 gm of sodiumcyano borohydride in the presence of 30 ml of acetic acid with the temperature range of about -5°C to about 25-30°C under nitrogen atmosphere. 46 gm of 4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]

triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (Formula III) was isolated by aqueous ammonia work-up .

HPLC purity : NLT = 85 %.

b) 4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-a] PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUOROPHENYL)BUTAN-2-AMINE :

3.5 gm of 4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine isolated as solid from 9.2 gm of crude with 117 ml of toluene crystallization with the temperature range from about 70-75°C to about 25-30°C. The isolated compound has been characterized by Melting points, Mass ^{1}H NMR and HPLC purity.

Mass : 408.43 $[M+H]^+$

^{1}H-NMR(300MHz,CDCl3) : 7.0(H,m),6.9(1H,m),4.9(2H,m,s),4.1(4H,m),3.6(1H,s), 3.5(1H,bs) 2.8~2.4(4H,bm),1.7(2H,bs)

Melting point : 97.03 °C

HPLC purity : NLT = 95 %.

[00111] Example 5: (2*R/2S*)-4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO [1,2,4]TRIAZOLO[4,3-a]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUOROPHENYL) BUTAN2-AMINE(-)DBLTA SALT

a) 41 gm of (2R/2S)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo [4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dissolved in 250 ml of acetone at 25-30°C was added to the solution of 35 gm of (-) Dibenzolyl -L-Tartaric acid dissolved in 1000 ml of diisopropyl ether at 25-30°C for 15-30 minutes followed by stirring the reaction mass to 2 hours. 61 gm of (R/S)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine. (-) DBLTA diastereomeric salt (Formula II) was obtained by filtration and followed by drying the solid at 50-55°C for 12 hours. The isolated solid has been characterized by chiral HPLC, XRD and DSC.

Chiral HPLC purity was 50:50(S/R diastereoisomer ratio).

b) PURIFICATIONS OF (2S/2R)-4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-*A*]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUORO PHENYL) BUTAN-2-AMINE (-) DBLTA DIASTREOMERIC SALT

5 gm of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo[4,3-*a*] pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine(-)DBLTA diastereomeric salt was obtained by repeated reflux-leaching with methanol at 65 °C to 25-30°C followed by two recrystallisations with 300 ml and 250 ml of methanol at the temperature of 65 °C to 25-30°C. The isolated solid has been characterized by chiral HPLC, XRD and DSC.
Chiral HPLC 95.42 % was observed.

c) PREPARATION CUM PURIFICATION OF (2*R*)-4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-*A*]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUOROPHENYL)BUTAN-2-AMINE(-)DBLTA

212 gm of (2R/2S)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dissolved in 848 ml of methanol was added to the solution of 172 gm of (-) dibenzolyl –L-tartaric acid dissolved in 848 ml of methanol at reflux temperature for about 15-30 minutes followed by stirring the reaction mass for about 2 hours at about 65-70°C. 252 gm of white solid was isolated by filtration at about 25-30°C. About 65-70 % chiral purity was observed.

The purity was further enhanced by recrystallisation with methanol-water system at 65-70°C with volume of 25 to35 of (2R/2S)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (Formula II) for twice ,106 gm of white solid was isolated by filtration . The isolated solid been characterized by chiral HPLC, XRD and DSC

The chiral purity observed was 85~90 %.

[00112] Example - 6: PREPARATION OF (2*R*)-4-OXO-4-[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-a]PYRAZIN-7(8*H*)-YL]-1-(2,4,5- TRIFLUOROPHENYL)BUTAN-2-AMINE

105 gm of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine(-)dibenzyl–L-tartaric acid was suspended in 525 ml of methylene dichloride and 525 ml of water. The pH of the suspension was adjusted to about 11 to 12 by addition of 10 % aqueous sodium hydroxide solution over about 15-20 minutes under stirring. Organic and aqueous layers were separated and the organic layer was distilled completely at about 30-35 °C under vacuum. The solid separated (Formula Ia) was isolated by toluene crystallization and was dried at about 55-60°C for 12 hours to afford 33.5 gm of the title compound.

Purity by HPLC: 99.36 %.

Purity by Chiral HPLC: 99.7 %.Melting point: 117.66°C.

[00113] Example -7 : PREPARATION OF (2*R*)-4-OXO-4-[3-(TRIFLUOROMETHYL) -5, 6-DIHYDRO[1,2,4]TRIAZOLO-[4,3-a]PYRAZIN-7(8*H*)-YL]-1-(2,4,5-TRIFLUORO PHENYL) BUTAN-2-AMINE DIHYDROGEN PHOSPHATE

5 gm of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*] pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and 150 ml of ethanol were charged into a clean and dry round bottom flask followed by heating to about 50-55 °C. 1.26 ml of 85 %v/v of phosphoric acid was added to the above solution at about 50-55 °C in one lot. The reaction mixture was maintained under stirring at about 75-78°C for about 30 minutes. The separated solid (Formula I) was filtered and the solid obtained was dried at about 50 -55°C for about 12 hours to afford 4 gm of the title compound.

Specific optical rotation [SOR]: -20 to -22 [C=1 % water)

Melting point: 212°C to 213.5 °C

Purity by Chiral HPLC: 99.92 %; Purity by HPLC: 99.95 %.

ABSTRACT

[00114] The present invention provides processes for the preparation of R-sitagliptin and its pharmaceutically acceptable salts thereof.

PRELIMINARY AMENDMENT

CLAIMS:

1. R-sitagliptin having an X-ray powder diffraction (XRPD) pattern with reflections at about: 7.3, 17.6, 18.8, 21.2, 21.5, 22.4, 24.1, 24.4, 24.7, 27.0, and 28.7 $\pm$ 0.2 degrees 2 theta.

2. R-sitagliptin having a differential scanning calorimetry (DSC) thermogram with sharp endotherm at about 117.66°C with onset at about 116.37°C and endset at about 119.58°C.

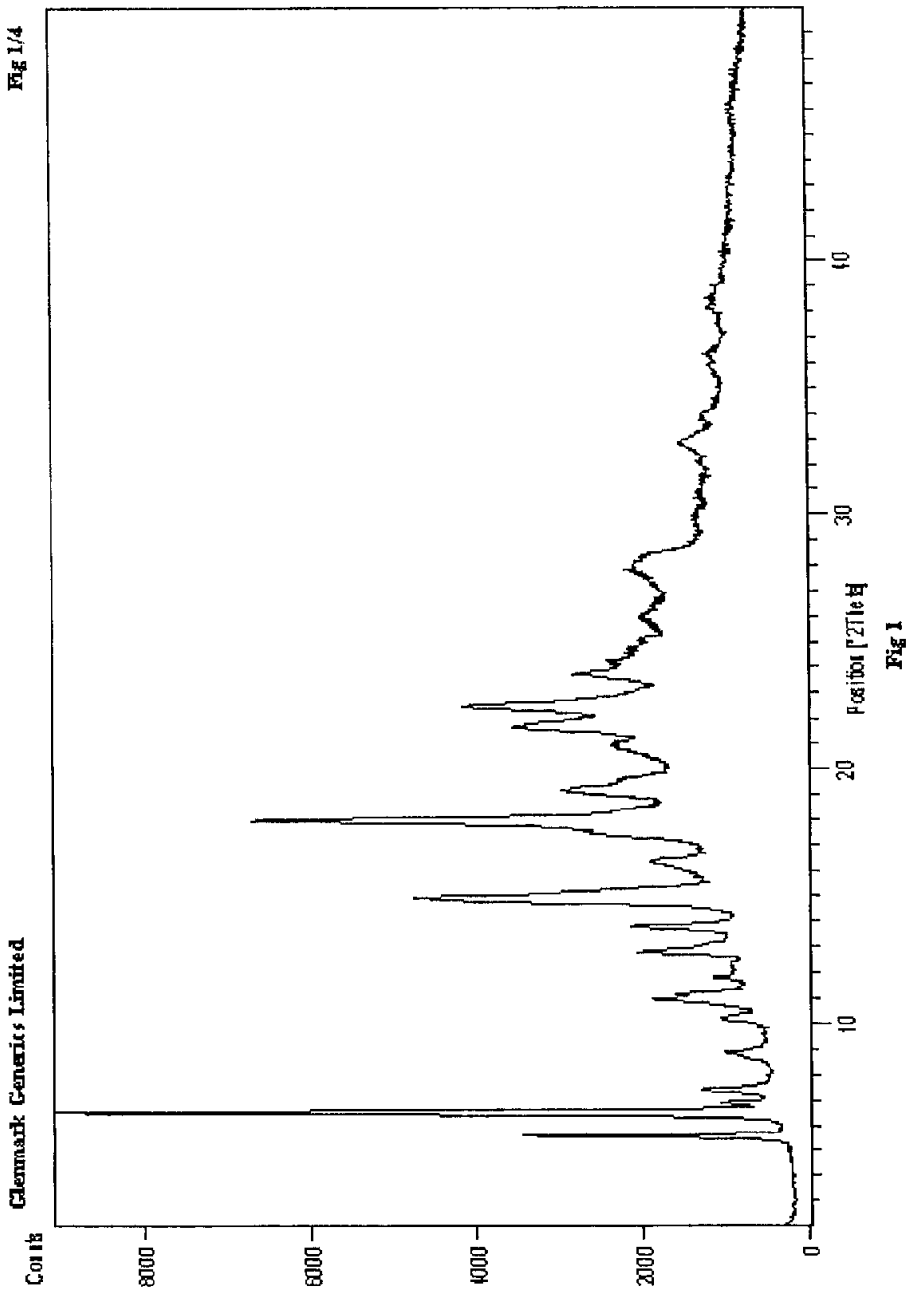


Fig 1/4
Glenmark Generics Limited
Counts
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6000
4000
2000
0
10
20
30
40
Position [°2Theta]

Fig 1

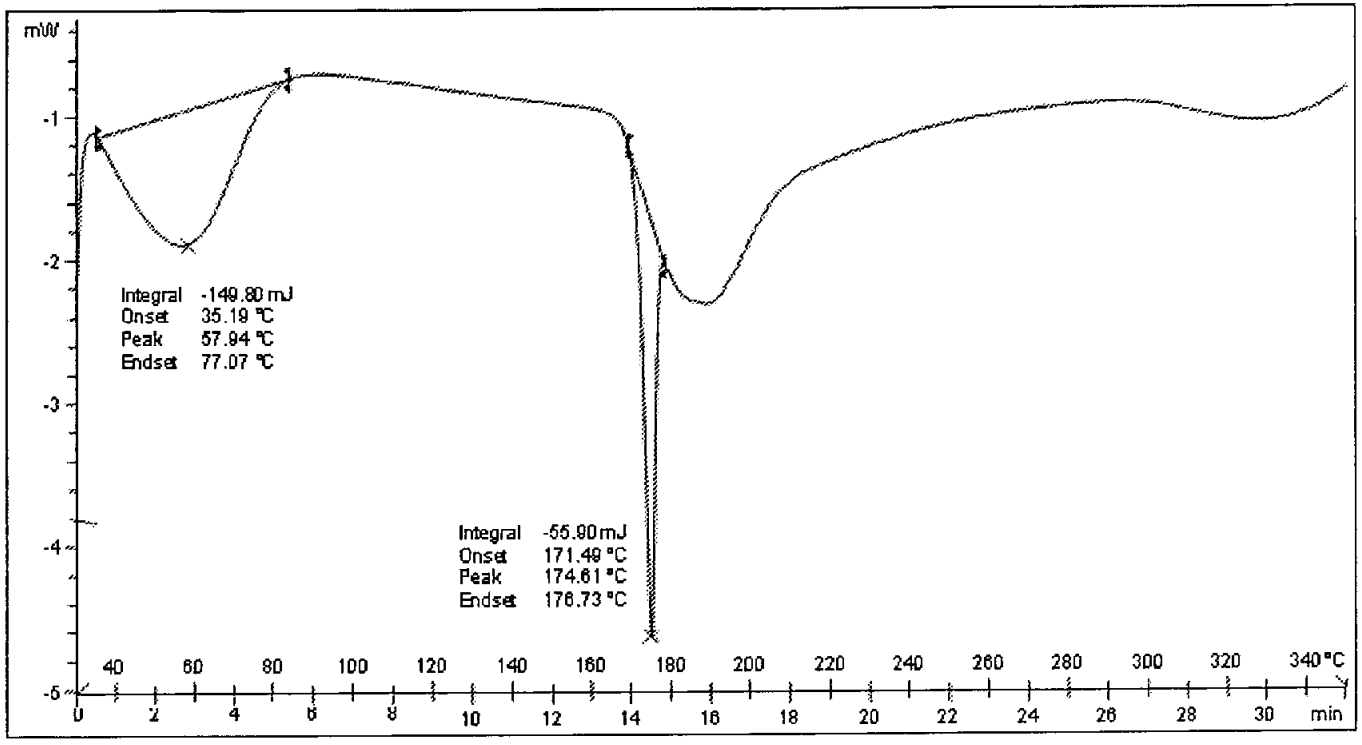

Fig 2

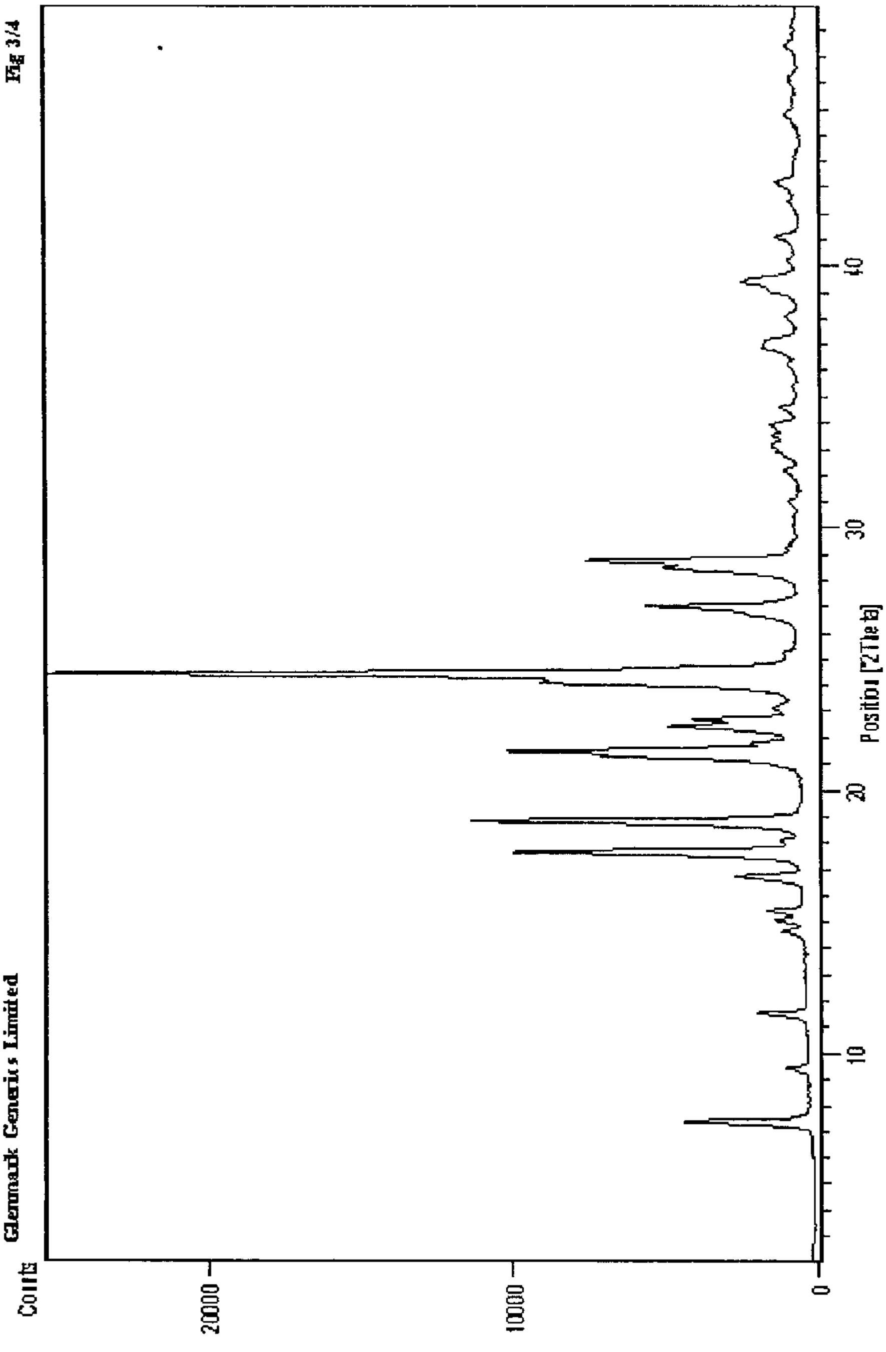
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Fig 3/4
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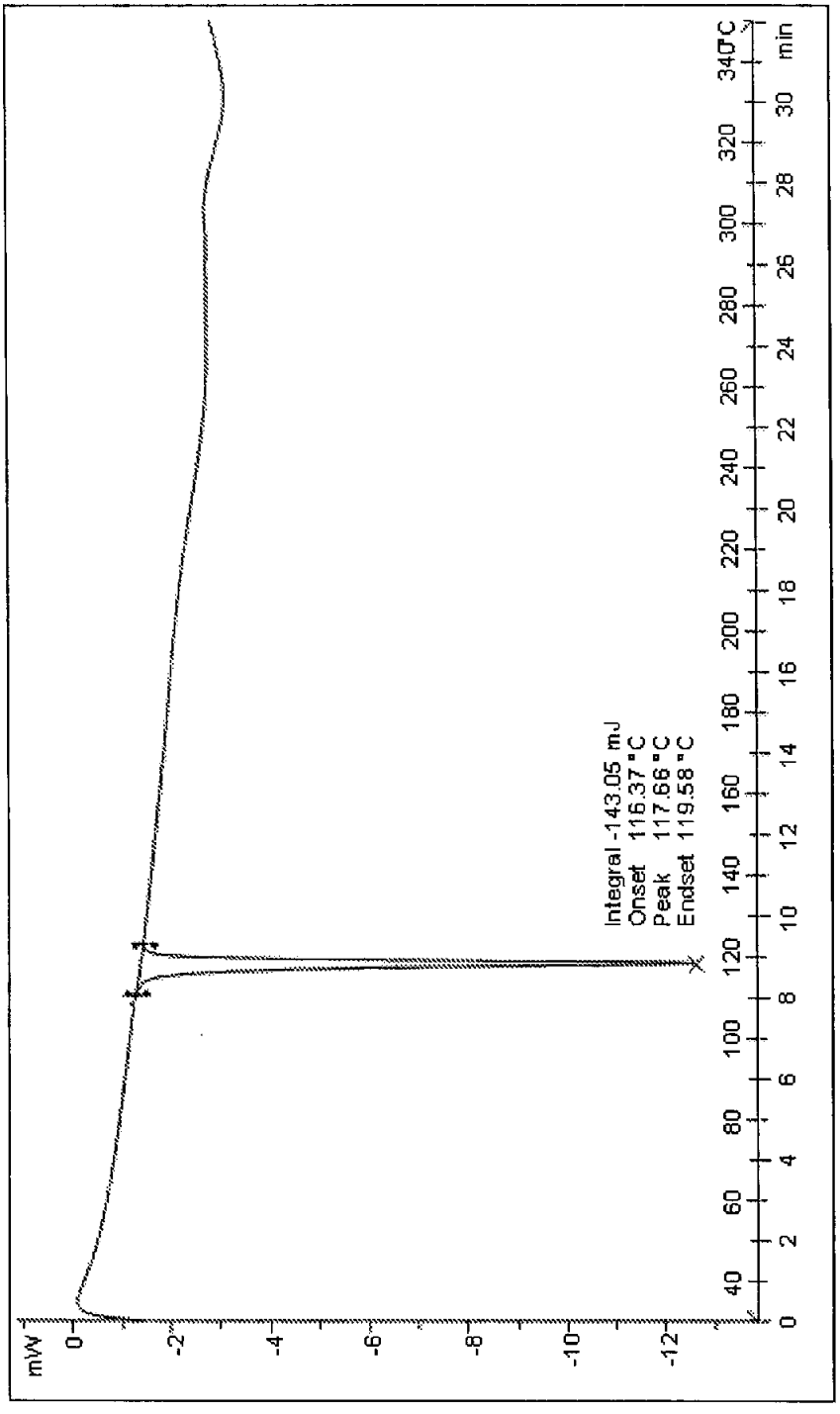
Position [°2Theta]

Fig 3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18
20
22
24
26
28
30
min
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340°C
Integral -143.05 mJ
Onset 116.37 °C
Peak 117.66 °C
Endset 119.58 °C

Fig 4

EXHIBIT D

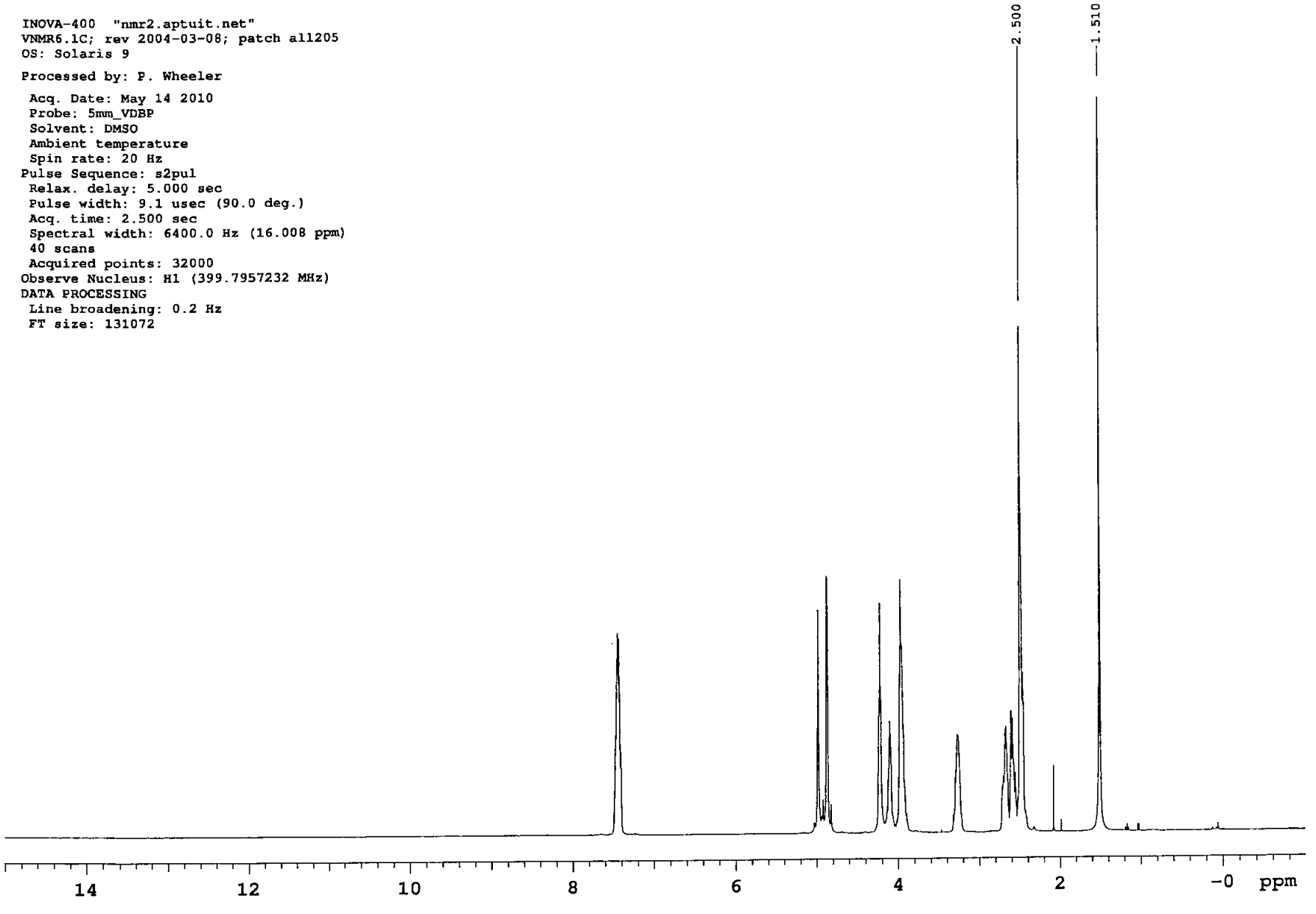
229438, Compound 184, Lot sal-069, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

File: 399497

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

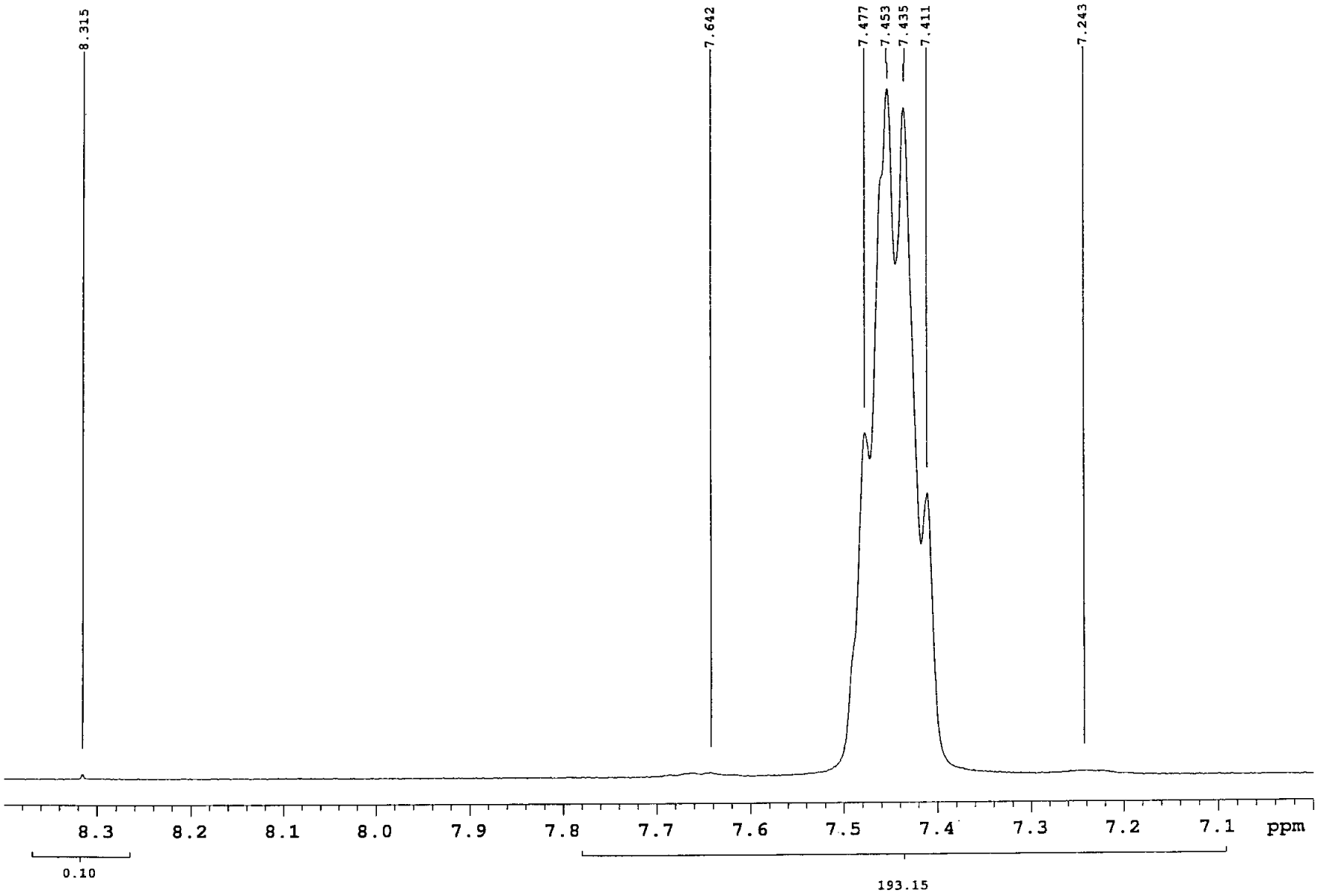
Acq. Date: May 14 2010
Probe: 5mm_VDBP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 9.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 399497-1

229438, Compound 184, Lot sal-069, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

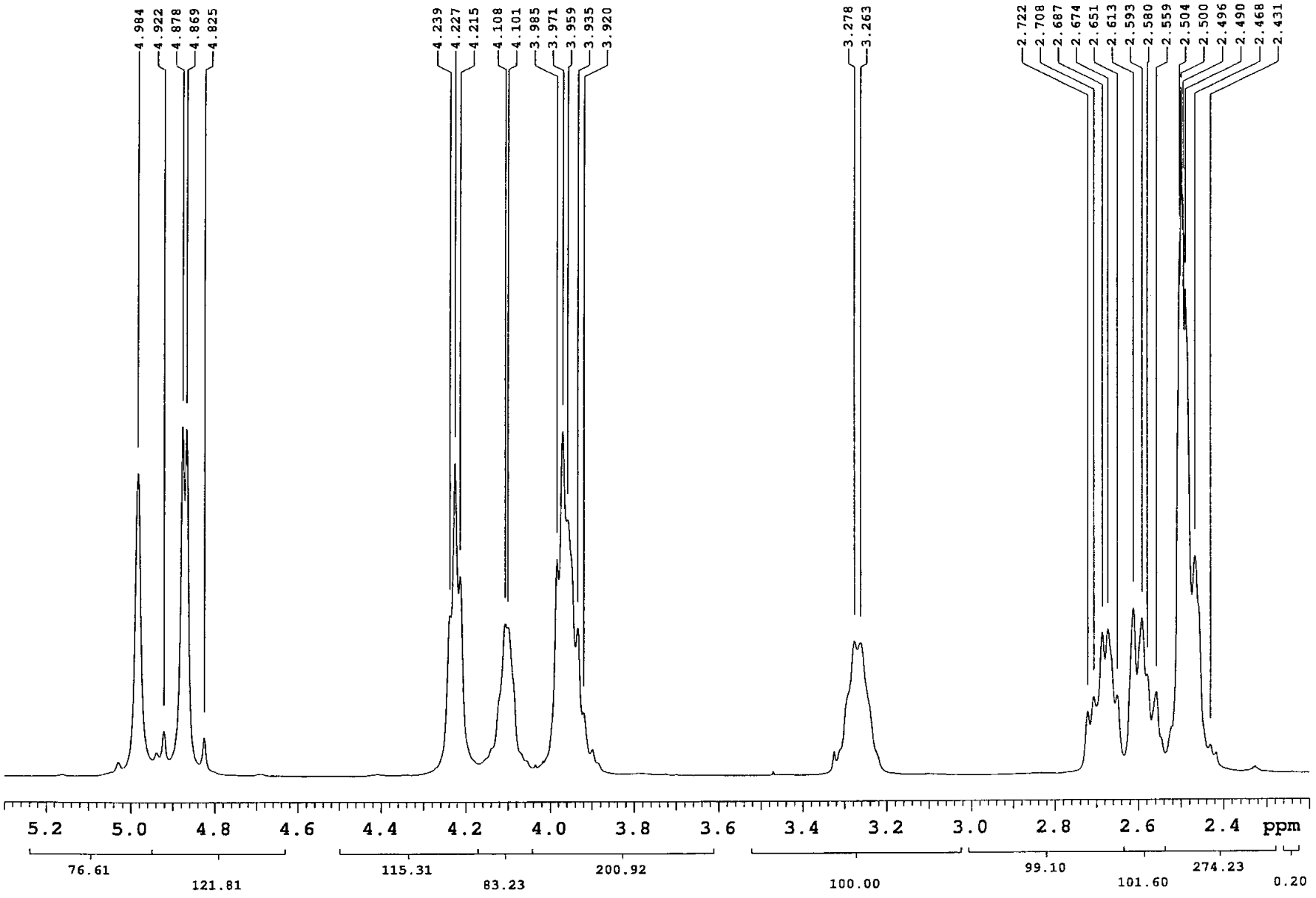
File: 399497



Plot file: 399497-2

229438, Compound 184, Lot sal-069, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

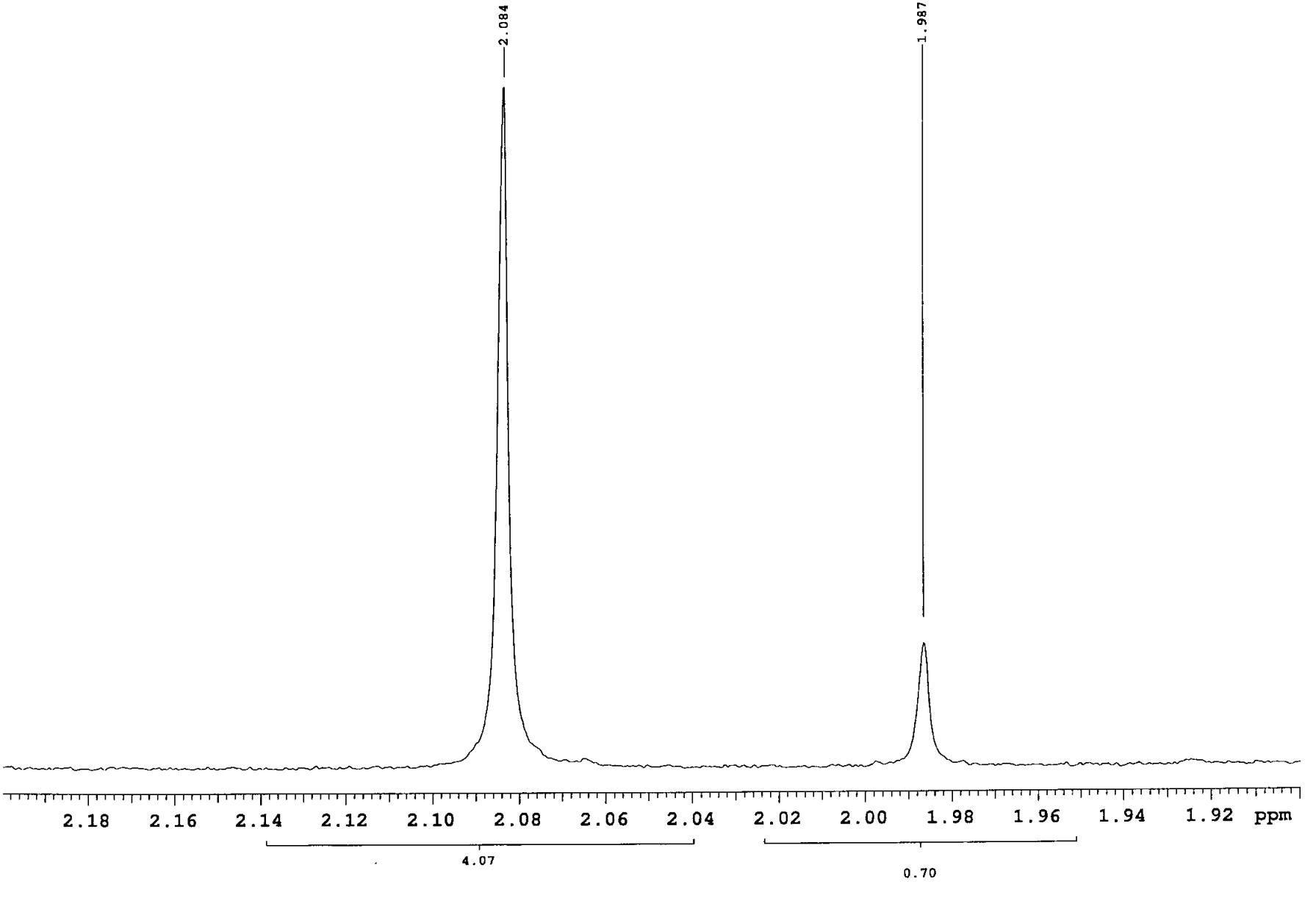
File: 399497



Plot file: 399497-3

229438, Compound 184, Lot sal-069, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

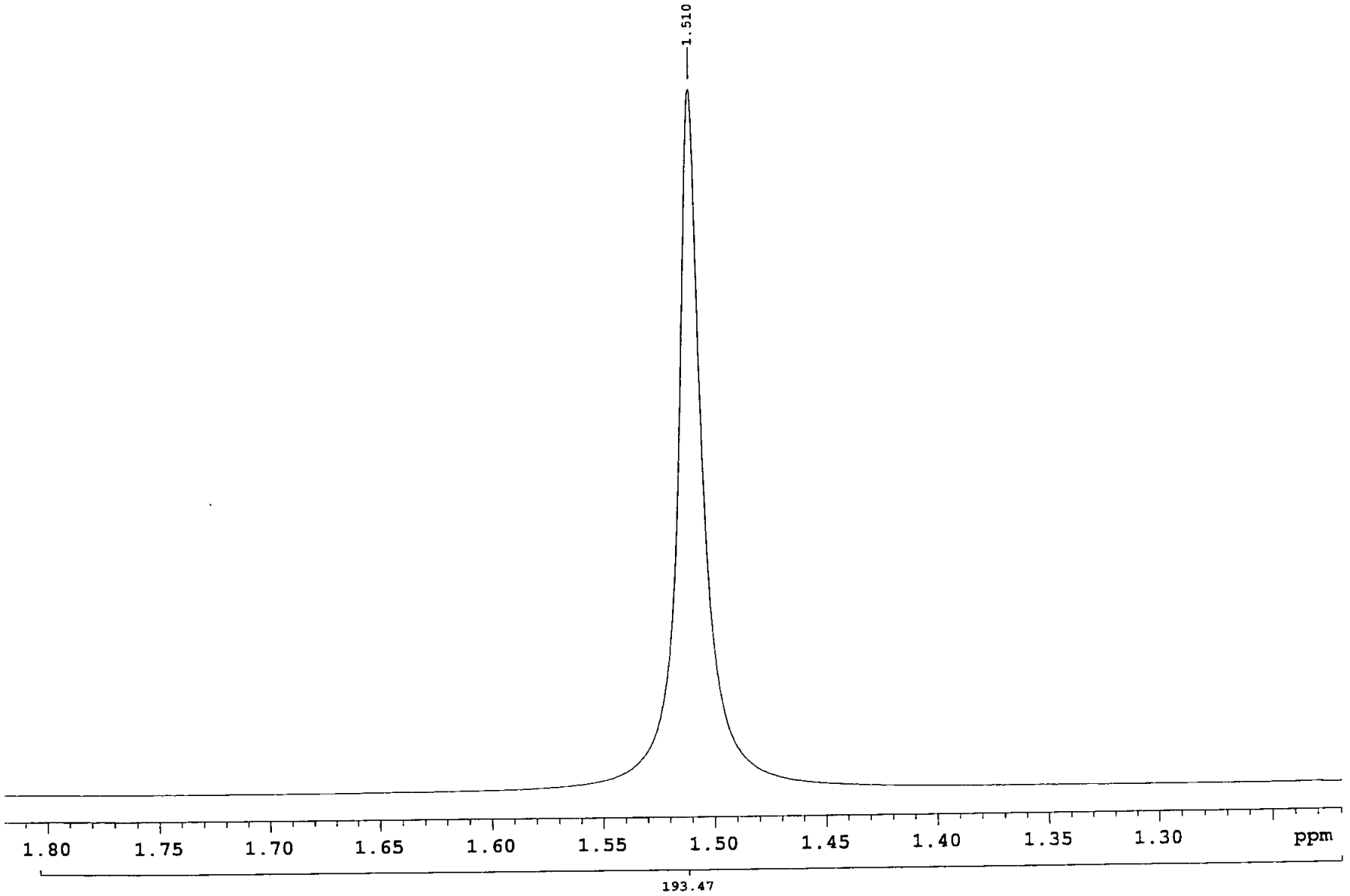
File: 399497



Plot file: 399497-4

229438, Compound 184, Lot sal-069, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

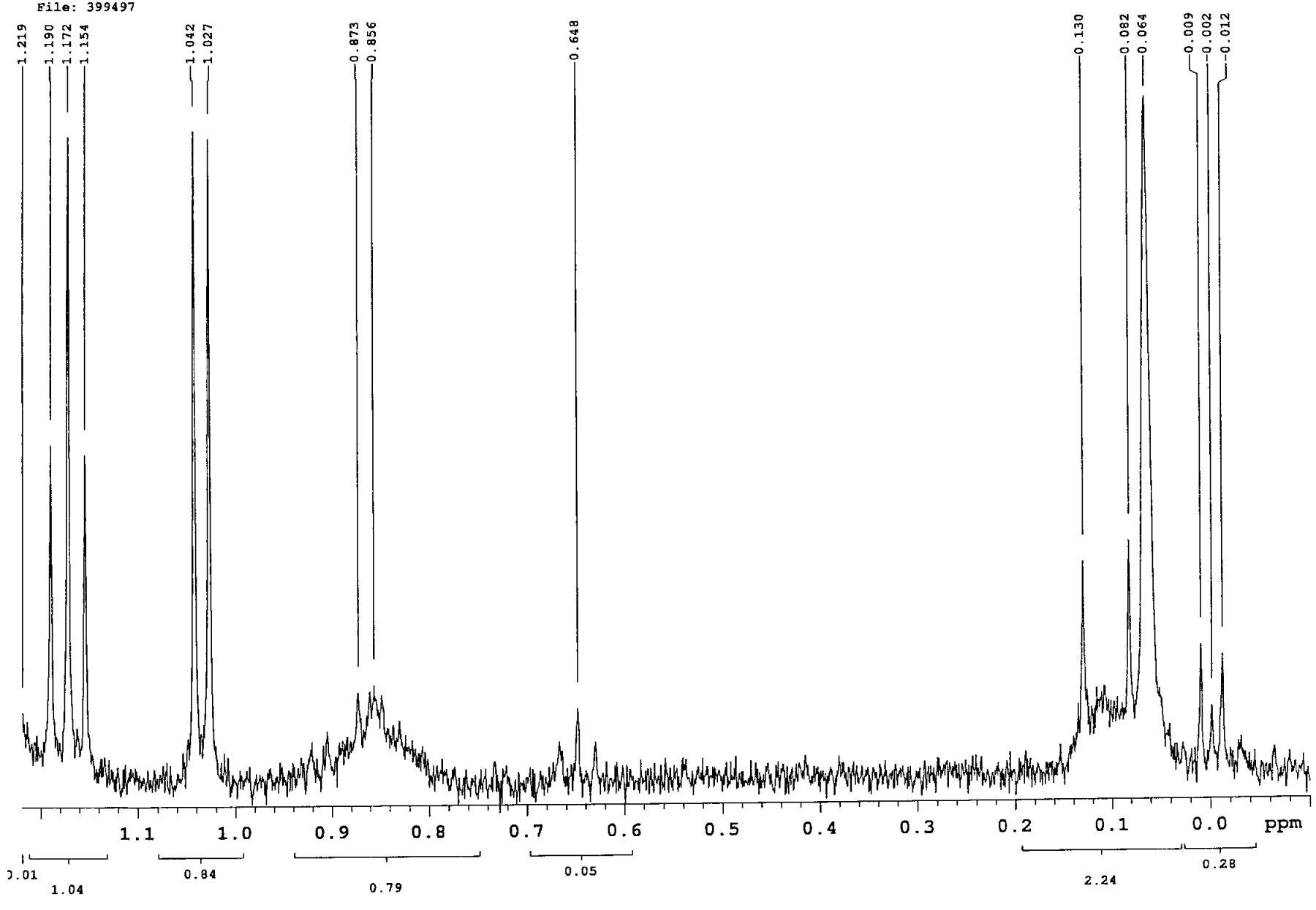
File: 399497



Plot file: 399497-5

229438, Compound 184, Lot sal-069, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

File: 399497



Plot file: 399497-6

Lot sal-069
LIMS 229438
file 397466-011

Lot sal-069
LIMS 229438
file 397466-012

Lot sal-069
LIMS 229438
file 398646-1

233285, Compound 184, Lot SAL-088,087, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 408900

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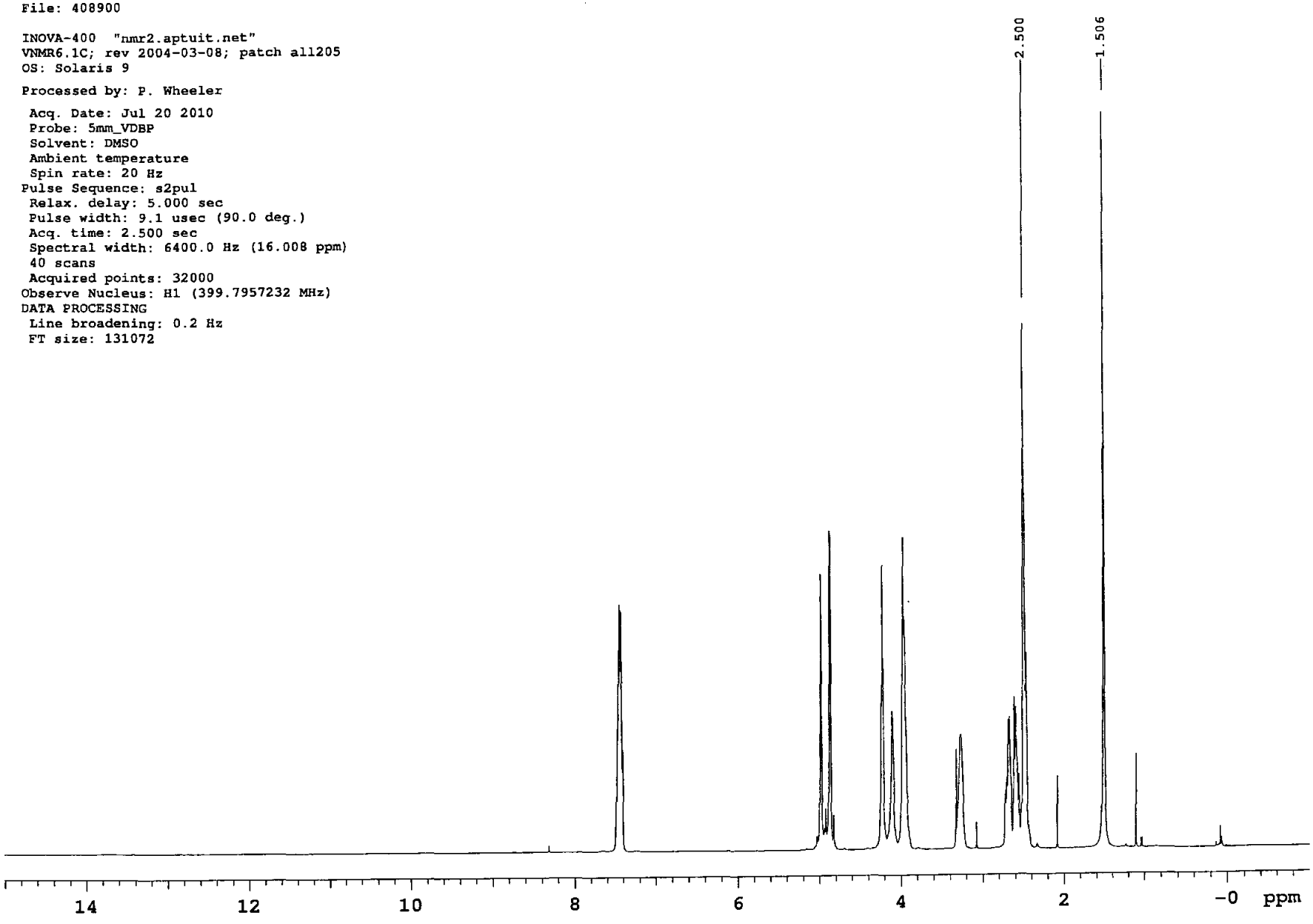
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

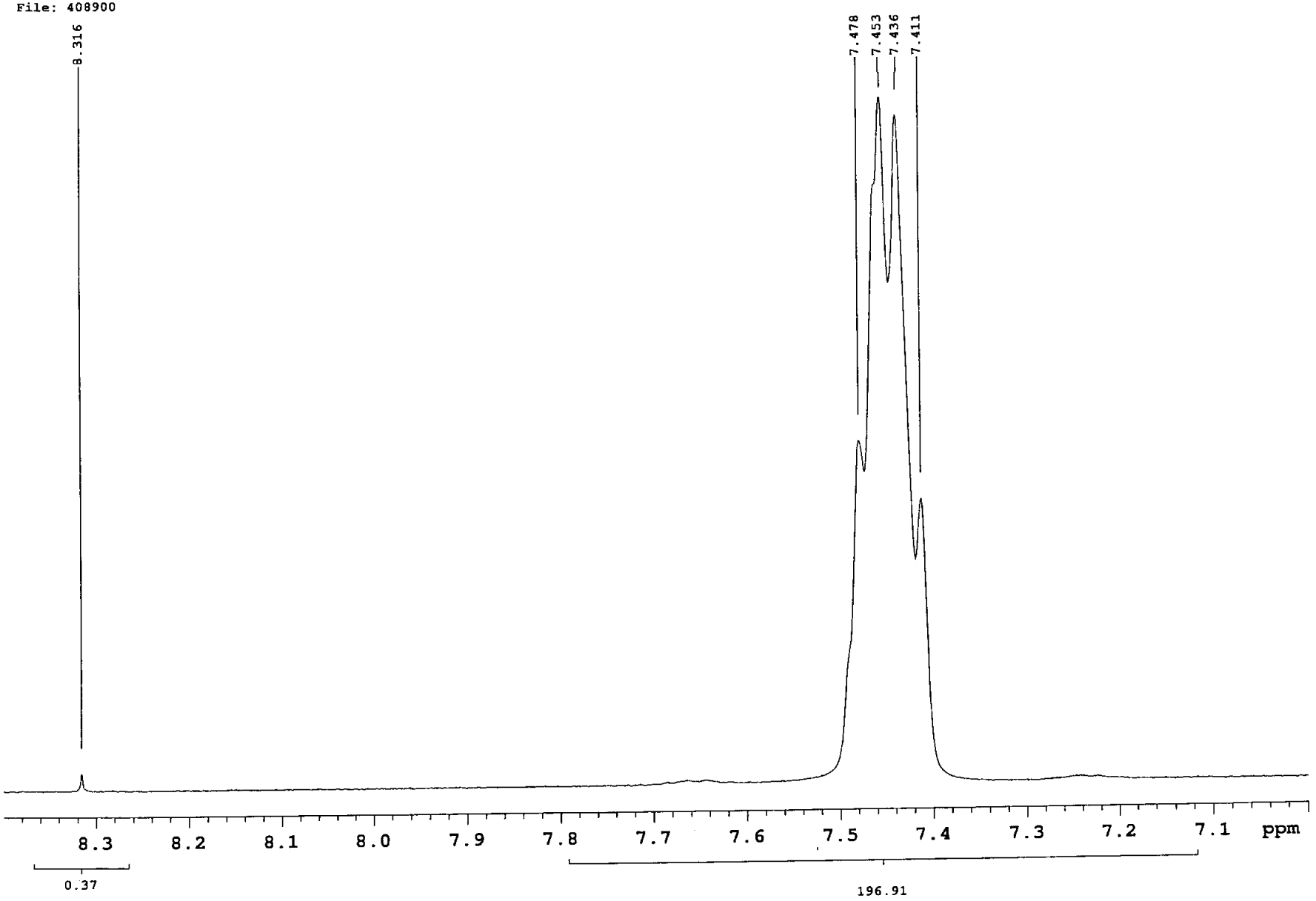
Acq. Date: Jul 20 2010
Probe: 5mm_VDBP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 9.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 408900-1

233285, Compound 184, Lot SAL-088,087, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

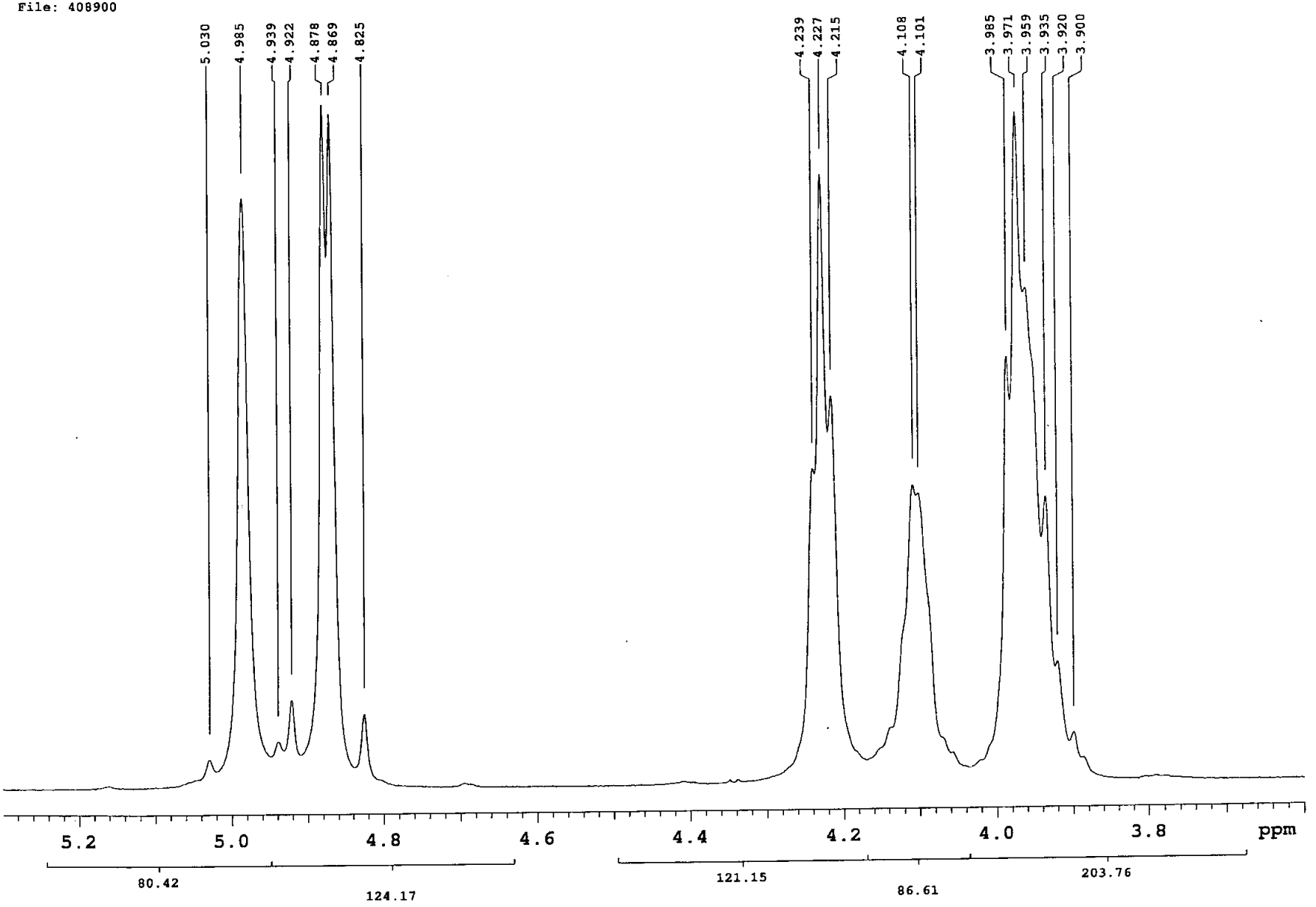
File: 408900



Plot file: 408900-2

233285, Compound 184, Lot SAL-088,087, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

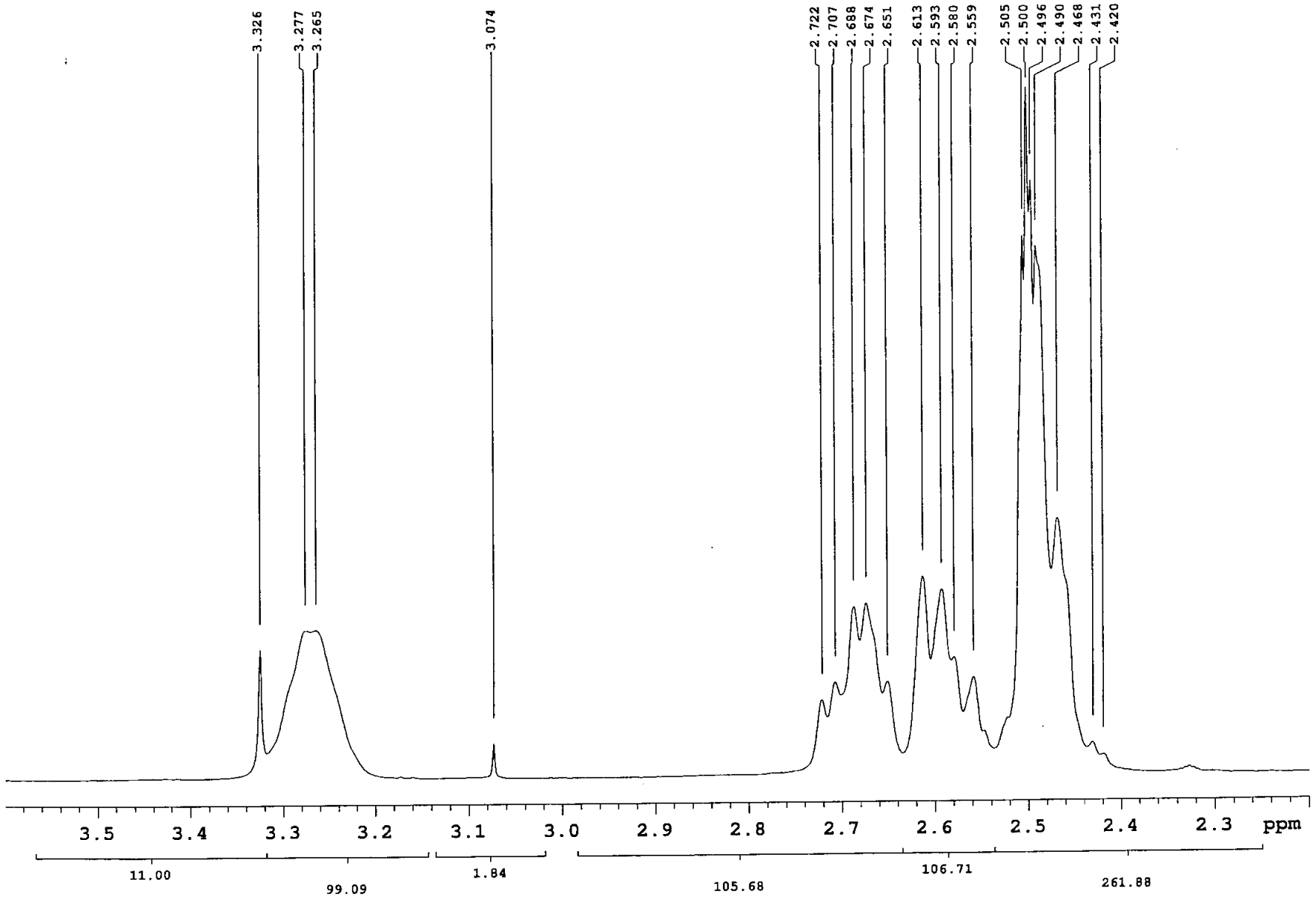
File: 408900



Plot file: 408900-3

233285, Compound 184, Lot SAL-088,087, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

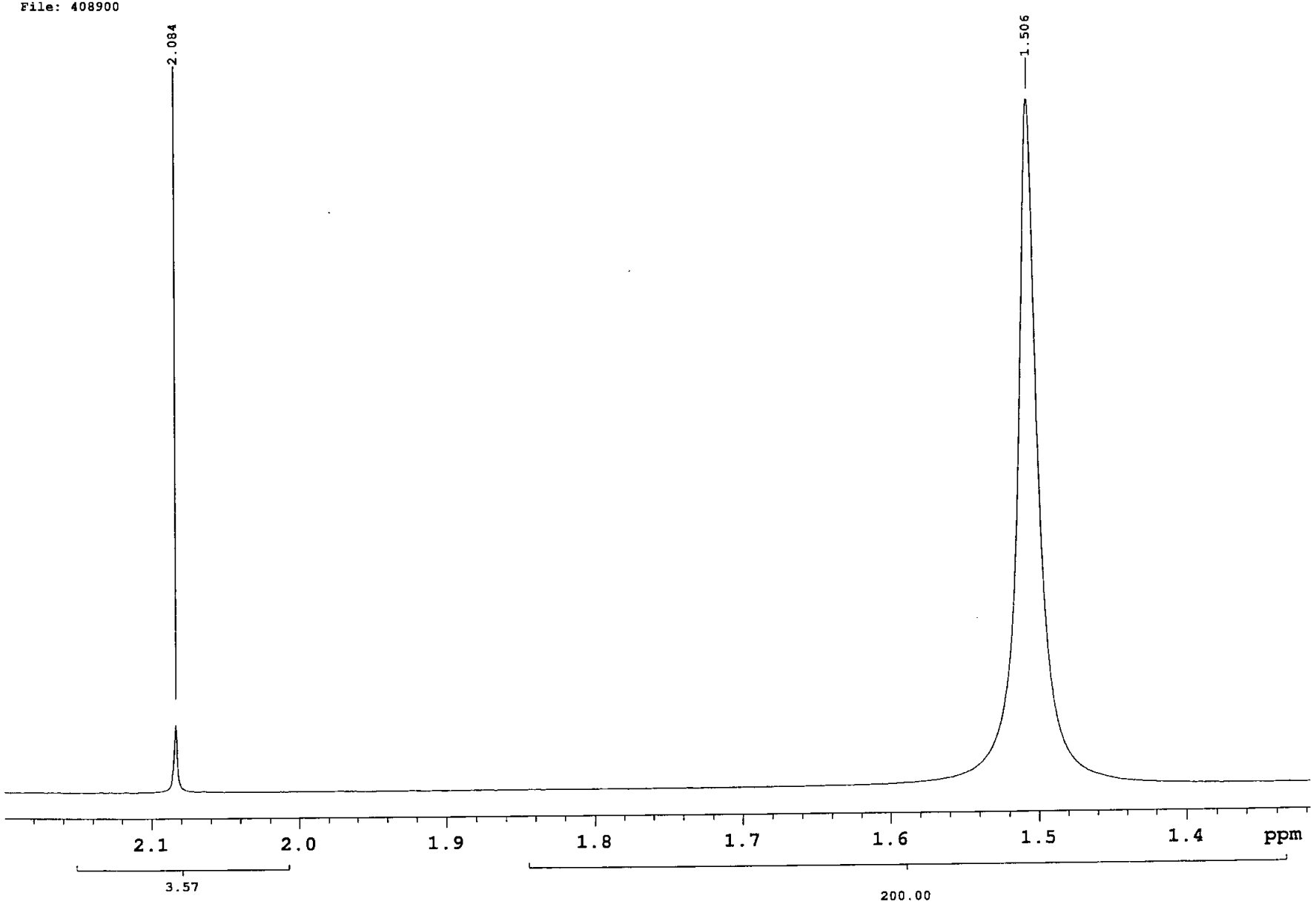
File: 408900



Plot file: 408900-4

233285, Compound 184, Lot SAL-088,087, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 408900



Plot file: 408900-5

233285, Compound 184, Lot SAL-088,087, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

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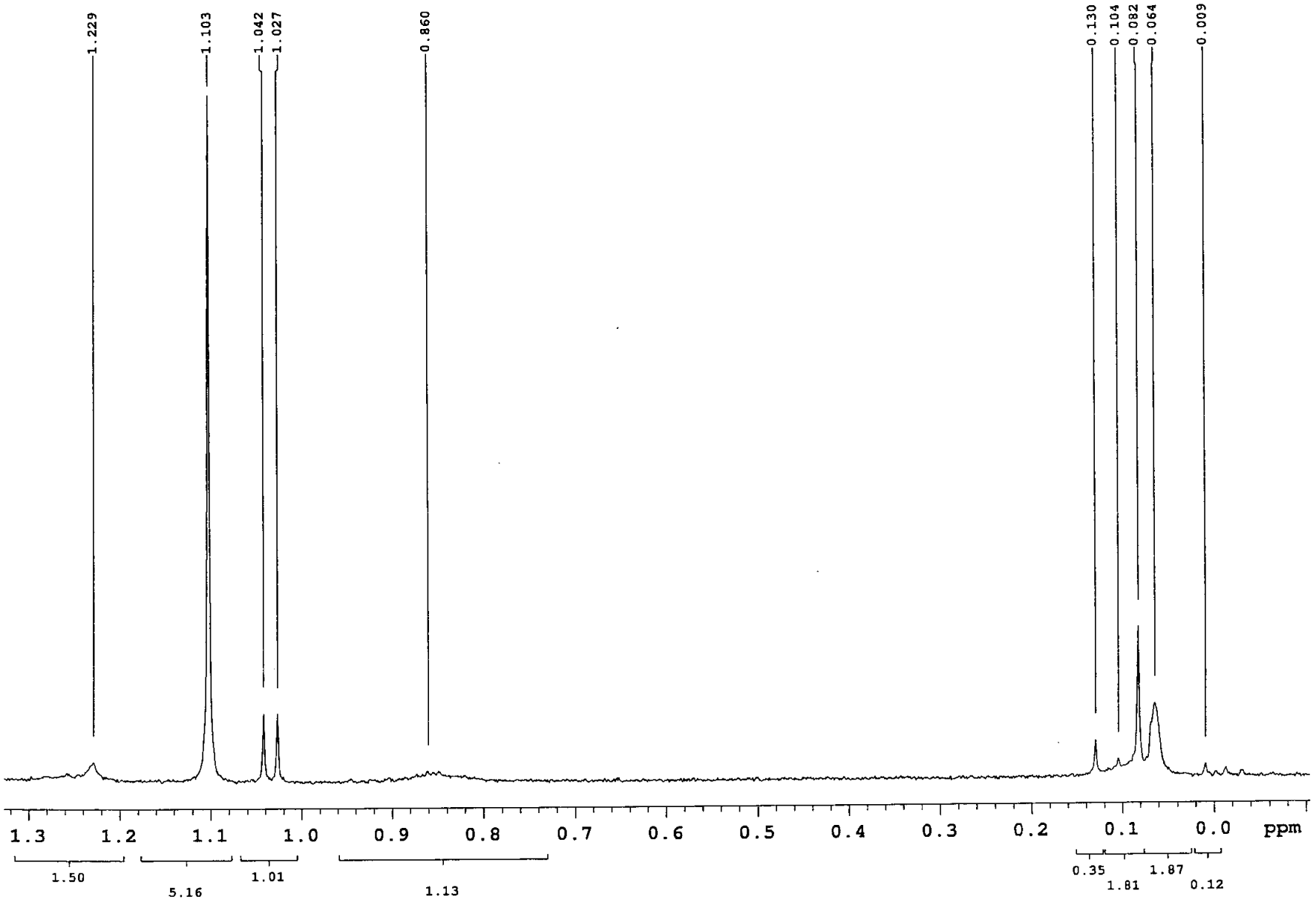


Plot file: 408900-6

Lot sal-0[illegible],087
LIMS 233285
file 409158-01

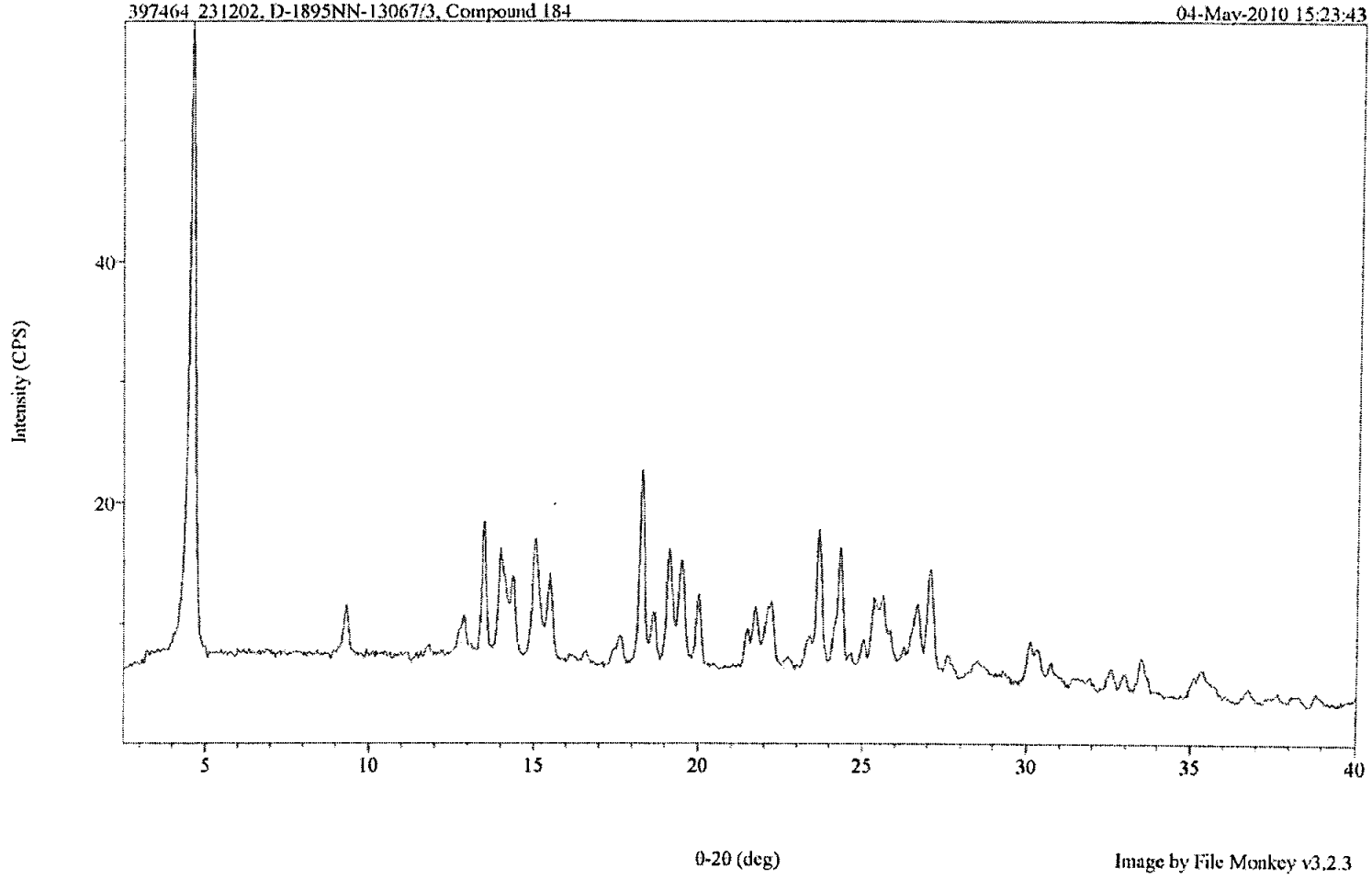
Lot sal-088,087
LIMS 233285
file 409158-02

Lot sal-088,087
LIMS 233285
file 409158-03

Lot sal-088,087
LIMS 233285
file 409158-04

EXHIBIT E

INEL XRG-3000
X-ray Tube: 1.54187000 Å Voltage: 40 (kV) Amperage: 30 (mA) Acquisition Time: 300 sec
Spinning capillary. Step size: approximately 0.03 °2θ.



231202, Compound 184, Lot D-1895NN-13067/3, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

File: 399864

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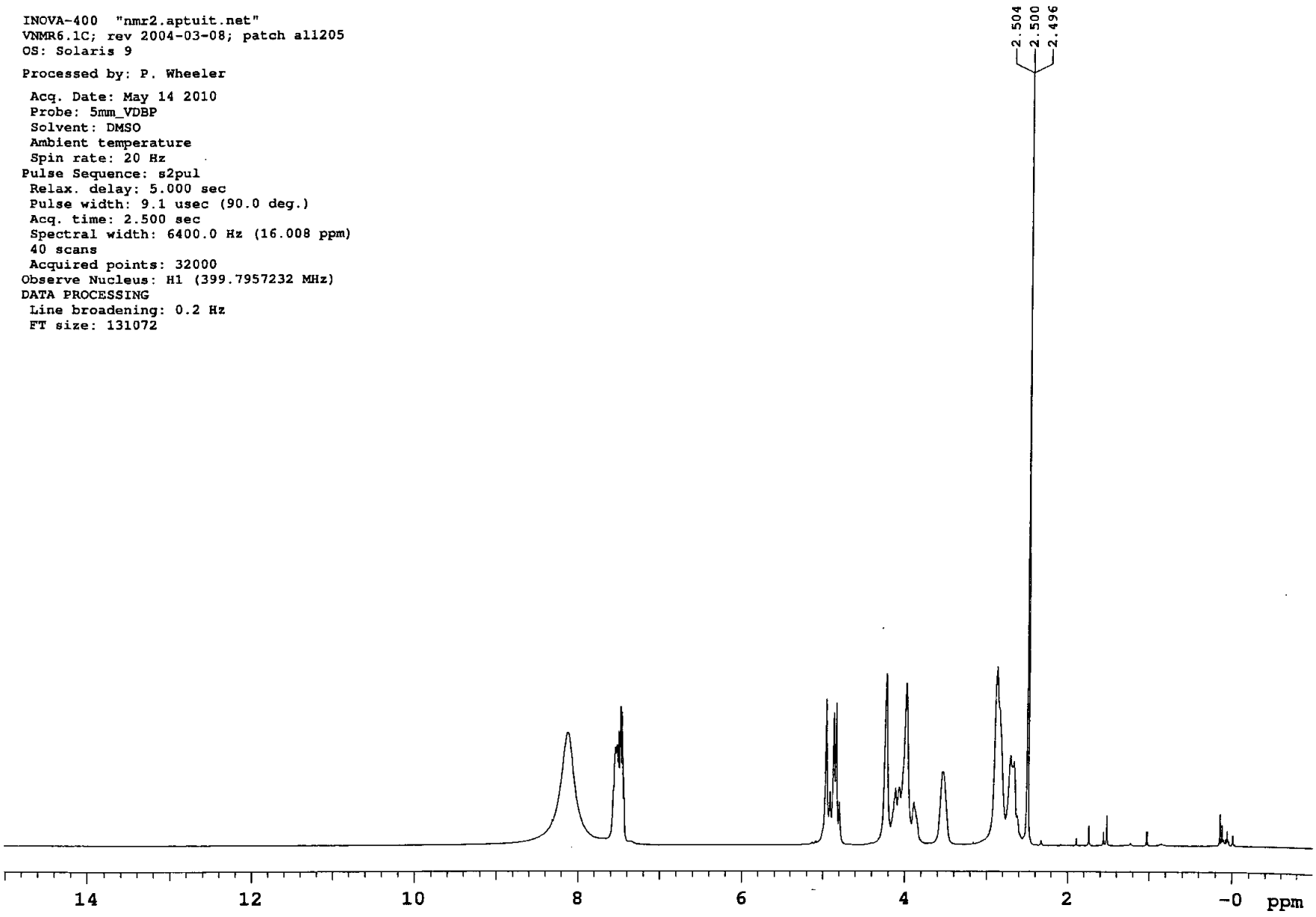
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

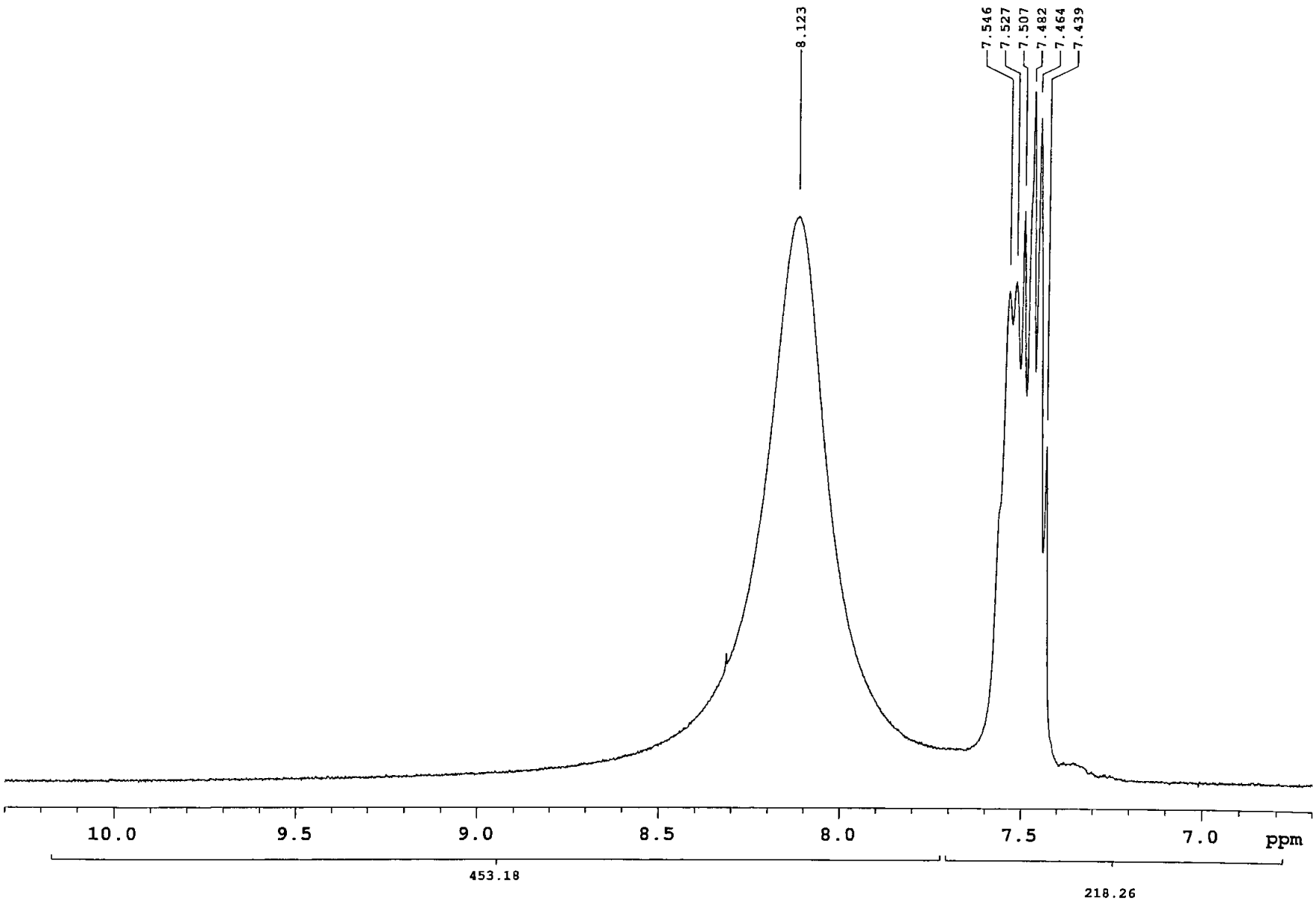
Acq. Date: May 14 2010
Probe: 5mm_VDBP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 9.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 399864-1

231202, Compound 184, Lot D-1895NN-13067/3, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

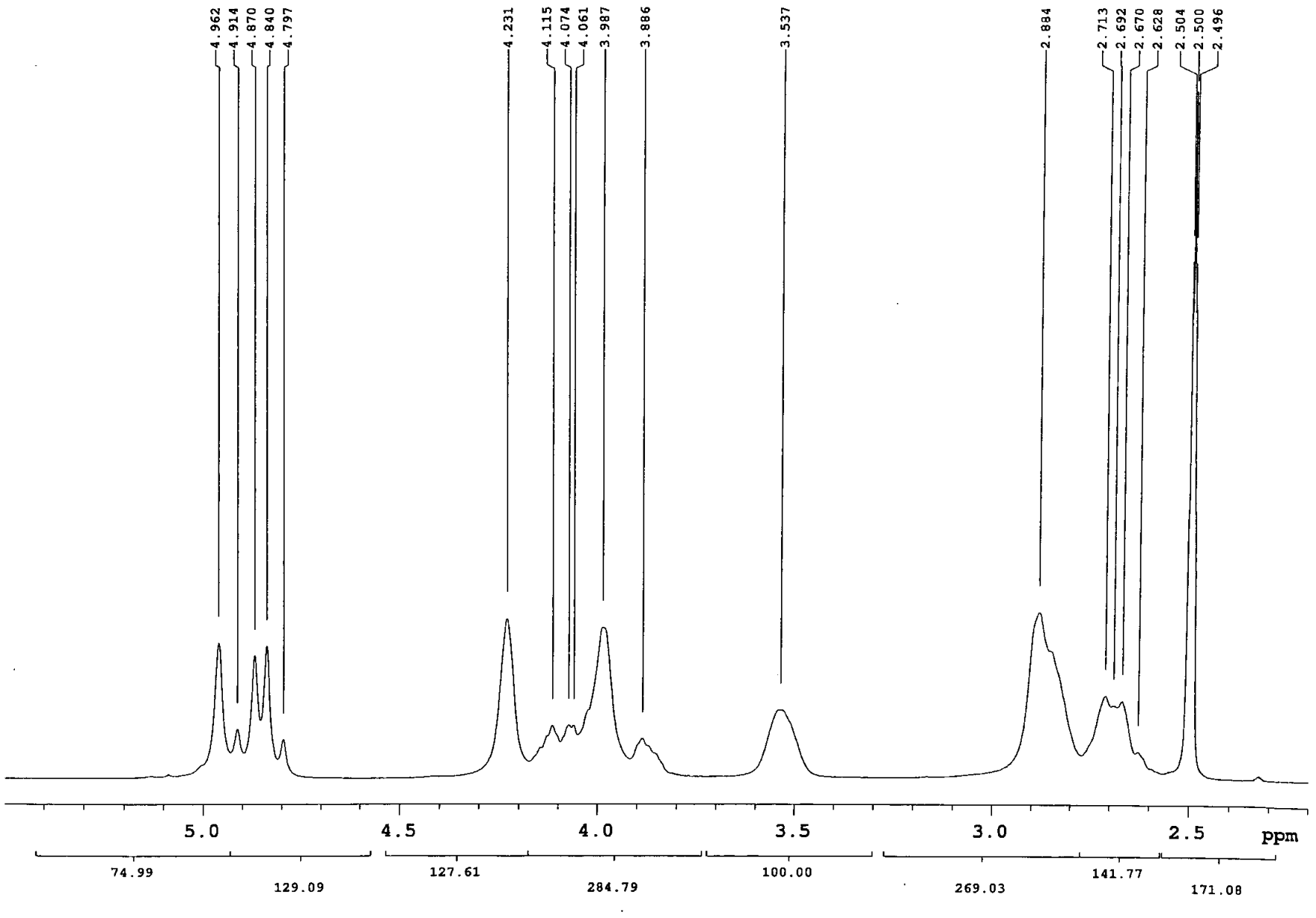
File: 399864



Plot file: 399864-2

231202, Compound 184, Lot D-1895NN-13067/3, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

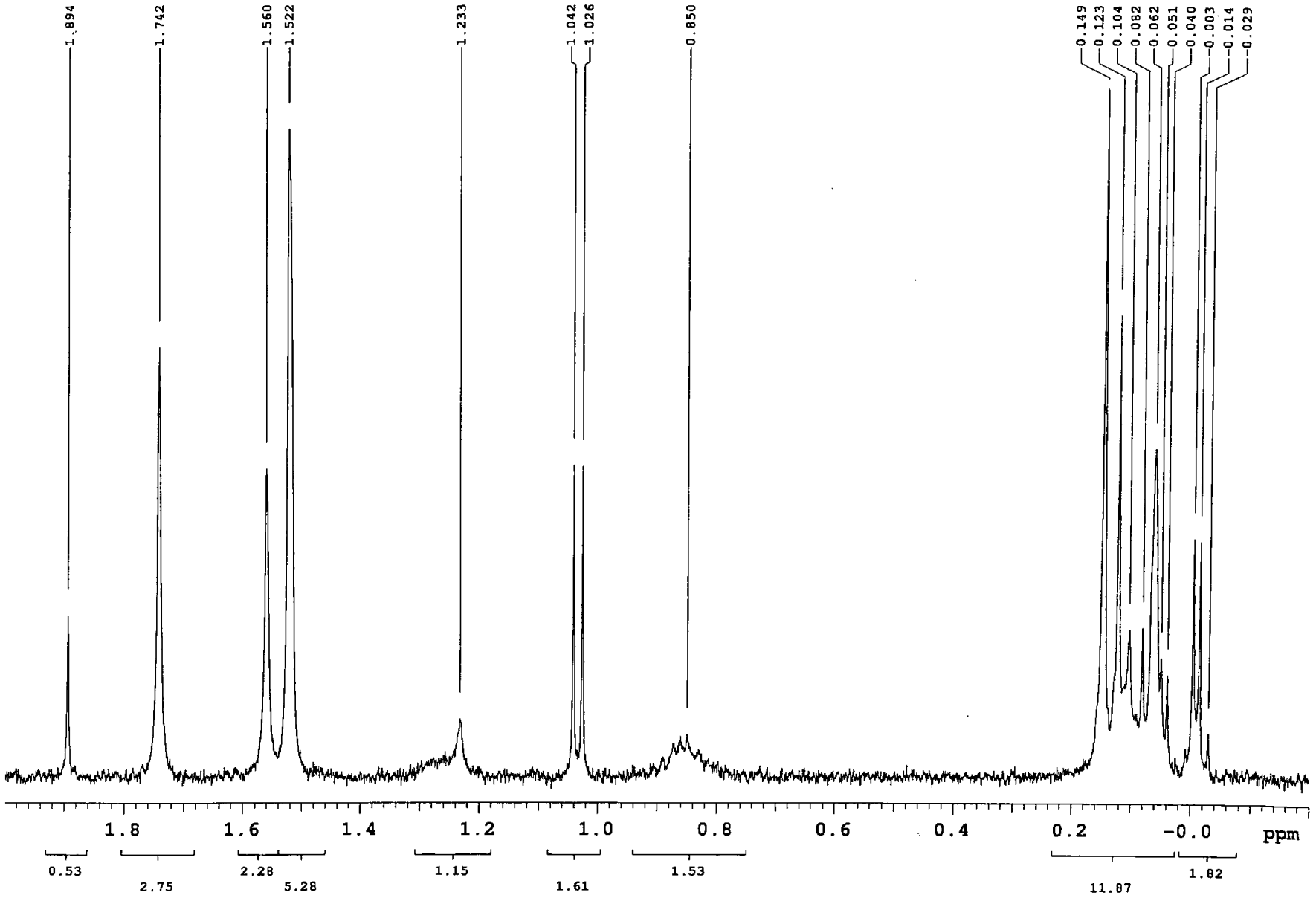
File: 399864



Plot file: 399864-3

231202, Compound 184, Lot D-1895NN-13067/3, in DMSO-d6 w/ TMS, 1H NMR, referenced to solvent at 2.5 ppm

File: 399864



Plot file: 399864-4

EXHIBIT F

US 20100041885A1

(19) United States

(12) Patent Application Publication

(10) Pub. No.: US 2010/0041885 A1

(43) Pub. Date: Feb. 18, 2010

Perlman et al.

(54) CRYSTALLINE FORMS OF SITAGLIPTIN PHOSPHATE

(76) Inventors: **Nurit Perlman**, Kfar Saba (IL); **Revital Ramaty**, Ramat-Hasharon (IL); **Mili Abramov**, Givataim (IL); **Nina Finkelstein**, Herzliya (IL); **Eli Lancry**, Modiin (IL); **Shay Asis**, Rishon Lezion (IL); **Ariel Mittelman**, Elad (IL)

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KENYON & KENYON LLP
ONE BROADWAY
NEW YORK, NY 10004 (US)

(21) Appl. No.: **12/410,738**

(22) Filed: **Mar. 25, 2009**

Related U.S. Application Data

(60) Provisional application No. 61/154,491, filed on Feb. 23, 2009, provisional application No. 61/201,304, filed on Dec. 8, 2008, provisional application No. 61/190,868, filed on Sep. 2, 2008, provisional application No. 61/092,555, filed on Aug. 28, 2008, provisional application No. 61/090,736, filed on Aug. 21, 2008, provisional application No. 61/189,128, filed on Aug. 14, 2008, provisional application No. 61/070,866, filed on Mar. 25, 2008, provisional application No. 61/201,860, filed on Dec. 15, 2008, provisional application No. 61/191,933, filed on Sep. 11, 2008, provisional application No. 61/091,759, filed on Aug. 26, 2008, provisional application No. 61/137,489, filed on Jul. 30, 2008, provisional application No. 61/134,598, filed on Jul. 10, 2008.

Publication Classification

(51) **Int. Cl.**
C07D 471/08 (2006.01)

(52) **U.S. Cl.** **544/350**

(57) **ABSTRACT**

A Sitagliptin phosphate characterized by data selected from the group consisting of: a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 4.7, 13.5, and 15.5±0.2 degrees two theta and at least another two peaks selected from the following list: 14.0, 14.4, 18.3, 19.2, 19.5 and 23.7±0.2 degrees two theta; and a powder XRD pattern with peaks at about 13.5, 19.2, and 19.5±0.2 degrees two theta and at least another two peaks selected from the following list: 4.7, 14.0, 15.1, 15.5, 18.3, and 18.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 13.5, 15.5, 19.2, 23.7, and 24.4±0.2 degrees two theta; and a powder XRD pattern with peaks at about 4.65, 13.46, 17.63, 18.30, and 23.66±0.10 degrees two theta, processes for preparing said Sitagliptin crystalline form, and pharmaceutical compositions thereof, are provided.

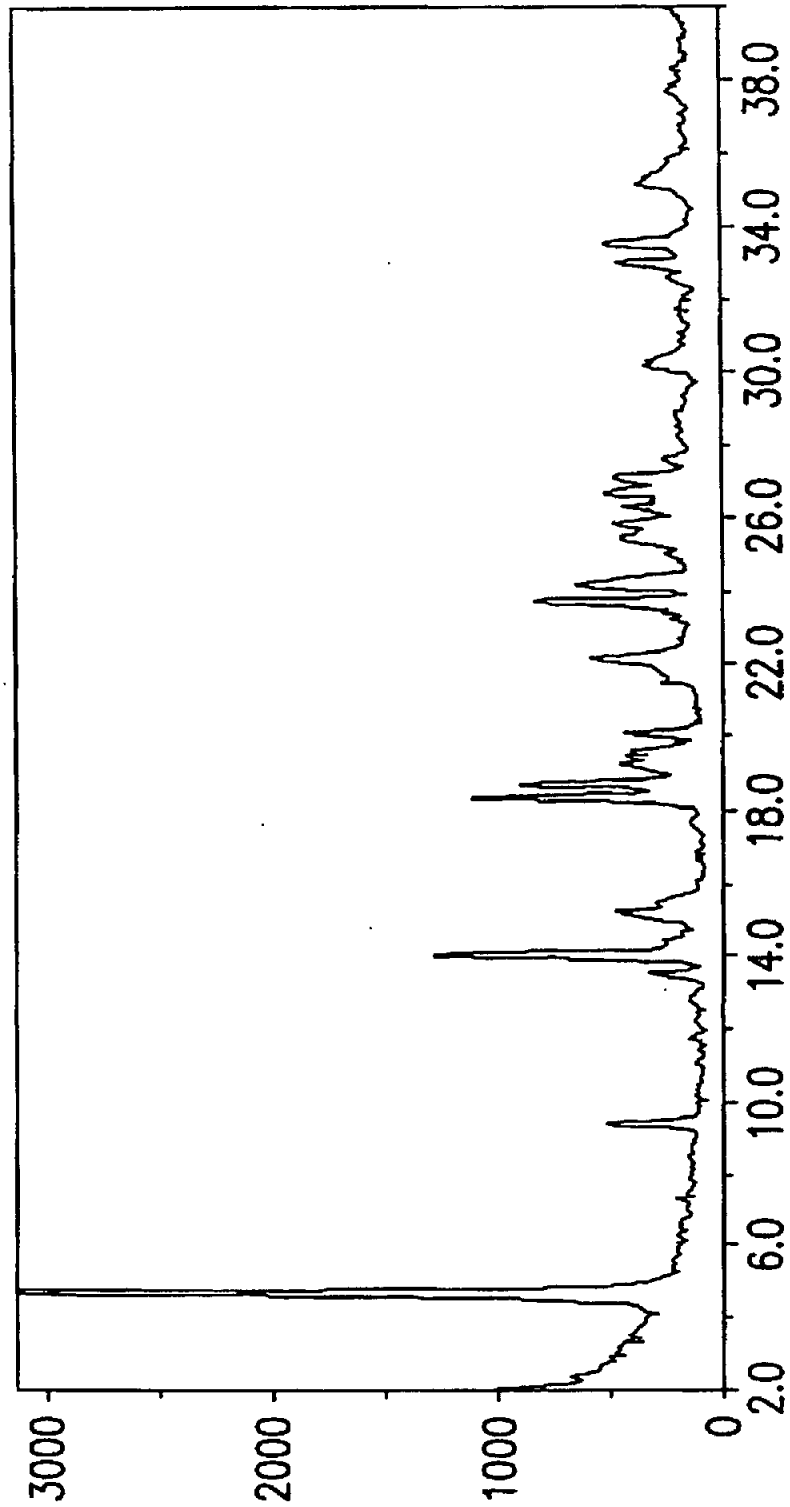


FIG. 1

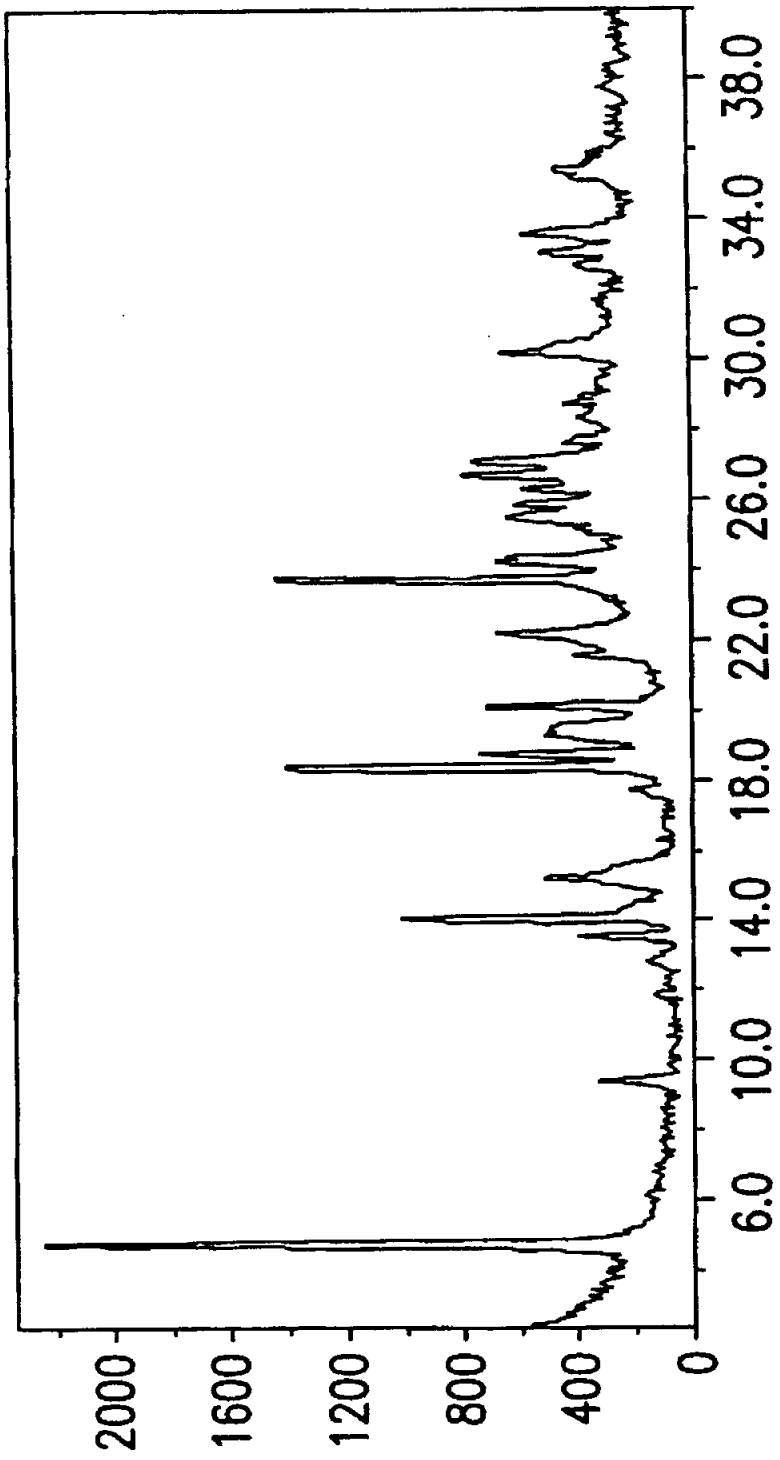


FIG.2

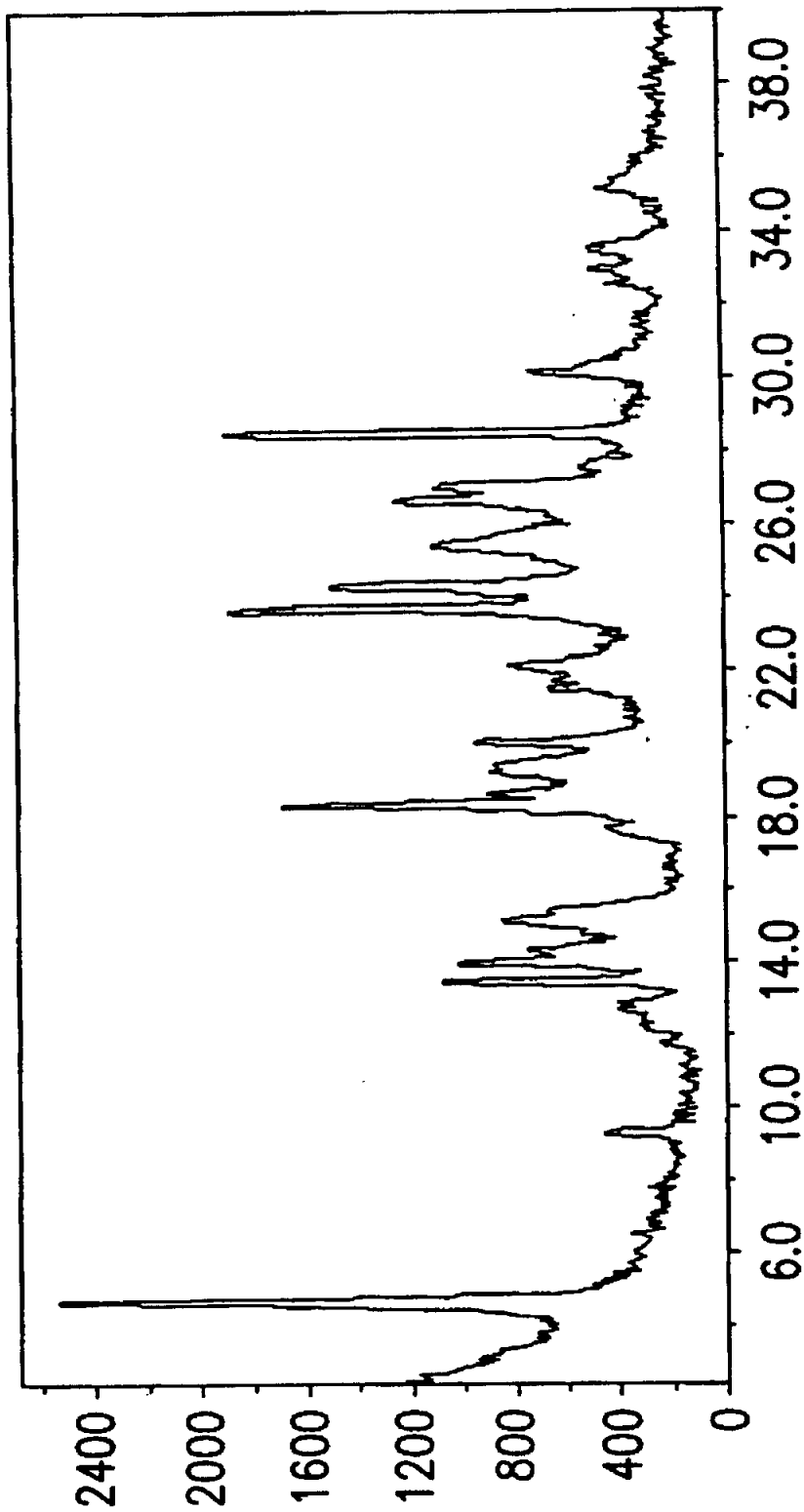


FIG. 3

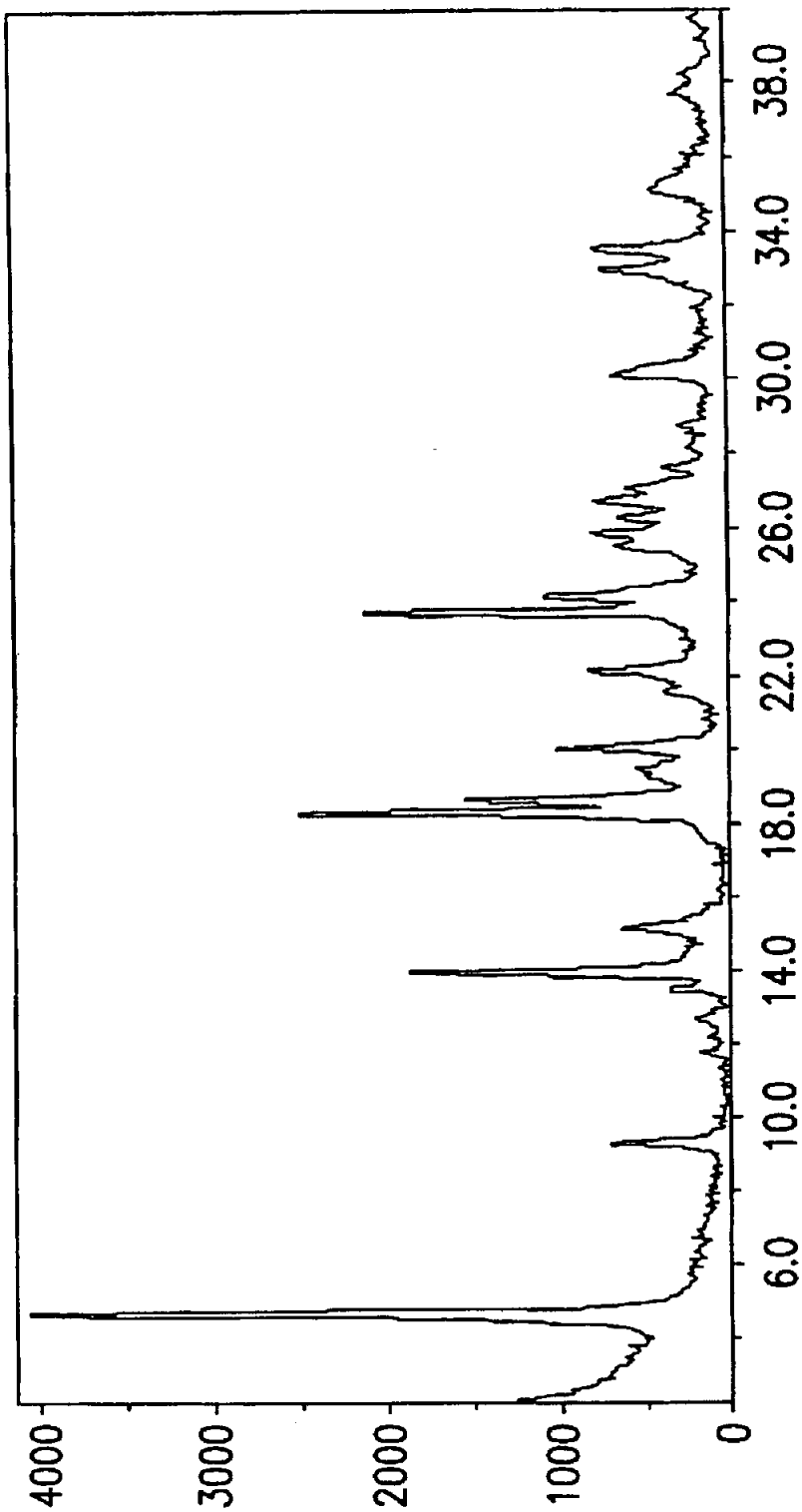


FIG. 4

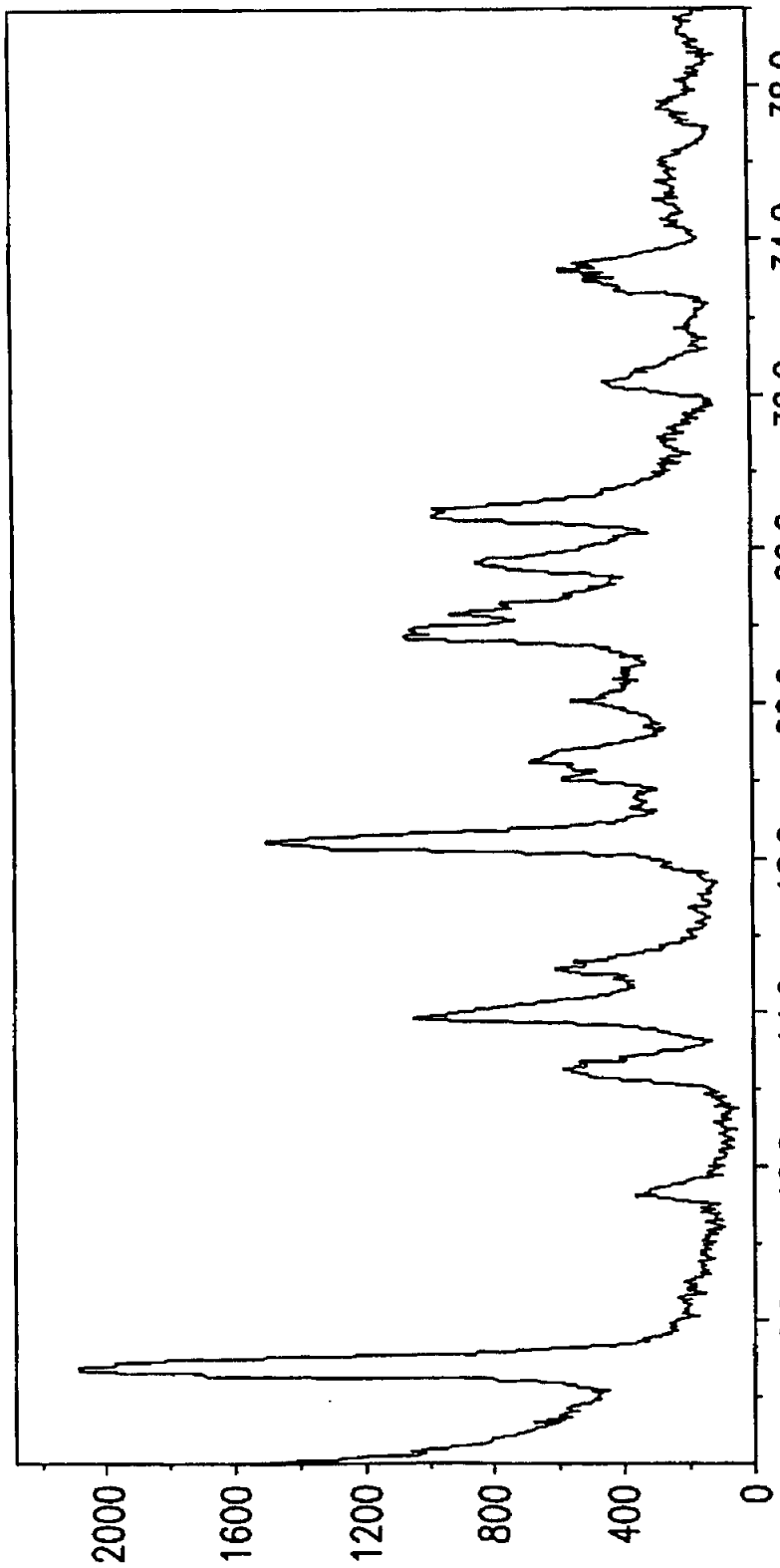


FIG.5a

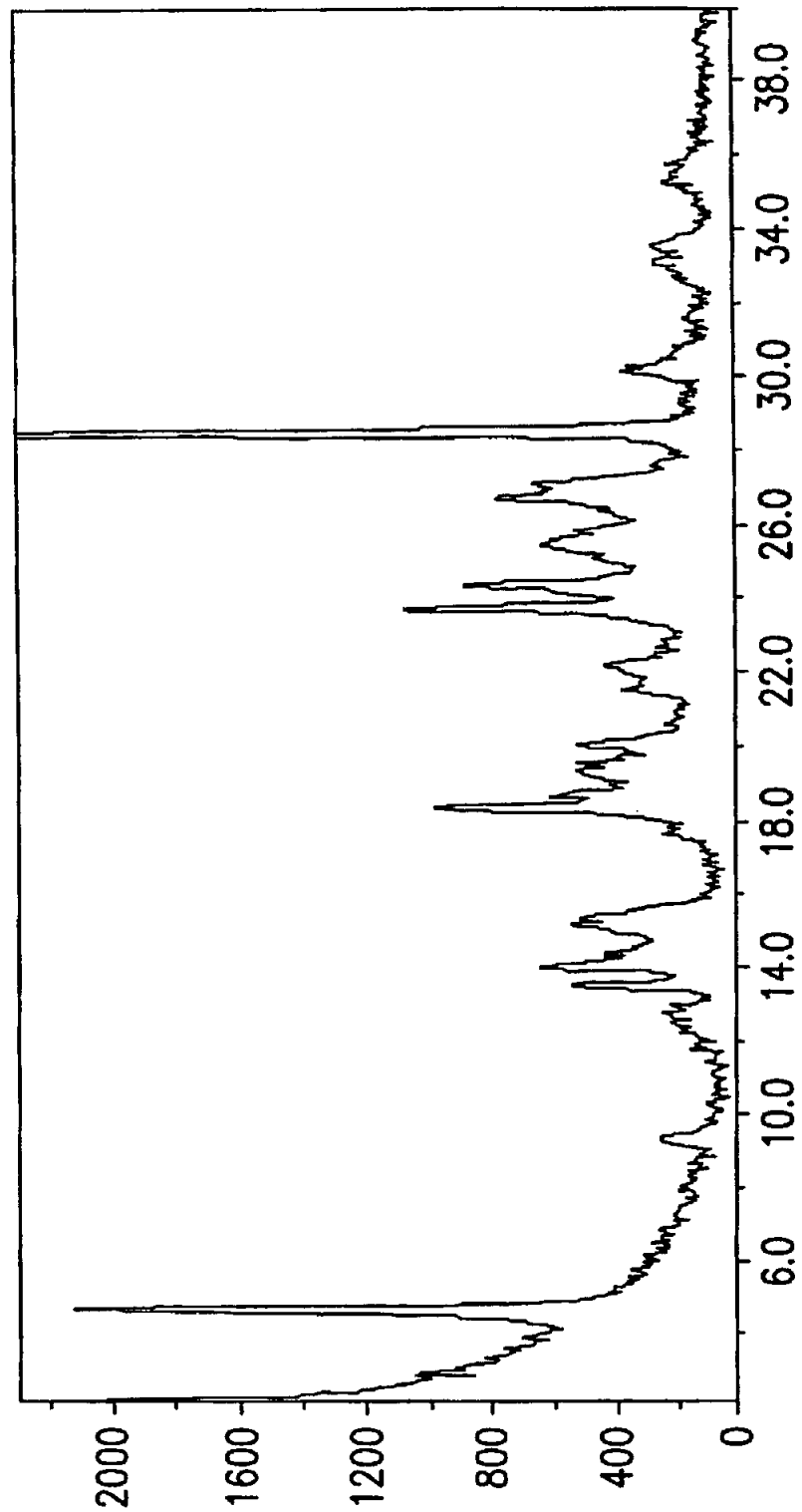


FIG.5b

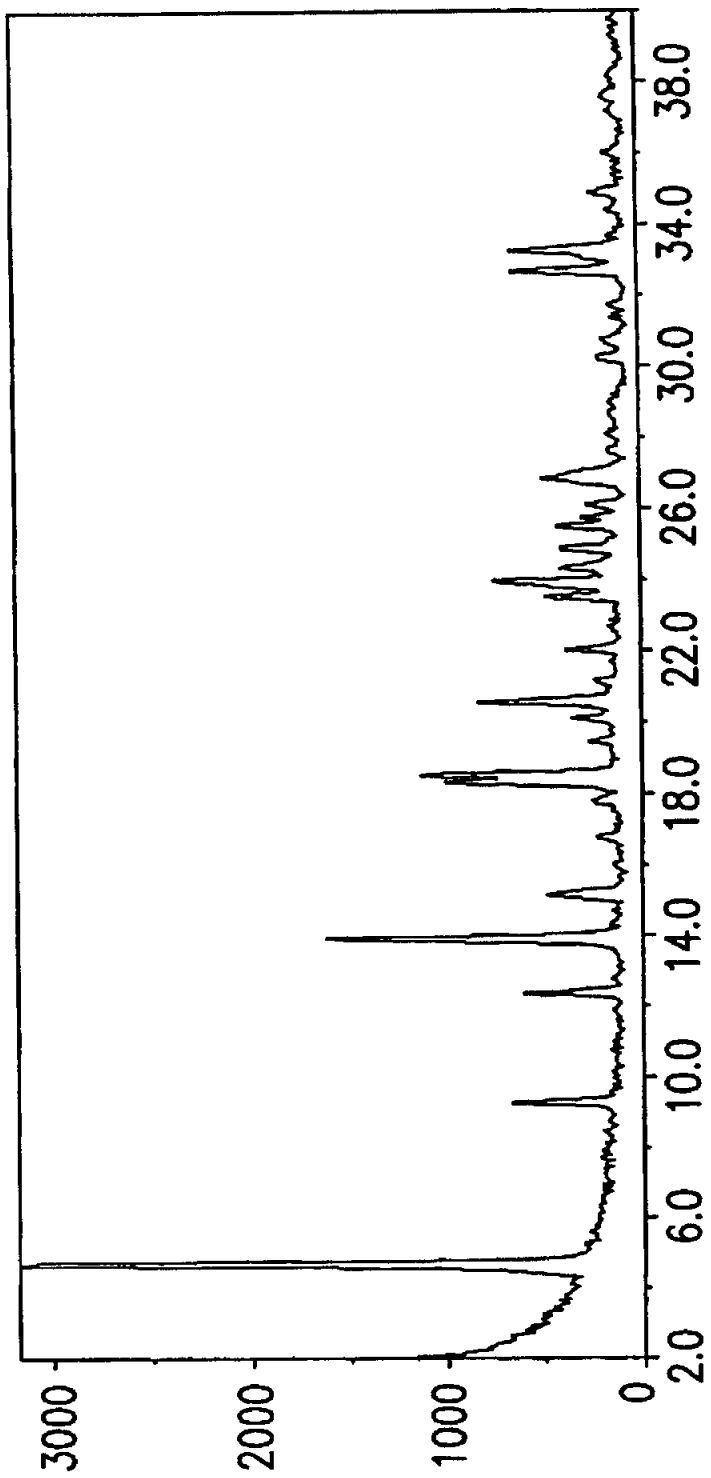


FIG.6

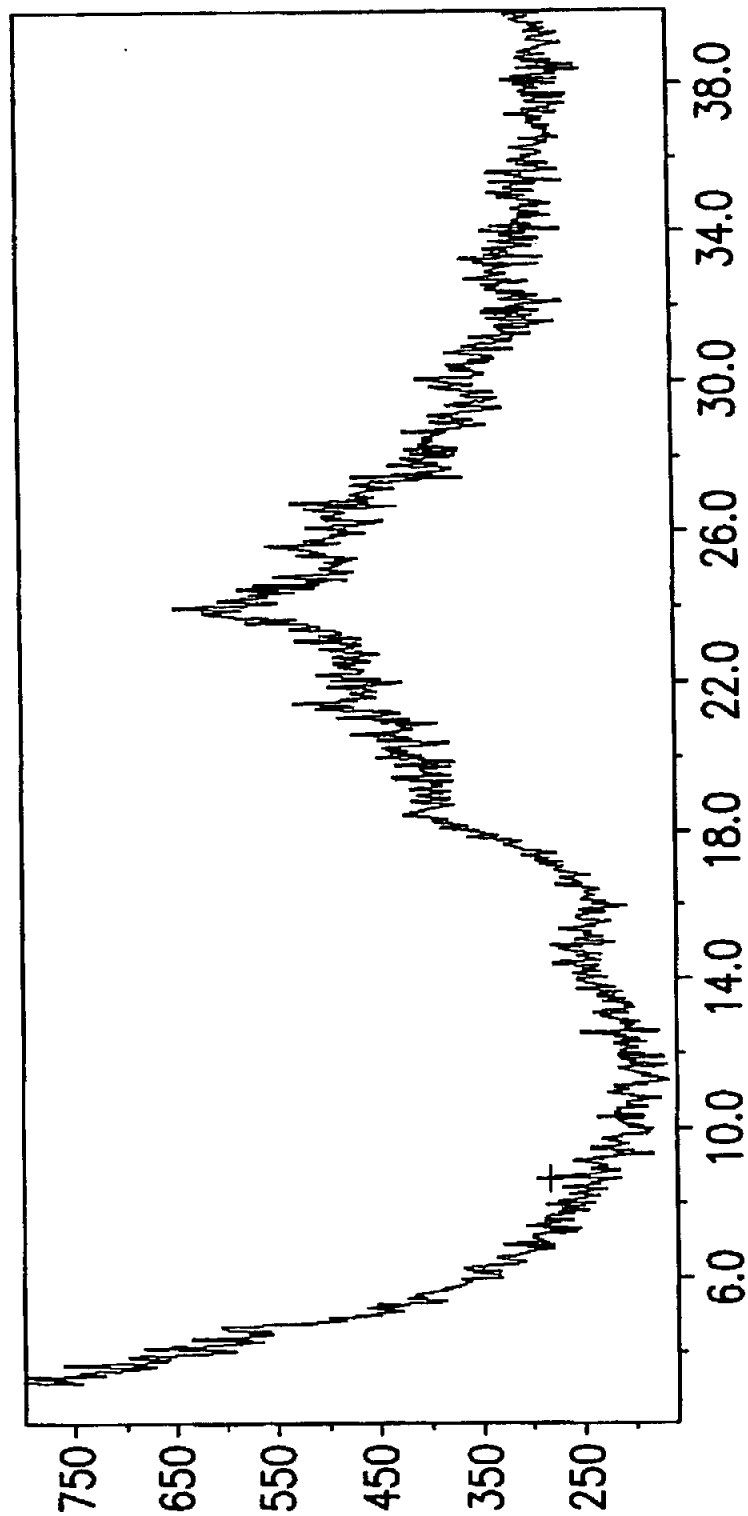


FIG. 7

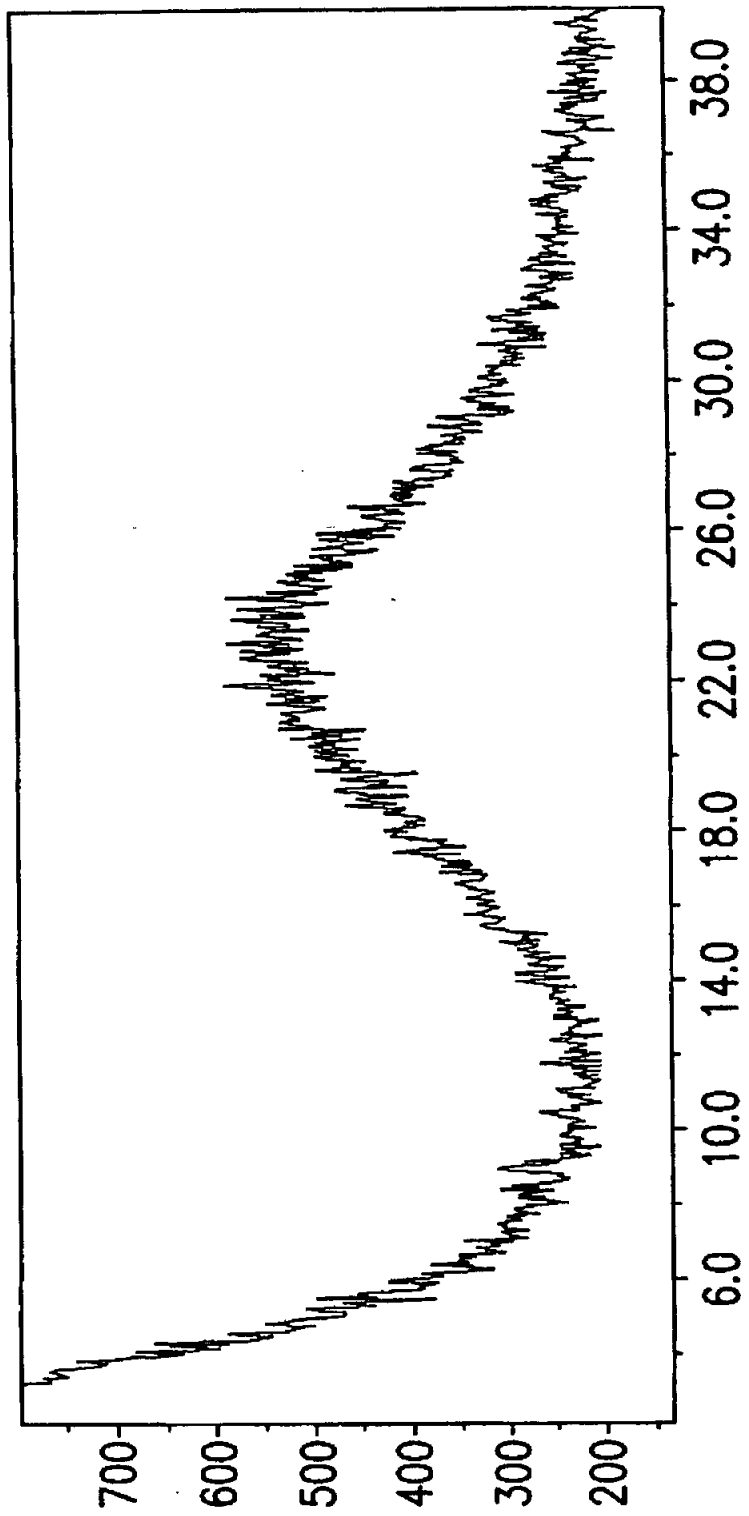


FIG.8

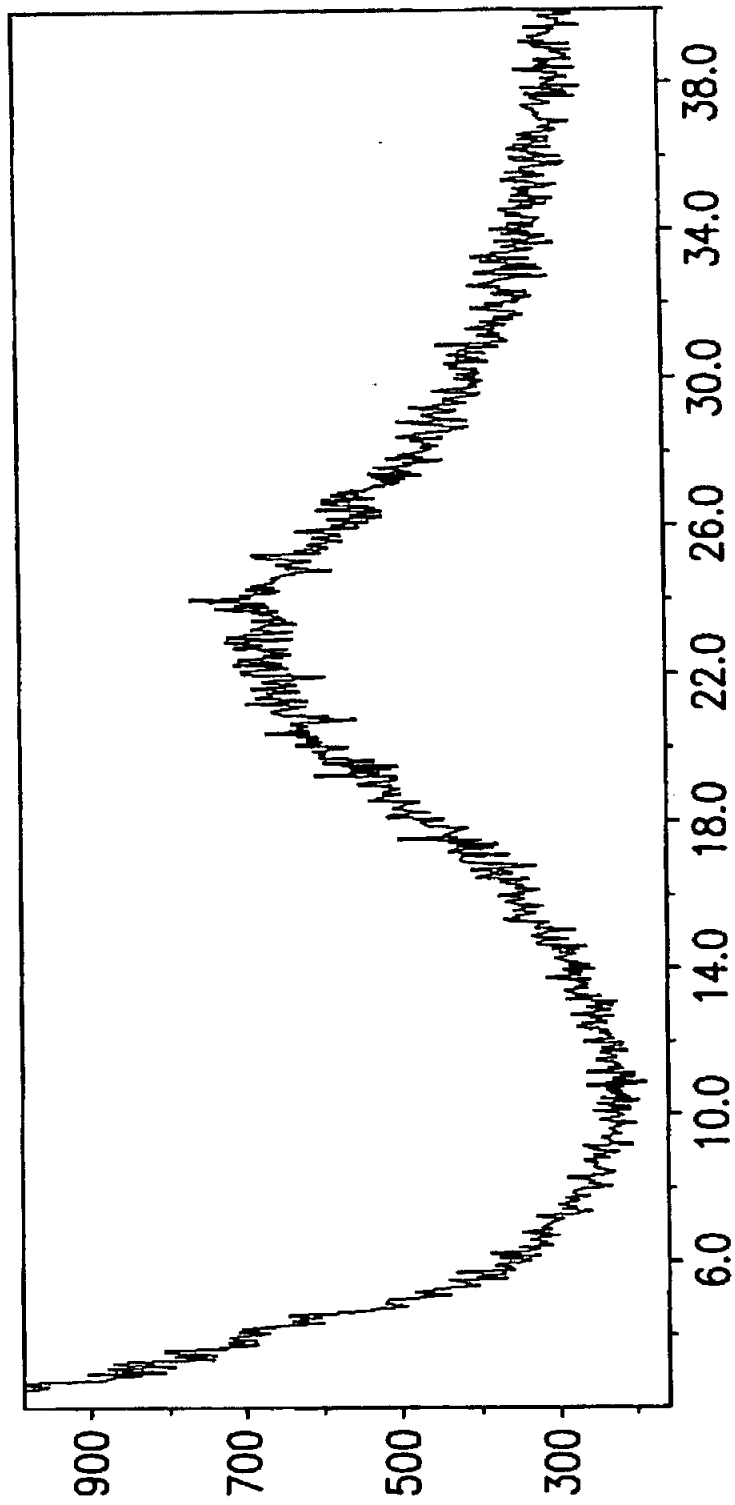


FIG.9

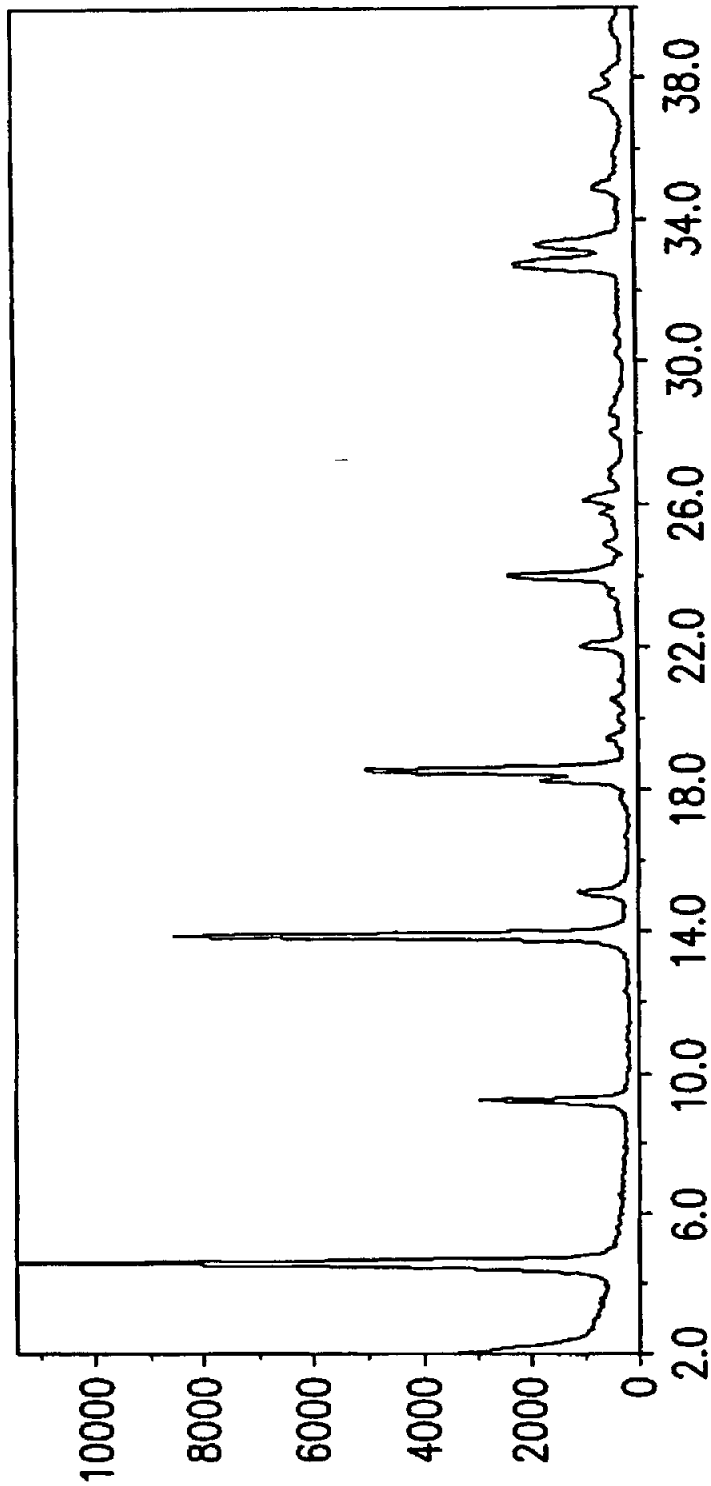

10000
8000
6000
4000
2000
0
2.0
6.0
10.0
14.0
18.0
22.0
26.0
30.0
34.0
38.0

FIG.10

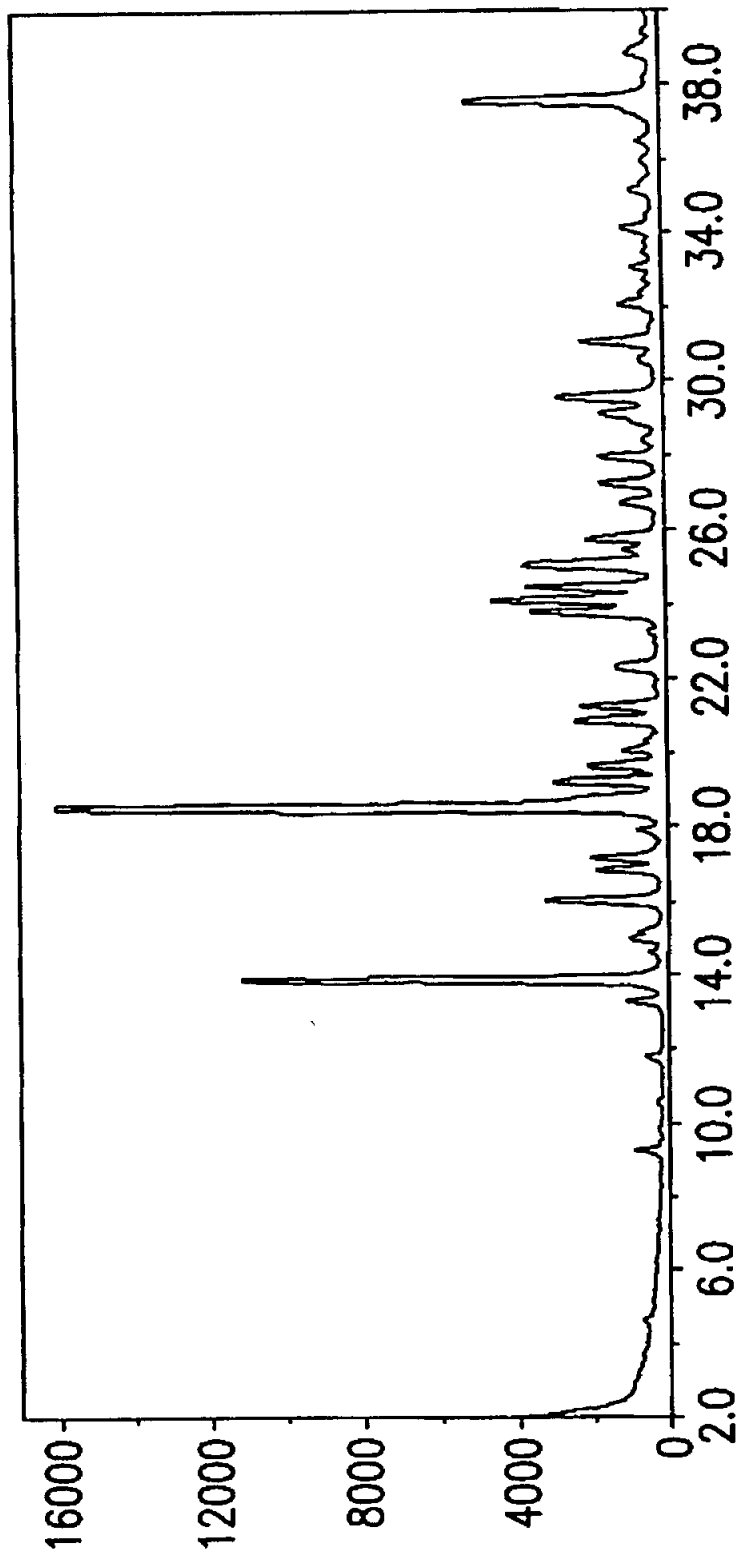


FIG. 11

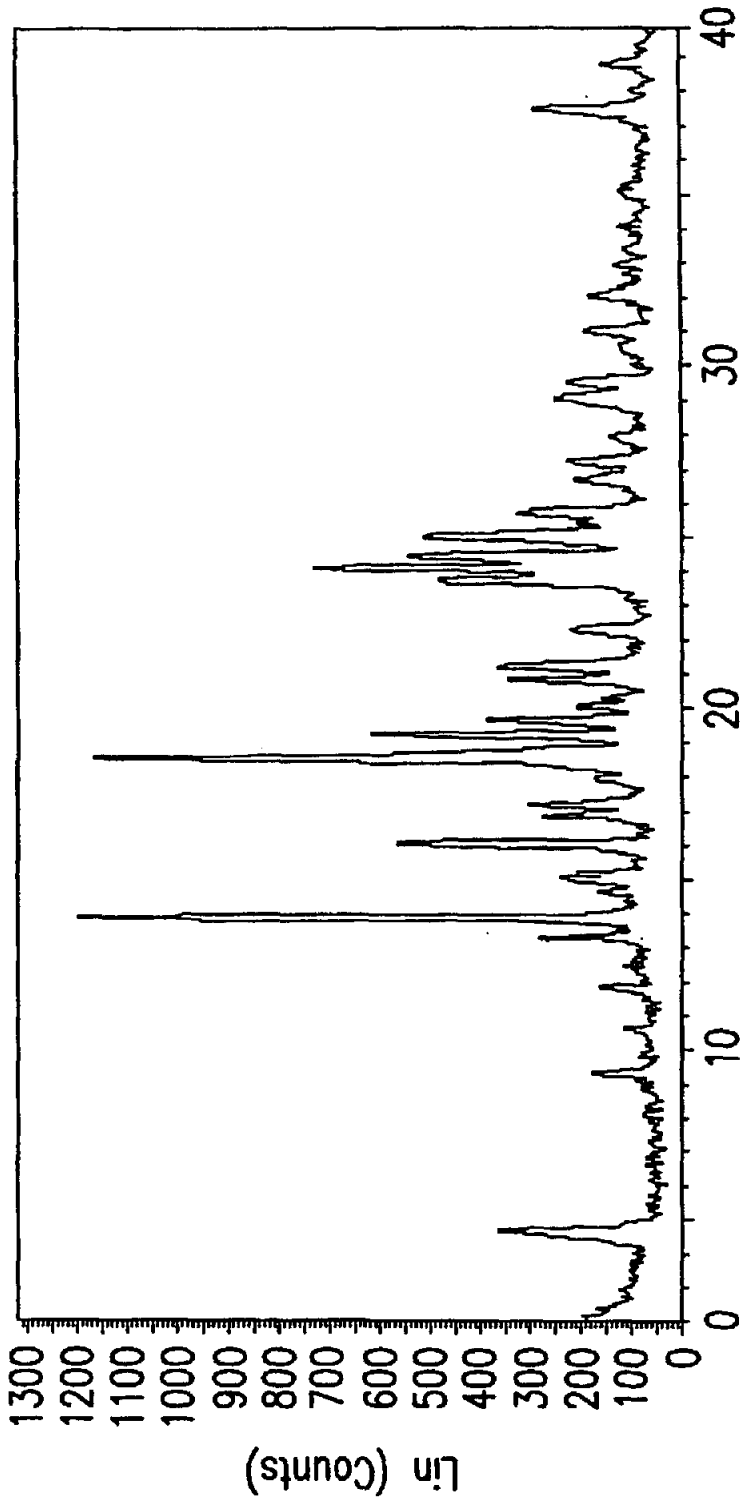

Lin (Counts)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40

FIG. 12

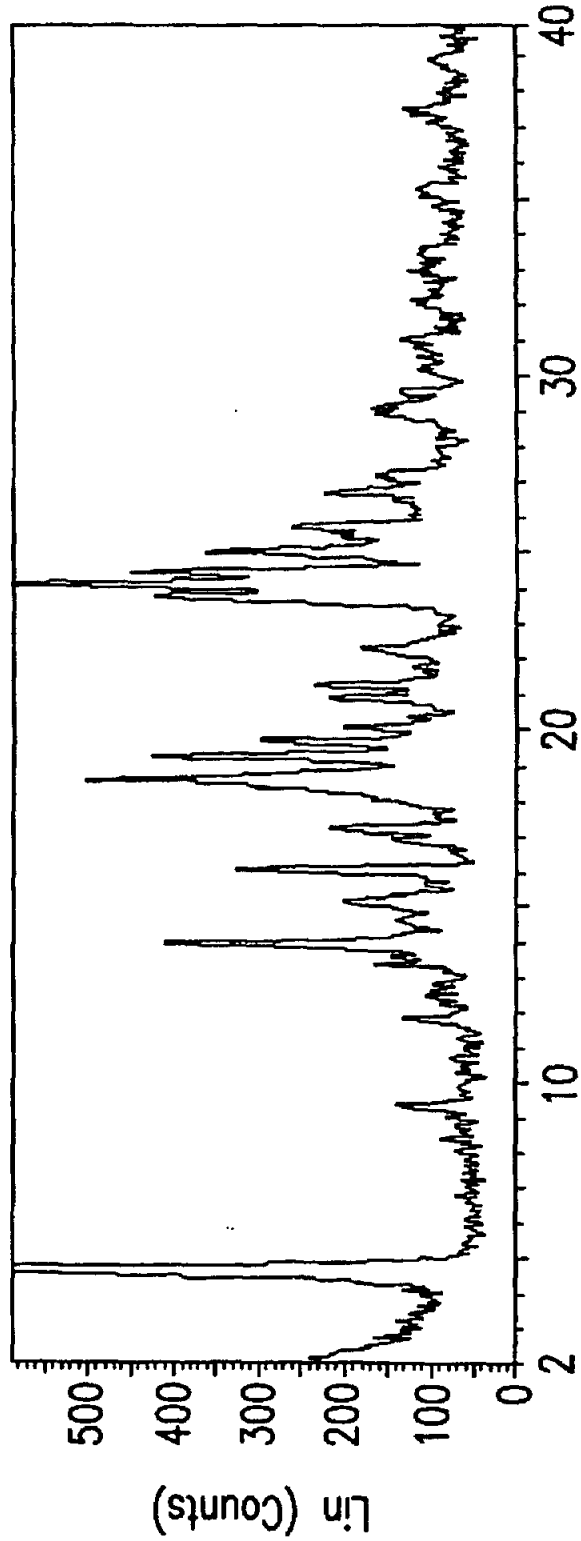


FIG. 13

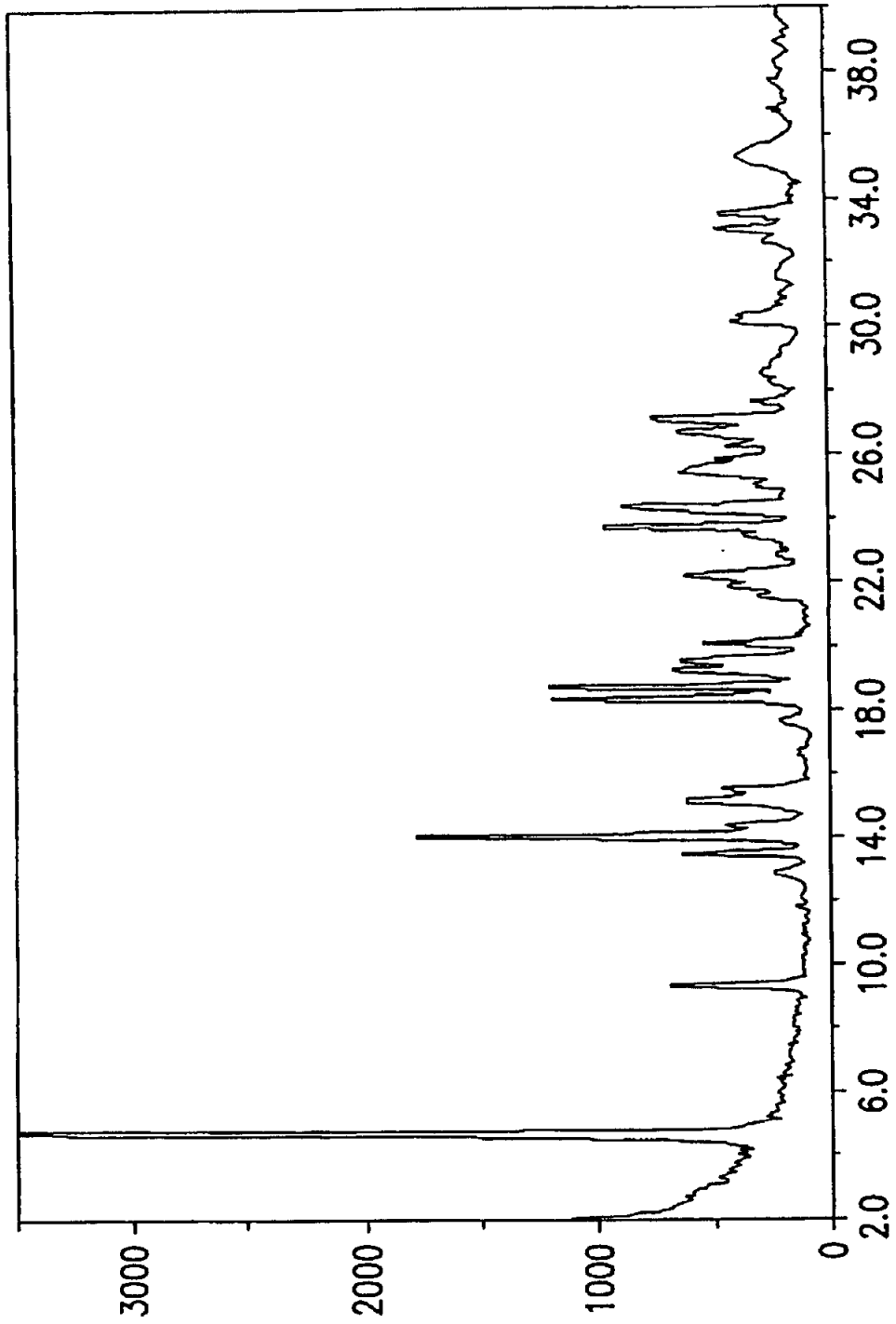


FIG. 14a

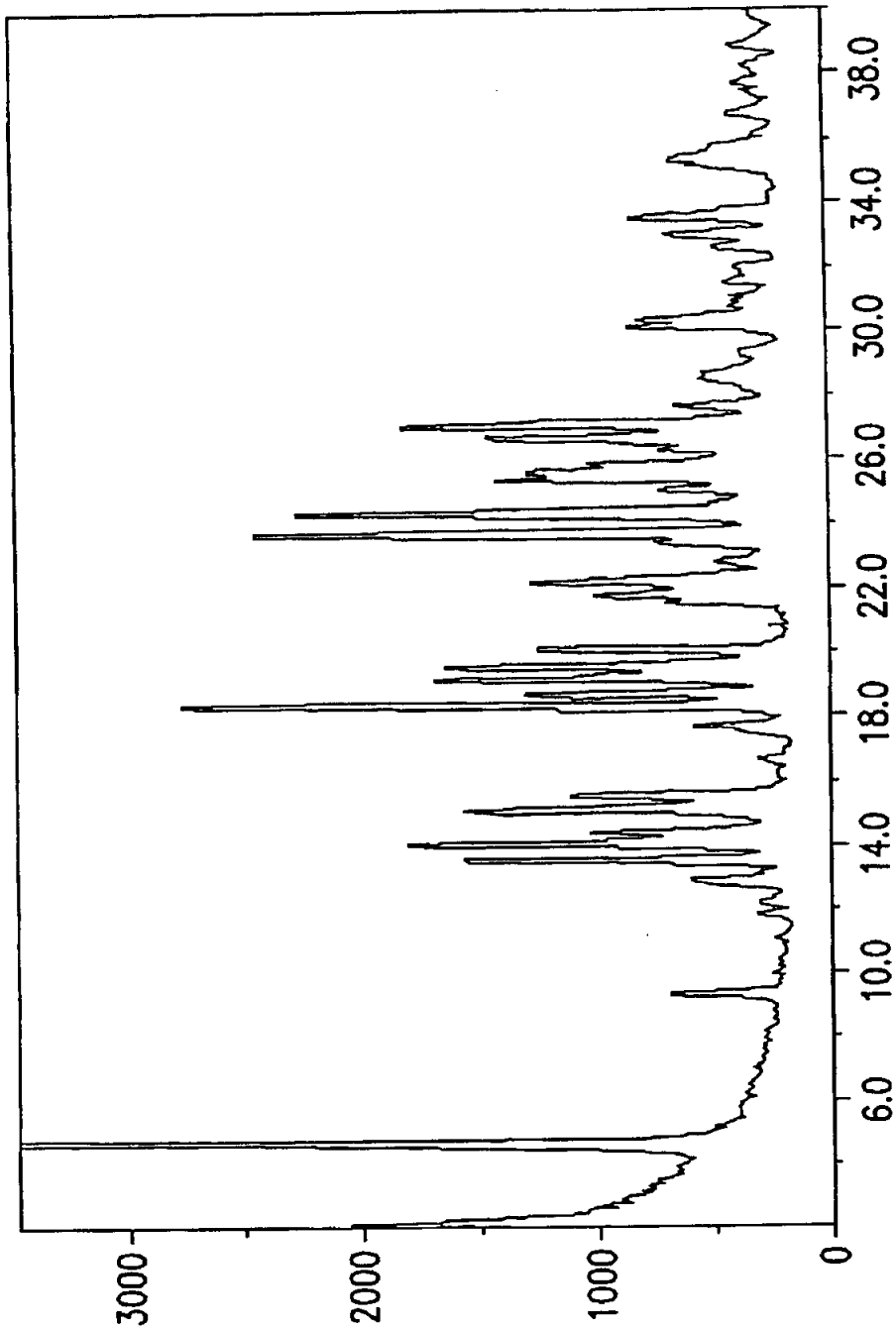


FIG. 14b

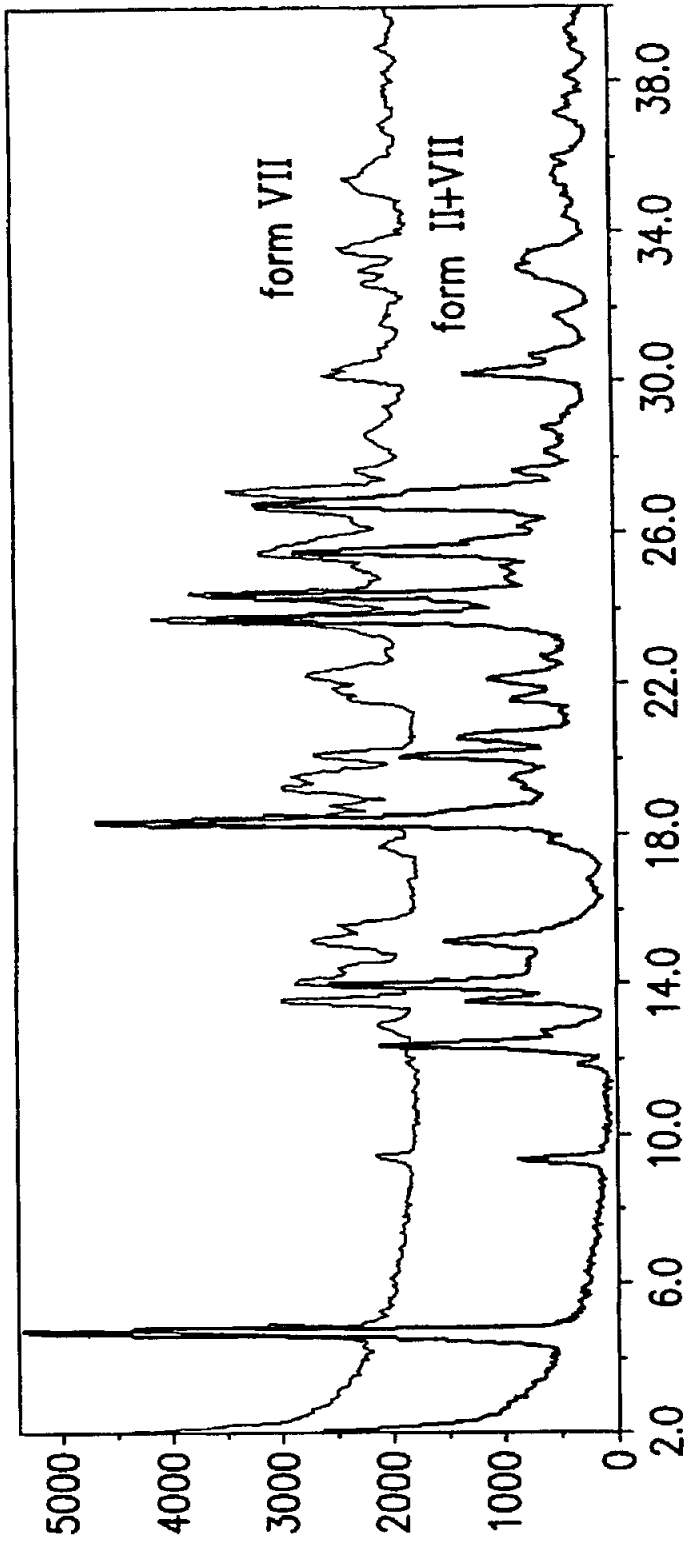

form VII
form II+VII
5000
4000
3000
2000
1000
0
2.0
6.0
10.0
14.0
18.0
22.0
26.0
30.0
34.0
38.0

FIG. 15

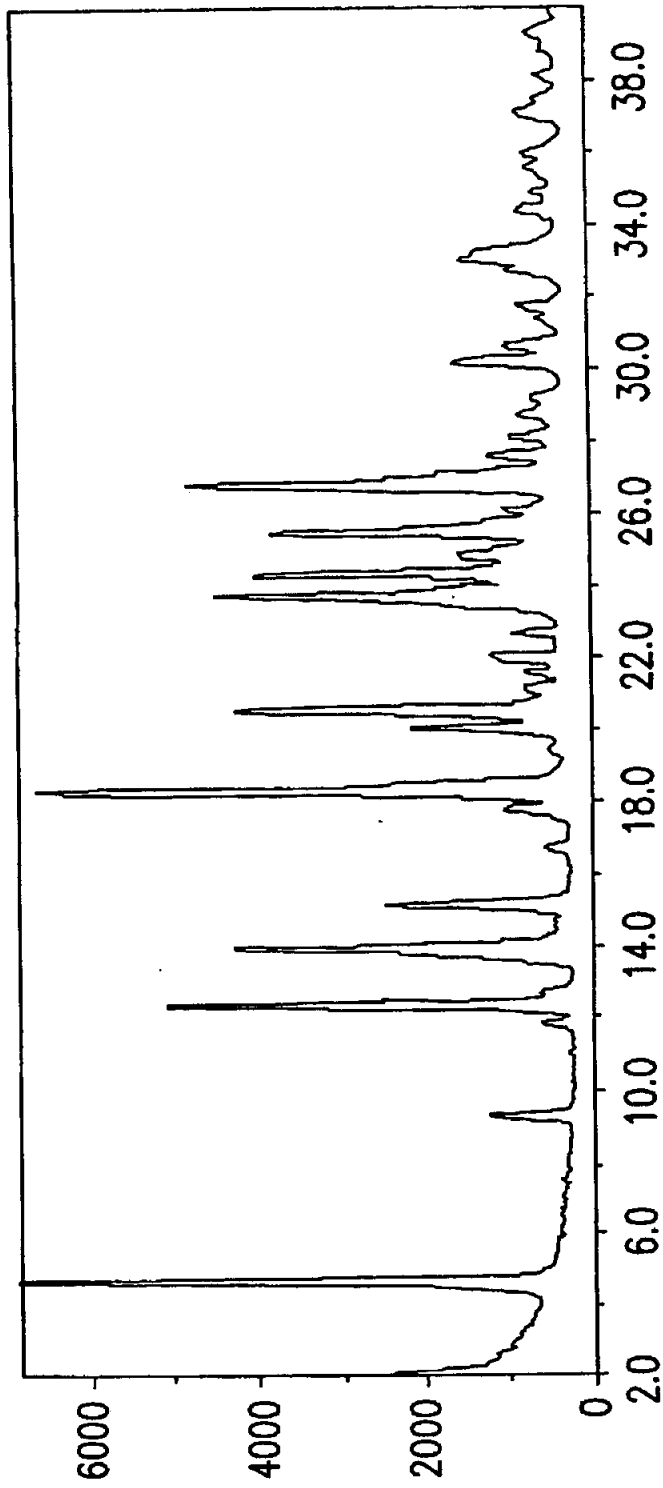


FIG. 16

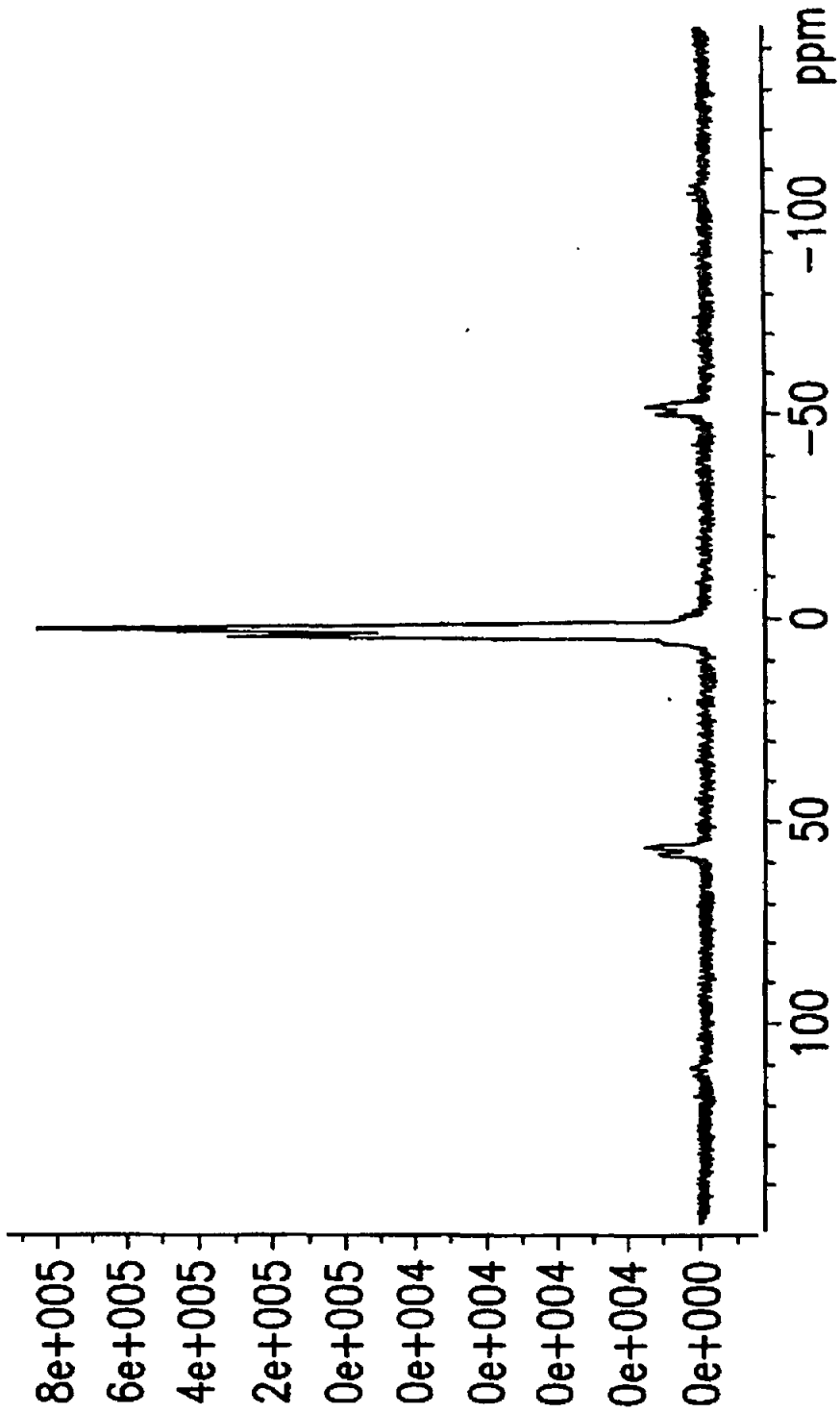

8e+005
6e+005
4e+005
2e+005
0e+005
0e+004
0e+004
0e+004
0e+004
0e+000
100
50
0
-50
-100
ppm

FIG.17

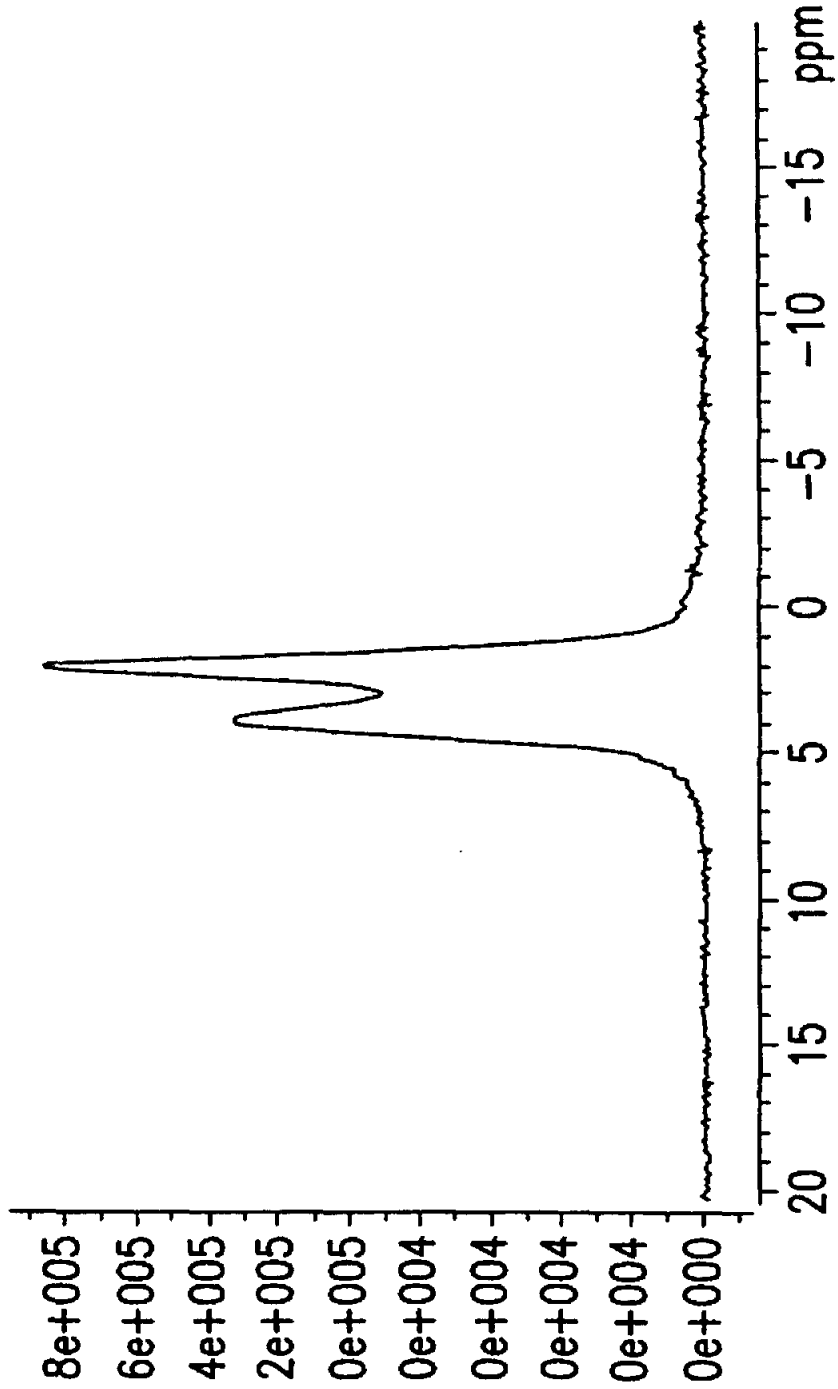

8e+005
6e+005
4e+005
2e+005
0e+005
0e+004
0e+004
0e+004
0e+004
0e+000
20
15
10
5
0
-5
-10
-15
ppm

FIG.18

CRYSTALLINE FORMS OF SITAGLIPTIN PHOSPHATE

RELATED APPLICATIONS

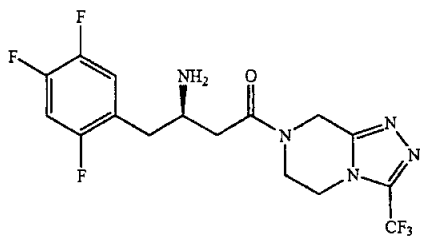
[0001] This application claims benefit of U.S. Provisional Patents Application No. 61/154,491, filed Feb. 23, 2009, 61/201,304, filed Dec. 8, 2008, 61/190,868, filed Sep. 2, 2008, 61/092,555, filed Aug. 28, 2008, 61/090,736, filed Aug. 21, 2008, 61/189,128, filed May 14, 2008, and 61/070,866, filed Mar. 25, 2008, the contents of which are incorporated herein in their entirety by reference. This application also claims benefit of U.S. Provisional Patents Application No. 61/201,860, filed Dec. 15, 2008, 61/191,933, filed Sep. 11, 2008, 61/091,759, filed Aug. 26, 2008, 61/137,489, filed Jul. 30, 2008, and 61/134,598, filed Jul. 10, 2008, the contents of which are incorporated herein in their entirety by reference.

FIELD OF THE INVENTION

[0002] The invention encompasses crystalline forms of Sitagliptin phosphate, processes for preparing the crystalline form, and pharmaceutical compositions thereof.

BACKGROUND OF THE INVENTION

[0003] Sitagliptin, (3R)-3-amino-1-[9-(trifluoromethyl)-1,4,7,8-tetrazabicyclo[4.3.0]nona-6,8-dien-4-yl]-4-(2,4,5-trifluorophenyl)butan-1-one, has the following chemical structure:



Sitagliptin

[0004] Sitagliptin phosphate is a glucagon-like peptide 1 metabolism modulator, hypoglycemic agent, and dipeptidyl peptidase IV inhibitor. Sitagliptin is currently marketed in its phosphate salt in the United States under the tradename JANUVIA™ in its monohydrate form. JANUVIA™ is indicated to improve glycemic control in patients with type 2 diabetes mellitus.

[0005] The following PCT Publications describe the synthesis of Sitagliptin via stereoselective reduction: WO 2004/087650, WO 2004/085661, and WO 2004/085378.

[0006] Several crystalline forms of Sitagliptin phosphate are described in the literature. WO 2005/020920 describes crystalline forms I, II, III and ethanol solvate; WO 2005/030127 describes crystalline form IV; WO 2005/003135 describes a monohydrate form, and WO 2006/033848 described the amorphous form.

[0007] Polymorphism, the occurrence of different crystal forms, is a property of some molecules and molecular complexes. A single molecule, like Sitagliptin, may give rise to a variety of crystalline forms having distinct crystal structures and physical properties like melting point, x-ray diffraction pattern, infrared absorption fingerprint, and solid state NMR spectrum. One crystalline form may give rise to thermal behavior different from that of another crystalline form. Thermal behavior can be measured in the laboratory by such techniques as capillary melting point, thermogravimetric analysis ("TGA"), and differential scanning calorimetry ("DSC"), which have been used to distinguish polymorphic forms.

[0008] The difference in the physical properties of different crystalline forms results from the orientation and intermolecular interactions of adjacent molecules or complexes in the bulk solid. Accordingly, polymorphs are distinct solids sharing the same molecular formula yet having distinct advantageous physical properties compared to other crystalline forms of the same compound or complex.

[0009] One of the most important physical properties of pharmaceutical compounds is their solubility in aqueous solution, particularly their solubility in the gastric juices of a patient. For example, where absorption through the gastrointestinal tract is slow, it is often desirable for a drug that is unstable to conditions in the patient's stomach or intestine to dissolve slowly so that it does not accumulate in a deleterious environment. Different crystalline forms or polymorphs of the same pharmaceutical compounds can and reportedly do have different aqueous solubilities.

[0010] The discovery of new polymorphic forms and solvates of a pharmaceutically useful compound provides a new opportunity to improve the performance characteristics of a pharmaceutical product. It enlarges the repertoire of materials that a formulation scientist has available for designing, for example, a pharmaceutical dosage form of a drug with a targeted release profile or other desired characteristic. Therefore, there is a need for additional crystalline forms of Sitagliptin.

SUMMARY OF THE INVENTION

[0011] The present invention provides a crystalline Sitagliptin phosphate characterized by data selected from the group consisting of: a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 4.7, 13.5, and 15.5±0.2 degrees two theta and at least another two peaks selected from the following list: 14.0, 14.4, 18.3, 19.2, 19.5 and 23.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 13.5, 19.2, and 19.5±0.2 degrees two theta and at least another two peaks selected from the following list: 4.7, 14.0, 15.1, 15.5, 18.3, and 18.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 13.5, 15.5, 19.2, 23.7, and 24.4±0.2 degrees two theta; and a powder XRD pattern with peaks at about 4.65, 13.46, 17.63, 18.30, and 23.66±0.10 degrees two theta, and processes for preparing thereof.

[0012] The present invention also provides a crystalline Form VI of Sitagliptin phosphate characterized by data selected from the group consisting of: a PXRD pattern having peaks at about 13.6, 14.3, 15.6, 16.9, and 19.1±0.2 degrees two theta or peaks at about 17.9, 20.3, 24.8, 26.3, and 28.9±0.2 degrees two theta; a solid-state ^{13}C NMR spectrum with signals at about 103.0, 121.5 and 173.2±0.2 ppm; and a solid-state ^{13}C NMR spectrum having chemical shifts differences between the signal exhibiting the lowest chemical shift and another in the chemical shift range of 100 to 180 ppm of about 0.0, 18.5 and 70.2±0.1 ppm, wherein, the signal exhibiting the lowest chemical shift in the chemical shift area of 100 to 180 ppm is typically at about 103.0±1 ppm, and processes for preparing thereof.

[0013] The present invention further provides processes for the preparation of crystalline Sitagliptin phosphate Form II, Sitagliptin phosphate monohydrate, and amorphous Sitagliptin.

[0014] The invention further provides a pharmaceutical formulation comprising the above described Sitagliptin phosphate crystalline forms. This pharmaceutical composition may additionally comprise at least one pharmaceutically acceptable excipient.

[0015] The invention further provides a pharmaceutical formulation comprising the above described Sitagliptin phosphate crystalline forms made by the processes of the present invention, and one or more pharmaceutically acceptable excipients.

BRIEF DESCRIPTION OF THE FIGURES

[0016] FIG. **1** shows a powder XRD pattern of a crystalline form of Sitagliptin phosphate, obtained in Example 1.

[0017] FIG. **2** shows a powder XRD pattern of a crystalline form of Sitagliptin phosphate, obtained in Example 2.

[0018] FIG. **3** shows a powder XRD pattern of a dry crystalline form of Sitagliptin phosphate, obtained in Example 3.

[0019] FIG. **4** shows a powder XRD pattern of a crystalline form of Sitagliptin phosphate, obtained in Example 4.

[0020] FIG. **5***a* shows a powder XRD pattern of wet crystalline Form II of Sitagliptin phosphate, obtained in Example 5.

[0021] FIG. **5***b* shows a powder XRD pattern of a dry crystalline form of Sitagliptin phosphate, obtained in Example 5.

[0022] FIG. **6** shows a powder XRD pattern of a crystalline Form II of Sitagliptin phosphate, obtained in Example 33.

[0023] FIG. **7** shows a powder XRD pattern of amorphous Sitagliptin phosphate, obtained in Example 63.

[0024] FIG. **8** shows a powder XRD pattern of amorphous Sitagliptin phosphate, obtained in Example 73.

[0025] FIG. **9** shows a powder XRD pattern of amorphous Sitagliptin phosphate, obtained in Example 74.

[0026] FIG. **10** shows a powder XRD pattern of a crystalline Form II of Sitagliptin phosphate, obtained in Example 78.

[0027] FIG. **11** shows a powder XRD pattern of Sitagliptin phosphate monohydrate, obtained in Example 84.

[0028] FIG. **12** shows a powder XRD pattern of Sitagliptin phosphate monohydrate, obtained in Example 86.

[0029] FIG. **13** shows a powder XRD pattern of Sitagliptin phosphate monohydrate, obtained in Example 87.

[0030] FIG. **14***a* shows a powder XRD pattern of a crystalline form of Sitagliptin phosphate, obtained in Example 88.

[0031] FIG. **14***b* shows a powder XRD pattern of a crystalline form of Sitagliptin phosphate, obtained in Example 88.

[0032] FIG. **15** shows a powder XRD pattern of a crystalline form of Sitagliptin phosphate, obtained in Example 92.

[0033] FIG. **16** shows a powder XRD pattern of crystalline Form II of Sitagliptin phosphate, obtained in Example 96.

[0034] FIG. **17** shows a solid-state ^{31}P NMR spectrum of a crystalline form of Sitagliptin phosphate in the (−150)-(150) ppm range.

[0035] FIG. **18** shows a solid-state ^{31}P NMR spectrum of a crystalline form of Sitagliptin phosphate in the (−20)-(20) ppm range.

DETAILED DESCRIPTION OF THE INVENTION

[0036] As used herein, Sitagliptin base Form I refers to crystalline Sitagliptin base characterized by data selected from the group consisting of: a PXRD pattern having any 5 peaks selected from the group consisting of 7.4, 11.5, 16.7, 17.7, 18.9, 24.1, 24.5, 27.0, 28.5 and 28.8±0.2 degrees 2-theta, wherein any combination of peaks selected includes the peak at 7.4±0.2 degrees two theta; a powder XRD pattern with peaks at about 7.4, 16.7, 17.7, 28.5 and 28.8±0.2 degrees 2-theta; a powder XRD pattern with peaks at about 7.4, 11.5, 16.7, 17.7 and 18.9±0.2 degrees 2-theta; a powder XRD pattern with peaks at about 7.4, 11.5, 16.7, 28.5 and 28.8±0.2 degrees 2-theta and a powder XRD pattern with peaks at about 7.4, 24.1, 24.5, 27.0, and 28.8±0.2 degrees 2-theta.

[0037] As used herein, Sitagliptin phosphate Form II refers to crystalline Sitagliptin base characterized by a powder XRD pattern with peaks at about 4.7, 9.3, 12.3, 13.9, 15.1, 20.5±0.2 degrees two theta.

[0038] As used herein, Sitagliptin phosphate monohydrate refers to crystalline Sitagliptin base characterized by a powder XRD pattern with peaks at about 11. 8, 13.9, 16.0, 18.5, 19.6, 22.5±0.2 degrees two theta.

[0039] As used herein, the terms "Sitagliptin phosphate" and "Sitagliptin dihydrophosphate" may be both used to describe Sitagliptin phosphate having a 1:1 ratio of Sitagliptin and phosphate.

[0040] As used herein, the term "slurry" refers to a thin mixture of a liquid and a finely divided substance, such as any form of Sitagliptin phosphate. Typically, the solvent is used in an amount that does not result in the full dissolution of the substance.

[0041] As used herein, an "antisolvent" refers to a liquid that, when added to a solution of Sitagliptin bas, and phosphoric acid, or a solution of Sitagliptin phosphate in a solvent, induces precipitation of Sitagliptin phosphate.

[0042] As used herein, a "wet crystalline form" refers to a polymorph that was not dried using any conventional techniques.

[0043] As used herein, a "dry crystalline form" refers to a polymorph that was dried using any conventional techniques. For example, drying at elevated temperature under reduced pressure. Preferably, the crystalline form is dried at about 40° C. to about 60° C., more preferably, between about 45° C. and about 55° C., and, most preferably, about 50° C. Preferably the drying is carried out under reduced pressure (for example less than 1 atmosphere, more preferably, about 10 mbar to about 100 mbar, more preferably, about 10 mbar to about 25 mbar). Preferably the drying takes place over a period of about 8 hours to about 36 hours, more preferably, about 10 hours to about 24 hours, and, most preferably, about 12 hours.

[0044] As used herein, the term "room temperature" preferably refers to a temperature of about 20° C. to about 35° C., more preferably, about 25° C. to about 35° C., even more preferably, about 25° C. to about 30° C., and, most preferably, about 25° C.

[0045] As used herein, the term "overnight" preferably refers to about 14 hours to about 24 hours, more preferably about 14 hours to about 20 hours, and most preferably about 16 hours.

[0046] The present invention provides a crystalline Sitagliptin phosphate characterized by data selected from the group consisting of: a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 4.7, 13.5, and 15.5±0.2 degrees two theta and at least another two peaks selected from the following list: 14.0, 14.4, 18.3, 19.2, 19.5, and 23.7±0.2 degrees two theta; and a powder XRD pattern with peaks at

about 13.5, 19.2, and 19.5±0.2 degrees two theta and at least another two peaks selected from the following list: 4.7, 14.0, 15.1, 15.5, 18.3, and 18.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 13.5, 15.5, 19.2, 23.7, and 24.4±0.2 degrees two theta; and a powder XRD pattern with peaks at about 4.65, 13.46, 17.63, 18.30, and 23.66±0.10 degrees two theta.

[0047] In another embodiment, the crystalline form of Sitagliptin phosphate is characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0048] In another embodiment, the crystalline form of Sitagliptin phosphate is characterized by a powder XRD pattern with peaks at about 13.5, 15.5, 19.2, 23.7, and 24.4±0.1 degrees two theta.

[0049] In another embodiment, the crystalline form of Sitagliptin phosphate is further characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0050] In another embodiment, the crystalline form of Sitagliptin phosphate is characterized by a powder XRD pattern with peaks at about 4.65, 13.46, 17.63, 18.30, and 23.66±0.10 degrees two theta.

[0051] The crystalline form of Sitagliptin phosphate is also characterized by the XRD diffractograms shown in FIGS. **1** to **4**, **6**, **14**, and **15**.

[0052] The crystalline form of Sitagliptin phosphate, which is characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is substantially free of the (S)-enantiomer of Sitagliptin phosphate. By "substantially free" is meant 10% (w/w) or less, more preferably 5% (w/w) or less, most preferably 2% (w/w) or less, particularly 1% (w/w) or less, more particularly 0.5% (w/w) or less, and most particularly 0.2% (w/w) or less.

[0053] The crystalline form of Sitagliptin phosphate, which is characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is also substantially free of any other polymorph forms. By "substantially free" is meant 20% (w/w) or less, preferably 10% (w/w) or less, more preferably 5% (w/w) or less, most preferably 2% (w/w) or less, particularly 1% (w/w) or less, more particularly 0.5% (w/w) or less, and most particularly 0.2% (w/w) or less.

[0054] In another embodiment, the present invention encompasses a crystalline Form VI of Sitagliptin phosphate characterized by data selected from the group consisting of: a PXRD pattern having peaks at about 13.6, 14.3, 15.6, 16.9, and 19.1±0.2 degrees two theta or peaks at about 17.9, 20.3, 24.8, 26.3, and 28.9±0.2 degrees two theta; a solid-state ^{13}C NMR spectrum with signals at about 103.0, 121.5 and 173.2±0.2 ppm; and a solid-state ^{13}C NMR spectrum having chemical shifts differences between the signal exhibiting the lowest chemical shift and another in the chemical shift range of 100 to 180 ppm of about 0.0, 18.5 and 70.2±0.1 ppm, wherein, the signal exhibiting the lowest chemical shift in the chemical shift area of 100 to 180 ppm is typically at about 103.0±1 ppm.

[0055] Form VI is preferably obtained as a mixture of from about 50% to about 85% of the enantiomer R, and from about 15% to about 50% of the enantiomer S, more preferably from about 50% to about 80% of the enantiomer R, and from about 20% to about 50% of the enantiomer S, more preferably about 60% to about 80% of the enantiomer R, and from about 20% to about 40% of the enantiomer S. In one specific embodiment, Form VI is obtained as a mixture of about 77% of the enantiomer R and about 23% of the enantiomer S.

[0056] In another example, the crystalline form, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is obtained in a process comprising combining Sitagliptin base and phosphoric acid and a solvent selected from the group consisting of ethyl acetate, dioxane, methyl isobutyl ketone, isobutyl acetate, butyl acetate, a mixture of acetonitrile and toluene, or a mixture of tetrahydrofuran and water, forming a slurry; and obtaining the crystalline form of Sitagliptin phosphate. The obtained slurry is formed either by adding the phosphoric acid to a slurry of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a slurry of the phosphoric acid in the organic solvent.

[0057] Preferably the acetonitrile:toluene and the tetrahydrofuran:water ratio is about 1:1 to about 1:15, and most preferably about 3:10. Preferably, the solution is heated to a temperature of about 45° C. to about 80° C., more preferably about 50° C. to about 70° C., preferably, for about 10 minutes to about 5 hours, more preferably for about 20 minutes to about 3 hours. To promote precipitation, the solution can be cooled. Preferably, solution is gradually cooled to a temperature of about room temperature, and stirred until a precipitate is obtained. Preferably, the solution is stirred overnight. The precipitate is further recovered by any conventional method known in the art, for example by filtration. The precipitate may be further dried at about 40° C. to about 60° C., preferably between about 45° C. and about 55° C., most preferably about 50° C. Preferably the drying is carried out under reduced pressure (for example less than 1 atmosphere, more preferably, about 10 mbar to about 100 mbar, more preferably, about 10 mbar to about 25 mbar). Preferably the drying takes place over a period of about 8 hours to about 36 hours, more preferably, about 10 hours to about 24 hours, and, most preferably, about 12 hours.

[0058] In another embodiment, the present invention encompasses another process for preparing the crystalline form of Sitagliptin phosphate, which is characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, comprising combining Sitagliptin base and phosphoric acid and a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone:n-hexane, acetone:n-heptane, acetone:cyclopentyl methyl ether, acetone:dibutyl ether, acetone:isopropylacetate, dimethylsulfoxide:methyl isobutyl ketone, and dimethylsulfoxide:methyl tert butyl ether; forming a mixture, and crystallizing Sitagliptin phosphate from the mixture. Where acetone:cyclopentyl methyl ether, acetone:isopropylacetate, and dimethylsulfoxide:methyl tert butyl ether are used, the obtained precipitate is further dried.

[0059] Preferably, the first organic solvent and the second organic solvent ratio is about 1:1 to about 1:15, and most preferably about 3:10. Alternatively, Sitagliptin phosphate can be used instead of Sitagliptin base and phosphoric acid.

[0060] Preferably, the mixture is heated to a temperature of about 45° to about 80° C., preferably to about 70° C., preferably for about an hour to about 4 hours, more preferably, for about 2 hours. To promote precipitation, the solution can be cooled. Preferably, mixture is gradually cooled to about room temperature with stirring overnight to allow the product to precipitate out. The precipitate is further recovered by any conventional method known in the art, for example by filtration.

[0061] The obtained mixture is formed either by adding the phosphoric acid to a mixture of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a mixture of the phosphoric acid in the organic solvent.

[0062] In another embodiment, the present invention encompasses another process for preparing the crystalline form of Sitagliptin phosphate of the present invention, comprising drying wet Form II.

[0063] Preferably, wet Form II is dried at about 40° to about 100° C., more preferably, at about 40° C. to about 60° C., even more preferably, between about 45° C. and about 55° C., and, most preferably at about 50° C. Preferably, the drying is carried out under reduced pressure (for example less than 1 atmosphere, more preferably, about 10 mbar to about 100 mbar, and, most preferably, about 10 mbar to about 25 mbar). Preferably, the drying takes place over a period of about 8 hours to about 36 hours, more preferably, about 10 hours to about 24 hours, and, most preferably, about 12 hours.

[0064] Wet Form II can be prepared by any method known in the art.

[0065] For example, wet Form II is obtained in a process comprising combining Sitagliptin base and phosphoric acid and an organic solvent selected from the group consisting of dimethyl carbonate, tetrahydrofuran, propylene glycol methyl ether, methyl ethyl ketone, ethanol, methyl acetate, dimethylformamide, diethyl carbonate, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, isopropanol, a mixture of acetonitrile and n-butanol, acetonitrile, dimethyl carbonate, forming a slurry; and obtaining Sitagliptin phosphate Form II.

[0066] Preferably, the slurry is maintained at a temperature of about room temperature to about 70° C. Preferably, the slurry is heated to a temperature of about 50° C. to about 70° C., preferably, for about 10 minutes to about 5 hours, and, more preferably, for about 10 minutes to about 3 hours. Preferably, when the slurry is heated, it is gradually cooled to about 0° C. to about room temperature, more preferably about 10° C. to about room temperature, and, most preferably, about room temperature, and, preferably, stirred overnight to allow the product to precipitate out. The precipitate is further recovered by any conventional method known in the art, for example by filtration.

[0067] The obtained slurry is formed either by adding the phosphoric acid to a slurry of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a slurry of the phosphoric acid in the organic solvent.

[0068] In another example, wet Form II is prepared in a process comprising combining Sitagliptin base and phosphoric acid and a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone: isopropylacetate, acetone:cyclohexane, acetone:isobutyl acetate, acetonitrile:n-butanol, and acetone:n-butanol, forming a mixture; crystallizing Sitagliptin phosphate from the mixture; and obtaining Sitagliptin phosphate Form II.

[0069] Preferably, the first organic solvent and the second organic solvent ratio is about 1:1 to about 1:15, and most preferably about 3:10.

[0070] Preferably, the mixture is heated to a temperature of about 45° C. to about 70° C., preferably to about 70° C., preferably for about an hour to about 4 hours, more preferably, for about 2 hours. To promote precipitation, the solution can be cooled. Preferably, the mixture is gradually cooled to about 0° C. to about room temperature, more preferably, about 10° C. to about room temperature, and, most preferably, to about room temperature with stirring overnight to allow the product to precipitate out. The precipitate is recovered by any conventional method known in the art, for example by filtration.

[0071] The obtained mixture is formed either by adding the phosphoric acid to a mixture of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a mixture of the phosphoric acid in the organic solvent.

[0072] In one specific embodiment, the crystalline form of Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is prepared in a process comprising drying wet Form II, wherein the wet Form II comprises a solvent selected from the group consisting of methyl isobutyl ketone, dimethyl carbonate, tetrahydrofuran, acetonitrile, propylene glycol methyl ether, methanol, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, butyl acetate, isopropanol, dimethyl carbonate, n-hexane, acetone, cyclohexane, isobutyl acetate, and mixtures thereof.

[0073] In another embodiment, the present invention encompasses a process for preparing crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, comprising heating a mixture of Sitagliptin phosphate Form II and the crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta to a temperature of about 40° C. to about 100° C., and, more preferably, about 40° C. to about 60° C., under reduced pressure (for example less than 1 atmosphere, more preferably, about 10 mbar to about 100 mbar, and, most preferably, about 10 mbar to about 25 mbar). Preferably, the mixture of Sitagliptin phosphate Form II and crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is heated over a period of about 8 hours to about 36 hours, more preferably, about 10 hours to about 24 hours, and, most preferably, about 12 hours.

[0074] In another embodiment, the present invention encompasses another process for preparing crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, comprising drying a mixture of Sitagliptin phosphate Form II and the crystalline Sitagliptin phosphate characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, in a fluidized bed dryer at a temperature of about 30° C. to about 60° C., more preferably about 35° C. to about 50° C.

[0075] In another embodiment, the present invention encompasses a crystalline form of Sitagliptin phosphate, characterized by data selected from the group consisting of: a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 4.7, 13.5, and 15.5±0.2 degrees two theta and at least another two peaks selected from the following list: 14.0, 14.4, 18.3, 19.2, 19.5 and 23.7±0.2 degrees two theta; and a powder XRD pattern with peaks at about 13.5, 19.2, and 19.5±0.2 degrees two theta and at least another two peaks selected from the following list: 4.7, 14.0, 15.1, 15.5, 18.3, and 18.7±0.2 degrees two theta; a powder XRD pattern with peaks at about 13.5, 15.5, 19.2, 23.7, and 24.4±0.2 degrees two theta; and a powder XRD pattern with peaks at about 4.65, 13.46, 17.63, 18.30, and 23.66±0.10 degrees two theta, made by the processes described above.

[0076] In another embodiment, the present invention encompasses a process for preparing Form II comprising providing a slurry of Sitagliptin phosphate characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, and a solvent selected from the group consisting of acetonitrile, methanol, ethanol, 1-propanol, isopropanol, acetone, tetrahydrofuran, n-butanol, isobutanol, toluene, propylene glycol, propylene glycol methyl ether, chloroform, diethyl carbonate, dimethylformamide, or mixtures of dimethylformamide with methyl isobutyl ketone, or n-butanol; heating the slurry; and recovering the obtained Form II.

[0077] Preferably, the mixture is heated at a temperature of about 50° C. to about 80° C., more preferably, about 60° C. to about 75° C., even more preferably, about 65° C. to about 75° C., and, most preferably, about 70° C. The mixture is preferably stirred at this temperature for about 5 minutes to about 5 hours, and, more preferably, about 10 minutes to about 3 hours. Preferably, the mixture is gradually cooled to about 0° C. to about room temperature, more preferably about 10° C. to about room temperature, and, most preferably, to about room temperature. The mixture is stirred at this temperature overnight. The precipitate is further recovered by any conventional method known in the art, for example by filtration.

[0078] In another the present invention encompasses another process for preparing Form II comprising combining Sitagliptin base and phosphoric acid and an organic solvent selected from the group consisting of dimethyl carbonate, tetrahydrofuran, propylene glycol methyl ether, methyl ethyl ketone, ethanol, methyl acetate, dimethylformamide, diethyl carbonate, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, isopropanol, a mixture of acetonitrile and n-butanol, acetonitrile, dimethyl carbonate, and a mixture of dimethyl carbonate and n-hexane, forming a slurry; and obtaining Form II.

[0079] Preferably, the slurry is maintained at a temperature of about room temperature to about 70° C. More preferably, the slurry is heated to a temperature of about 50° C. to about 70° C., preferably for about 10 minutes to about 5 hours, more preferably for about 10 minutes to about 3 hours. Preferably, when the slurry is heated, it is gradually cooled to a temperature of about 0° C. to about room temperature, more preferably about 10° C. to about room temperature, and most preferably to about room temperature and stirring, preferably overnight to allow the product to precipitate out. The precipitate is further recovered by any conventional method known in the art, for example by filtration.

[0080] The obtained slurry is formed either by adding the phosphoric acid to a slurry of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a slurry of the phosphoric acid in the organic solvent.

[0081] In another embodiment, the present invention encompasses another process for preparing Form II comprising combining Sitagliptin base and phosphoric acid and a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone:isopropylacetate, acetone:cyclohexane, acetone:isobutyl acetate, acetonitrile:n-butanol, and acetone:n-butanol, forming a mixture; crystallizing Sitagliptin phosphate from the mixture; and recovering Sitagliptin phosphate Form II.

[0082] Preferably, the first organic solvent and the second organic solvent ratio is about 1:1 to about 1:15, and most preferably about 3:10.

[0083] Preferably, the mixture is heated to a temperature of about 45° C. to about 70° C., preferably to about 70° C., preferably for about an hour to about 4 hours, more preferably, for about 2 hours. To promote precipitation, the solution can be cooled. Preferably, mixture is gradually cooled to about 0° C. to about room temperature, more preferably, about 10° C. to about room temperature, and, most preferably, to about room temperature and stirring overnight to allow the product to precipitate out. The precipitate is recovered by any conventional method known in the art, for example by filtration.

[0084] The obtained mixture is formed either by adding the phosphoric acid to a mixture of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a mixture of the phosphoric acid in the organic solvent.

[0085] In another embodiment, the present invention encompasses another process for preparing Sitagliptin phosphate Form II, comprising dissolving Sitagliptin phosphate in dimethylsulfoxide; adding an antisolvent selected from the group consisting of iso-butanol, acetonitrile, diethyl ether, diethyl carbonate, and tert-butyl ether; and recovering Sitagliptin phosphate Form II.

[0086] Preferably, the solvent/antisolvent ratio is about 1:1 to about 1:20, and most preferably about 3:10.

[0087] Preferably, the starting Sitagliptin phosphate is crystalline Sitagliptin phosphate characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0088] In order to promote precipitation, the mixture may be cooled to about 0° C. to about 20° C., preferably, for about 2 hours to about 24 hours.

[0089] In another embodiment, the present invention encompasses another process for preparing Sitagliptin phosphate Form II, comprising granulating Sitagliptin phosphate in the presence of isopropanol. Preferably, the starting Sitagliptin phosphate is crystalline Sitagliptin phosphate characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0090] The term "granulation" broadly refers to a process comprising mixing the solid with a minimal amount of solvent, and stirring the mixture at about room temperature for the time needed to cause the desired transformation. A mechanical stirrer can be used in the process. Typically, about 0.1 to about 0.2 ml of solvent is used per 1 gram of compound. Preferably, the mixture is granulated using a rotary evaporator.

[0091] In another embodiment, the present invention encompasses a process for preparing Form II, comprising exposing Sitagliptin phosphate characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta to a C_1-C_4 alcohol, where the alcohol is preferably selected from the group consisting of ethanol, methanol, and isopropanol.

[0092] In another embodiment, the present invention encompasses a process for preparing the crystalline form VI of Sitagliptin phosphate of the present invention, comprising providing a slurry of Sitagliptin phosphate, and an organic solvent selected from the group consisting of acetonitrile (ACN), and C_1-C_4 alcohols, most preferably isopropanol; heating the slurry; cooling the resulting mixture; and recovering the obtained Form VI of Sitagliptin phosphate. Optionally, Sitagliptin phosphate can be formed in situ starting from Sitagliptin base and phosphoric acid. The Sitagliptin base or the Sitagliptin phosphate are introduced as a mixture of from

about 50% to about 85% of the enantiomer R, and from about 15% to about 50% of the enantiomer S, more preferably from about 50% to about 80% of the enantiomer R, and from about 20% to about 50% of the enantiomer S, more preferably about 60% to about 80% of the enantiomer R, and from about 20% to about 40% of the enantiomer S.

[0093] Preferably, from about 10 ml to about 70 ml of acetonitrile, and, more preferably, about 25 ml to about 60 ml are used per gram of the Sitagliptin phosphate. Preferably, from about 2 ml to about 12 ml, and, more preferably, about 4 ml to about 10 ml of the organic solvent are used per gram of the Sitagliptin.

[0094] Preferably, the Sitagliptin or the Sitagliptin salt, which is combined with the ACN, is amorphous.

[0095] The obtained slurry is preferably heated to a temperature of about 40 to about reflux, more preferably, the slurry is heated to about 60 to about reflux, and, most preferably, the slurry is heated to about reflux. To induce precipitation, the slurry is then cooled to about 0° C. to about room temperature, more preferably to about 0° C. to about 4° C., and preferably maintained for about 1 day to about 5 days, and, more preferably, for about 3 days, to induce precipitation.

[0096] When phosphoric acid is introduced into a mixture of Sitagliptin and the organic solvent, preferably, it is added in a dropwise manner. Preferably, the acid is added to a heated solution or slurry of the Sitagliptin and the organic solvent, where the heated solution or slurry is at a temperature of about 40° C. to about 65° C., and, more preferably about 45° C. to about 60° C.

[0097] Preferably, the chemical purity of the obtained Form VI is more than 99.5%, and, more preferably, more than 99.9%.

[0098] In another embodiment, the present invention encompasses another process for preparing amorphous Sitagliptin phosphate, comprising dissolving Sitagliptin phosphate in dimethylsulfoxide; and adding an antisolvent selected form a group consisting of methyl tert-butyl ether, and tetrahydrofuran to obtain amorphous Sitagliptin phosphate.

[0099] The mixture is maintained at a temperature of about 0° C. for about 2 hours to induce precipitating.

[0100] In another embodiment, the present invention encompasses another process for preparing amorphous Sitagliptin phosphate comprising combining Sitagliptin base and phosphoric acid and an organic solvent selected from the group consisting of diethyl carbonate, dimethyl carbonate, and a mixture of cyclohexanone and methyl tert-butyl ether, forming a slurry; and recovering the precipitate from the mixture.

[0101] Preferably, the mixture is maintained at a temperature of about 15° C. to about 70° C., preferably about 20° C. to about 50° C. for about 10 minutes to about 7 days, more preferably for about 10 minutes to about an hour.

[0102] The obtained slurry is formed either by adding the phosphoric acid to a slurry of the Sitagliptin base in the organic solvent, or by adding the Sitagliptin base into a slurry of the phosphoric acid in the organic solvent.

[0103] In another embodiment, the present invention encompasses a process to obtain Sitagliptin phosphate monohydrate comprising heating a mixture of Sitagliptin phosphate with water and an organic solvent selected from a group consisting of methyl tert-butyl ether and acetonitrile; and recovering the precipitate. Alternatively, a mixture of Sitagliptin base and phosphoric acid can be introduced instead of Sitagliptin phosphate.

[0104] Preferably, the mixture is heated to about 50° C. to about 80° C., more preferably 60° C. to about 70° C., and then cooled to about 0° C. to about 25° C. Recovering the product may be carried out via any known method in the art, for example by filtration or evaporation.

[0105] The invention further provides a pharmaceutical formulation comprising the above described Sitagliptin phosphate crystalline forms. This pharmaceutical composition may additionally comprise at least one pharmaceutically acceptable excipient.

[0106] The invention further provides a pharmaceutical formulation comprising the above described Sitagliptin phosphate crystalline forms made by the processes of the present invention, and one or more pharmaceutically acceptable excipients. The compositions of the invention include powders, granulates, aggregates and other solid compositions comprising the present invention form of Sitagliptin solid crystalline.

[0107] The present invention also provides methods of treating type 2 diabetes mellitus in a patient, preferably a human, by administrating to the patient a pharmaceutical composition comprising Sitagliptin phosphate crystalline form as described herein. Preferably, the pharmaceutical composition comprises a therapeutically effective amount of Sitagliptin phosphate crystalline form.

[0108] The present invention also provides the use of the above mentioned Sitagliptin phosphate crystalline forms, for the manufacture of a pharmaceutical composition for the treatment of type 2 diabetes mellitus.

[0109] Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification. The invention is further defined by reference to the following examples describing in detail the preparation of the composition and methods of use of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

Examples

X-Ray Power Diffraction:

[0110] X-Ray powder diffraction data was obtained by using methods known in the art using a SCINTAG powder X-Ray diffractometer model X'TRA equipped with a solid-state detector. Copper radiation of 1.5418 Å was used. A round aluminum sample holder with zero background was used. The scanning parameters included: range: 2-40 degrees two-theta; scan mode: continuous scan; step size: 0.05 deg.; and a rate of 3 deg/min. All peak positions are within ±0.2 degrees two theta.

[0111] The PXRD peaks positions are calibrated using silicon powder as internal standard in an admixture with the sample measured. The position of the silicon (111) peak was corrected to be 28.45 degrees two theta. The positions of Sitagliptin phosphate form peaks were corrected respectively. (No correction was performed on the presented diffractograms in the figures).

[0112] FIGS. **12** and **13** were obtained by using methods known in the art using a Bruker X-Ray powder diffractometer

model D8 advance equipped with lynxEye. Scan range: 2-40°. Step size: 0.05°. Time per step: 5.2 seconds.

NMR Parameters

[0113] ^{31}P NMR at 202 MHz using Bruker Avance II+ 500

[0114] SB probe using 4 mm rotors

[0115] Magic angle was set using KBr

[0116] Homogeneity of magnetic field checked using adamantane

[0117] Parameters for Cross polarization optimized using glycine

[0118] Spectral reference set according to Ammonium Dihydrogeno Phosphate as external standard (0.00 ppm for signal)

Scanning Parameters:

[0119] Magic Angle Spinning Rate: 11 kHz

[0120] Pulse Program: cp with tppm15 during decoupling

[0121] Delay time: 25 s

[0122] STG (Sitagliptin) base form I can be obtained according to the procedures described in PCT application No. PCT/US08/01317.

Example 1

[0123] STG (Sitagliptin) base form I (100 mg) was dissolved in ethyl acetate (500 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 2

[0124] STG base form I (100 mg) was dissolved in tetrahydrofuran:water 2:1 (300 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 3

[0125] STG base form I (100 mg) was partially dissolved in methyl isobutyl ketone (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 1.5 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta. The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 4

[0126] STG base form I (100 mg) was partially dissolved in dioxane (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 1.5 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 5

[0127] STG base form I (100 mg) was partially dissolved in dimethyl carbonate (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0128] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II

[0129] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 6

[0130] STG base form I (100 mg) was dissolved in acetone (100 μL) at 25° C. Then, n-Hexane was added (500 μL) at 25° C. Two phases were formed. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0131] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 7

[0132] STG base form I (100 mg) was dissolved in acetone (100 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and n-Hexane (500 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0133] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 8

[0134] STG base form I (100 mg) was dissolved in acetone (100 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and n-Heptane (500 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0135] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 9

[0136] STG base form I (100 mg) was dissolved in acetone (100 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and cyclopentyl methyl ether (1000 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0137] The product was isolated by vacuum filtration to obtain a mixture of wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5,

17.7, 18.3, and 23.7±0.2 degrees two theta and form II. The sample was dried in vacuum oven at 50° C. 24 hours to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 10

[0138] STG base form I (100 mg) was dissolved in acetone (300 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and dibutyl ether (1000 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0139] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 11

[0140] STG base form I (100 mg) was dissolved in methyl ethyl ketone (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 12

[0141] STG base form I (100 mg) was dissolved in acetone (300 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and cyclohexane (1000 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0142] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 13

[0143] STG phosphate Form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (50 mg) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then iso-Butanol (1 ml) was added at 25° C. The solution formed was slurry (crystallization occurred) and was cooled in ice water bath for 2 hrs.

[0144] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0145] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 14

[0146] STG phosphate Form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (50 mg) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then Acetonitrile (1 ml) was added at 25° C. Crystallization occurred and the mixture was cooled in ice water bath for 16 hours.

[0147] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 15

[0148] STG phosphate Form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (50 mg) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then diethyl ether (1 ml) was added at 25° C. The solution formed was a slurry (crystallization occurred) and was cooled in ice water bath for 2 hours.

[0149] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 16

[0150] STG phosphate Form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (50 mg) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then diethyl carbonate (1 ml) was added at 25° C. The solution formed was a slurry (crystallization occurred) and was cooled in ice water bath for 2 hours.

[0151] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 17

[0152] STG base form I (100 mg) was partially dissolved in tetrahydrofuran (500 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0153] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 18

[0154] STG base form I (100 mg) was dissolved in acetonitrile (500 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0155] The product was isolated by vacuum filtration to obtain a mixture of wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta and form II.

[0156] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 19

[0157] STG base form I (100 mg) was dissolved in ethanol (500 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0158] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 20

[0159] STG base form I (100 mg) was dissolved in methyl acetate (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2.5 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0160] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 21

[0161] STG base form I (100 mg) was dissolved in propylene glycol methyl ether (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2.5 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0162] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0163] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 22

[0164] STG base form I (100 mg) was dissolved in dimethyl formamide (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by evaporation to obtain wet STG phosphate crystalline form II.

Example 23

[0165] STG base form I (100 mg) was dissolved in dimethylsulfoxide (200 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by evaporation, addition of methanol and vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 24

[0166] STG base form I (100 mg) was dissolved in dimethyl formamide (500 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 25

[0167] STG base form I (100 mg) was dissolved in acetone (100 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and iso-butyl acetate (500 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0168] The product was isolated by vacuum filtration to obtain STG phosphate crystalline form II.

Example 26

[0169] STG base form I (100 mg) was dissolved in acetone (100 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and iso-propyl acetate (1000 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0170] The product was isolated by vacuum filtration to obtain STG phosphate crystalline form II.

[0171] The sample was dried at 50° C. for about 24 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 27

[0172] STG base form I (100 mg) was dissolved in acetone (300 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) and n-butanol (1000 μL) were then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0173] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 28

[0174] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in acetonitrile (1 ml) at 25° C., then heated to 70° C., stirred at 70° C. for 5 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0175] The product was isolated by vacuum filtration to obtain STG phosphate crystalline form II.

Example 29

[0176] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in methanol (1 ml) at room temperature, then heated to 50° C., stirred at 50° C. for 5 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0177] The product was isolated by vacuum filtration to obtain STG phosphate crystalline form II.

Example 30

[0178] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in acetone (1 ml) at room temperature, then heated to 50° C., stirred at 50° C. 5 for hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0179] The product was isolated by vacuum filtration to obtain STG phosphate crystalline form II.

Example 31

[0180] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in tetrahydrofuran (1 ml) at room temperature, then heated to 50° C., stirred at 50° C. for 5 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0181] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 32

[0182] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in n-Butanol (1 ml) at room temperature, then heated to 95° C., stirred at that temperature for 5 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0183] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 33

[0184] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03g) was slurried in 0.3 ml n-butanol at 25° C., under magnetic stirring for 24 hours. The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 34

[0185] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in iso-Butanol (1 ml) at room temperature, then heated to 95° C., stirred at that temperature for 5 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0186] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 35

[0187] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.05g) was slurried in 1 ml iso-BuOH at 50° C., under magnetic stirring for 3 hours and at 10° C. for 16 hours. The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 36

[0188] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in toluene (1 ml) at room temperature, then heated to 95° C., stirred at that temperature for 5 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours.

[0189] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 37

[0190] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in N,N-Dimethyl Formamide (1 ml) at room temperature, then heated to 70° C., stirred at that temperature for 4 hours, cooled gradually to 10° C. and remained at 10° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 38

[0191] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in N,N-Dimethyl Formamide (0.5 ml) at room temperature. Then Methyl iso-Butyl Ketone (0.5 ml) was added at room temperature. The solution formed was slurry and stirred for 16 hours.

[0192] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 39

[0193] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in N,N-Dimethyl Formamide (0.5 ml) at room temperature. Then n-butanol (0.5 ml) was added at room temperature. The solution formed was slurry and stirred for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 40

[0194] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in propylene glycol (0.025 ml) at 25° C. for 16 hours.

[0195] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 41

[0196] STG base Form I (100 mg) was slurried in n-butanol (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0197] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0198] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 42

[0199] STG base Form I (100 mg) was slurried in iso-propanol (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2.5 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 43

[0200] STG base Form I (100 mg) was slurried in 1-propanol (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0201] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0202] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 44

[0203] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03g) was slurried in 0.3 ml 1-propanol at 25° C., under magnetic stirring for 24 hours.

The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 45

[0204] STG base Form I (100 mg) was slurried in isopropyl acetate (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 46

[0205] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in propylene glycol methyl ether (0.25 ml) at 25° C. for 16 hours.

[0206] The product was isolated by vacuum filtration to obtain a wet STG phosphate crystalline form II.

Example 47

[0207] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in chloroform (0.25 ml) at 25° C., then cooled gradually to 25° C. and stirred at 25° C. for 16 hours.

[0208] The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 48

[0209] STG base Form I (100 mg) was slurried in isopropyl acetate (1000 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by vacuum filtration to obtain a mixture of wet STG phosphate crystalline form II and form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0210] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain a mixture of STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 49

[0211] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03 g) was slurried in 1 ml acetonitrile at 50° C., under magnetic stirring for 3 hours and at 10° C. for 16 hours. The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 50

[0212] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03 g) was slurried in 0.3 ml ethanol at 25° C., under magnetic stirring for 24 hours. The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 51

[0213] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03 g) was slurried in 0.3 ml iso-propyl alcohol at 25° C., under magnetic stirring for 24 hours. The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 52

[0214] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03 g) was slurried in 0.3 ml diethylcarbonate at 25° C., under magnetic stirring for 24 hours. The product was isolated by filtration. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 53

[0215] Sitagliptin dihydrophosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03 g) was granulated with 0.006 ml isopropyl alcohol at 25° C., in a rotavapor for 9-12 hours. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate Form II.

Example 54

[0216] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then Methyl iso-Butyl Ketone (1 ml) was added at room temperature. The solution formed was slurry (crystallization occurred) and was cooled in ice water bath for 2 hours.

[0217] The product was isolated by vacuum filtration to obtain STG phosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 55

[0218] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then Tetrahydrofuran (1 ml) was added at 25° C. The solution formed was slurry (crystallization occurred) and was cooled in ice water bath for 16 hours.

[0219] The product was isolated by vacuum filtration to obtain wet amorphous STG phosphate.

Example 56

[0220] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was dissolved in dimethylsulfoxide (0.05 ml) at 25° C. Then methyl t-butyl ether (1 ml) was added at 25° C. The solution formed was slurry (crystallization occurred) and was cooled in ice water bath for 16 hours.

[0221] The product was isolated by vacuum filtration to obtain wet amorphous STG phosphate.

[0222] The sample was dried at 50° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 57

[0223] STG phosphate (50 mg, crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was slurried in water (300 μL) at 25° C., then heated to 60° C. and was dissolved at that temperature.

[0224] Then methyl t-butyl ether (250 μL) was added and the solution was cooled in an ice water bath, and stirred for 2 hours. Crystallization occurred.

[0225] The product was isolated by vacuum filtration to obtain wet STG phosphate monohydrate.

Example 58

[0226] STG base Form I (100 mg) was dissolved in acetonitrile:water 1:1 (300 μL) at 25° C. Phosphoric acid (85%, 17 μL, 1 eq) was then added and the mixture was heated to 70° C., stirred at 70° C. for 2 hours, then cooled gradually to 25° C. and stirred at 25° C. for 16 hours. The product was isolated by evaporation to obtain wet STG phosphate crystalline monohydrate.

Example 59

[0227] Sitagliptin dihydrophosphate form V, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (0.03 g) was granulated with 0.006 ml

[0228] Iso-propanol:water 1:1 at 25° C., in a rotavapor for 9-12 hours. The wet material was analyzed by XRD and found to be Sitagliptin dihydrophosphate monohydrate.

Example 60

[0229] STG base Form I (500 mg) was slurried in acetonitrile (2.5 mL) at 25° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 25° C. for 35 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 61

[0230] STG base Form I (500 mg) was slurried in toluene (2.5 mL) at 25° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 25° C. for 12 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0231] The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 62

[0232] STG base Form I (500 mg) was slurried in acetonitrile (1 mL) at 70° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 70° C. for 10 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0233] The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 63

[0234] STG base Form I (500 mg) was slurried in diethyl carbonate (2.5 mL) at 25° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 25° C. for 10 minutes.

[0235] The product was isolated by vacuum filtration to obtain wet STG phosphate amorphous.

Example 64

[0236] STG base Form I (500 mg) was slurried in isobutyl acetate (2.5 mL) at 25° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 25° C. for 10 minutes.

[0237] The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 65

[0238] STG base Form I (500 mg) was slurried in n-butanol (2.5 mL) at 25° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 25° C. for 25 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

Example 66

[0239] STG base Form I (500 mg) was slurried in 1-propanol (2.5 mL) at 25° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 25° C. for 18 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 67

[0240] STG base Form I (500 mg) was dissolved in dimethyl carbonate (2.5 mL) at 74° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 74° C. for 13 minutes.

[0241] The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 68

[0242] STG base Form I (500 mg) was dissolved in diethyl carbonate (2.5 mL) at 74° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 74° C. for 20 minutes.

[0243] The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 69

[0244] STG base Form I (500 mg) was slurried in isobutyl acetate (2.5 mL) at 74° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 74° C. for 30 minutes.

[0245] The product was isolated by vacuum filtration to obtain wet STG phosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 70

[0246] STG base Form I (500 mg) was slurried in n-Butanol (2.5 mL) at 74° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 74° C. for 18 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate form II. The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 71

[0247] STG base Form I (500 mg) was slurried in 1-propanol (2.5 mL) at 74° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 74° C. for 23 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form II.

[0248] The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 72

[0249] STG base Form I (500 mg) was slurried in methyl isobutyl ketone (2.5 mL) at 74° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 74° C. for 25 minutes.

[0250] The product was isolated by vacuum filtration to obtain wet STG phosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0251] The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 73

[0252] STG base Form I (500 mg) was slurried in dimethyl carbonate (5.5 mL) at 50° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 50° C. for 8 minutes.

[0253] The product was isolated by vacuum filtration to obtain wet STG phosphate amorphous.

Example 74

[0254] STG base Form I (500 mg) was slurried in diethyl carbonate (10 mL) at 50° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 50° C. for 15 minutes.

[0255] The product was isolated by vacuum filtration to obtain wet STG phosphate amorphous.

Example 75

[0256] STG base Form I (500 mg) was slurried in n-butanol (3.5 mL) at 50° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 50° C. for 1.25 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 76

[0257] STG base Form I (500 mg) was slurried in 1-propanol (3.5 mL) at 50° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 50° C. for 1.25 hours. The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 77

[0258] STG base Form I (500 mg) was slurried in acetonitrile (1.5 mL) at 50° C. Phosphoric acid (85%, 83 μL, 1 eq) was then added and the mixture was stirred at 50° C. for 10 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 78

[0259] STG base Form I (500 mg) slurried in acetonitrile (1.5 mL) at 70° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in acetonitrile (1.5 mL) at 70° C. The mixture was stirred at 70° C. for 10 minutes.

[0260] The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 79

[0261] STG base Form I (500 mg) slurried in acetonitrile (1 mL) at 70° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in toluene (2.5 mL) at 70° C. The mixture was stirred at 70° C. for 15 minutes.

[0262] The product was isolated by vacuum filtration to obtain wet STG phosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

[0263] The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 80

[0264] STG base Form I (500 mg) slurried in 1-propanol (1.5 mL) at 72° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in 1-propanol (1.5 mL) at 70° C. The mixture was stirred at 70° C. for 15 minutes.

[0265] The product was isolated by vacuum filtration to obtain wet STG phosphate form II.

Example 81

[0266] STG base Form I (500 mg) slurried in acetonitrile (2.5 mL) at 25° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in n-Butanol (5 mL) at 25° C. The mixture was stirred at 25° C. for 15 minutes.

[0267] The product was isolated by vacuum filtration to obtain wet STG phosphate form II. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate

crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 82

[0268] STG base Form I (500 mg) slurried in acetonitrile (2.5 mL) at 50° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in n-Butanol (5 mL) at 50° C. The mixture was stirred at 50° C. for 35 minutes.

[0269] The product was isolated by vacuum filtration to obtain a mixture of wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta and form II. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 83

[0270] STG base Form I (500 mg) slurried in dimethyl carbonate (2.5 mL) at 50° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in n-Hexane (2.5 mL) at 50° C. The mixture was stirred at 50° C. for 10 minutes.

[0271] The product was isolated by vacuum filtration to obtain a mixture of wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta and form II. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 84

[0272] STG base Form I (500 mg) slurried in cyclohexanone (5 mL) at 25° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in methyl tert-butyl ether (1 mL) at 25° C. The mixture crystallized after 30 minutes and was stirred for 45 minutes at 25° C.

[0273] The product was isolated by vacuum filtration to obtain wet amorphous STG phosphate. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate crystalline form monohydrate.

Example 85

[0274] STG base Form I (500 mg) was added in portions to phosphoric acid (85%, 83 μL, 1 eq) in cyclopentyl methyl ether (5 mL) at 25° C. The mixture was stirred at 25° C. for 25 minutes. The product was isolated by vacuum filtration to obtain a mixture of STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta and form II. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 86

[0275] STG base Form I (500 mg) slurried in cyclohexanone (5 mL) at 25° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in methyl tert-butyl ether (1 mL) at 25° C. The mixture crystallized after 30 minutes and was stirred for 3 hours and 20 minutes at 25° C. The product was isolated by vacuum filtration to obtain wet amorphous STG phosphate. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate crystalline form monohydrate.

Example 87

[0276] STG base Form I (500 mg) slurried in cyclohexanone (5 mL) at 25° C. was added dropwise to phosphoric acid (85%, 83 μL, 1 eq) in methyl tert-butyl ether (1 mL) at 25° C. The mixture crystallized after 30 minutes and was stirred for 1 week at 25° C.

[0277] The product was isolated by vacuum filtration to obtain wet amorphous STG phosphate. The sample was dried in vacuum oven at 40° C. 16 hours to obtain STG phosphate crystalline form monohydrate.

Example 88

[0278] STG base Form I (5.6 g, 13.8 mmol) was dissolved in ethanol-water (18 ml-13 ml) at 50° C. To that solution, 85%-H_3PO_4 (0.92 ml, 13.8 mmol) was added at once with stirring. The solution was at 64-68° C. for an hour, and then the stirred solution was cooled to 25° C. for 40 min. The product was precipitated after additional stirring at 25° C. for 20 minutes. Ethanol (90 ml) was added to suspension, and the suspension was stirred at 25° C. for 18 hours. The solid was filtered, washed with ethanol (12 ml), dried at 50° C. under vacuum for 7 hours to give STG phosphate (6.0 g). The solid was analyzed by XRD and found to be STG phosphate Form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta. The STG phosphate Form V, characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (30 mg) was placed in a 50 ml- beaker. The opened beaker was kept in closed 100 ml-vessel containing 20 ml of methyl tert-butyl ether at 25° C. for 40 days. The solid was analyzed by XRD and found to be STG phosphate Form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta with higher crystallinity.

Example 89

[0279] STG base (500 mg) was slurried in butyl acetate (2.5 mL) at 25° C., and was added drop-wise to phosphoric acid (85%, 83 μL, 1 eq) in butyl acetate (3.5 mL) at 25° C. The mixture was stirred at 25° C. for 20 minutes. The product was isolated by vacuum filtration to obtain wet STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta. The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 90

[0280] STG base (800 mg) was dissolved in methanol (2 mL) at 25° C., and heated to 50° C. Phosphoric acid (85%, 131 μL, 1 eq) in methanol (1 mL) was then added drop-wise, and the mixture was stirred at 50° C. The solution formed a very thick slurry. Therefore, 9 ml methanol was added in portions, and then stirred at 50° C. for 1 hour and at 25° C. for 16 hours. The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form

characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 91

[0281] STG base (600 mg) was slurried in isopropanol (3 mL) at 25° C., and heated to 50° C. Phosphoric acid (85%, 100 μL, 1 eq) in isopropanol (1 mL) was then added dropwise, and the mixture was stirred at 50° C. for 16 hours. The sample was dried at 40° C. for 16 hours under reduced pressure to obtain STG phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 92:

[0282] A 100 mg of a mixture of Form II and crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta was kept under relative humidity of 100% for one day, to obtain pure crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, as presented in FIG. **18**.

Example 93

[0283] Sitagliptin phosphate (9 gr, a dry mixture of crystalline Form II and a form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was dried in fluidized bed dryer at 40° C. at 40% humidity for four hours to obtain Sitagliptin phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta (6.8 gr).

Example 94

[0284] Sitagliptin phosphate (1 gr, a dry mixture of crystalline Form II and a form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was dried in vacuum oven at 80° C. for 24 hours to obtain Sitagliptin phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 95

[0285] Sitagliptin phosphate (1 gr, a dry mixture of crystalline Form II and a form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta) was dried in vacuum oven at 100° C. for 24 hours to obtain Sitagliptin phosphate crystalline form characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta.

Example 96

[0286] Sitagliptin phosphate from characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta was stored under ethanol vapors at 25° C. for 18 hours. It was then analyzed by PXRD, and identified as form II of Sitagliptin phosphate.

Example 97

[0287] Sitagliptin phosphate from characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta was stored under methanol vapors at 25° C. for 1 week. It was then analyzed by PXRD, and identified as form II of Sitagliptin phosphate.

Example 98

[0288] Sitagliptin phosphate from characterized by a powder XRD pattern with peaks at 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta was stored under iso-propanol vapors at 25° C. for 1 week. It was then analyzed by PXRD, and identified as form II of Sitagliptin phosphate.

Example 99

[0289] To 1 g of amorphous Sitagliptin-phosphate (97.8% purity and 81.9% R) was added 50 ml of acetonitrile (ACN). The slurry was heated to reflux and stirred for 1 hour, then cooled to 2° C., and stirred for 1 hour. The product was isolated by vacuum filtration at 2° C., and washed with 2 ml of ACN, and dried at 50° C. in a vacuum oven for 15 hours to yield 0.88 g of Sitagliptin-phosphate (100% purity and 75.5% R) form VI (88% yield).

Example 100

[0290] To 5 g of oily STG-base (75.1 % R) was added 20 ml of isopropanol (IPA). The slurry was heated to 50° C., than H_3PO_4 85% (1.13 g in 10 ml IPA) was added dropwise and stirred for 1 hour. The slurry reaction was cooled to room temperature, and stirred for three days. The product was isolated by vacuum filtration, and washed with 20 ml of IPA to yield STG-Phosphate form VI, as a white-grey solid (99.5% purity and 74.7% R). Further purification accepted by adding 50 ml of ACN to the product. The slurry mixture was heated to reflux and stirred for 1 to 2 hours, than cooled to room temperature and stirred over night. Vacuum filtration followed by washings with 40 ml ACN yield a white-grey solid that was dried at 40° C. in a vacuum oven for 15 hours to yield 4.74 g of STG-Phosphate (99.7% purity and 78.0% R) form VI (95% yield).

Example 101

[0291] To degaussed 2,2,2-trifluoroethanol (TFE) (30 mL) were added Rhodium(I) chloride 1,5-cycloocatadiene complex (18.3 mg, 0.05%) and (R)-(−)-1-[(S)-2-diphenylphosphino)ferrocenyl]ethyl di-tert-butylphosphine (44.2 mg, 0.11%). The solution was stirred at room temperature, degaussed three times, and then stirred for one hour at room temperature.

[0292] To 250 ml hydrogenator were added (Z)-3-amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a] pyrazyn-7(8H)-yl)-4-(2,4,5-trifluorophenyl)but-2-en-1-one (30 gr, 1 equivalent) and TFE (120 ml) at room temperature and the mixture was washed three times with nitrogen gas. The catalyst solution was added and the clear solution was washed three times with nitrogen gas and then with hydrogen gas. The mixture remained under hydrogen at constant pressure of 5 bar and heated to 55° C. The mixture was stirred at 55° C. for 26 hours to obtain Sitagliptin base in TFE solution (optical purity by HPLC 76.9%, purity by HPLC 91.5%)

[0293] Two reaction mixtures which were obtained according to the above procedure were combined and the solution was divided to 10 parts.

[0294] 7 parts of the solution, each contained ca ~6 gr Sitagliptin were concentrated and Sitagliptin base was precipitated by addition of MTBE then filtrated by vacuum filtration. The combined mother liqueur from the crystallization

experiments was concentrated. The residue was dissolved in isopropanol (40 mL) at room temperature, heated to 50° C. A solution of phosphoric acid (85%, 1.7 mL, ca ~1 eq) in isopropanol (20 mL) was added and the mixture kept stirring at 50° C. for one hour, then cooled gradually to 25° C., and stirred at 25° C. over night.

[0295] The product was isolated by vacuum filtration and dried at 40° C. vacuum oven over night to obtain Sitagliptin phosphate crystalline form VI (optical purity by HPLC 51.8%, purity by HPLC 99.20%).

What is claimed:

1. A process for preparing a crystalline form of Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, the process selected from the group consisting of:

a. a process comprising combining Sitagliptin base, phosphoric acid, and a solvent selected from the group consisting of ethyl acetate, dioxane, methyl isobutyl ketone, isobutyl acetate, butyl acetate, a mixture of acetonitrile and toluene, or a mixture of tetrahydrofuran and water to form a slurry; and obtaining a Sitagliptin phosphate precipitate;

b. a process comprising combining Sitagliptin phosphate or Sitagliptin base and phosphoric acid with a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone:n-hexane, acetone:n-heptane, acetone:cyclopentyl methyl ether, acetone:dibutyl ether, acetone:isopropylacetate, dimethylsulfoxide:methyl isobutyl ketone, and dimethylsulfoxide:methyl tert butyl ether, forming a mixture, and crystallizing Sitagliptin phosphate from the mixture, wherein, when acetone:cyclopentyl methyl ether, acetone:isopropylacetate, and dimethylsulfoxide:methyl tert butyl ether, are used, the crystallized Sitagliptin phosphate is further dried;

c. a process comprising drying wet Sitagliptin phosphate Form II;

d. a process comprising heating a mixture of Sitagliptin phosphate Form II and the crystalline form characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, to a temperature of about 40° C. to about 100° C. under reduced pressure; and

e. a process comprising drying a mixture of Sitagliptin phosphate Form II and a crystalline form, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, in a fluidized bed dryer at a temperature of about 30° C. to about 60° C.

2. The process of claim 1, wherein Sitagliptin phosphate or Sitagliptin base and phosphoric acid are combined with the mixture of the first organic solvent and the second organic solvent.

3. The process of claim 2, wherein the first organic solvent and the second organic solvent ratio is about 1:1 to about 1:15.

4. The process of claim 2, wherein Sitagliptin phosphate is combined with the mixture of the first organic solvent and the second organic solvent.

5. The process of claim 2, wherein the solution is heated to a temperature of about 45° C. to about 80° C.

6. The process of claim 1, wherein the wet Sitagliptin phosphate Form II is dried.

7. The process of claim 6, wherein the wet Sitagliptin phosphate Form II is dried at about 40° C. to about 100° C. under reduced pressure.

8. The process of claim 6, wherein the Sitagliptin phosphate Form II is prepared in a process, comprising combining Sitagliptin base and phosphoric acid and an organic solvent selected from the group consisting of dimethyl carbonate, tetrahydrofuran, propylene glycol methyl ether, methyl ethyl ketone, ethanol, methyl acetate, dimethylformamide, diethyl carbonate, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, isopropanol, and a mixture of acetonitrile and n-butanol, acetonitrile, dimethyl carbonate to form a slurry, and obtaining Sitagliptin phosphate Form II.

9. The process of claim 6, wherein the Sitagliptin phosphate Form II is prepared in a process comprising combining Sitagliptin base and phosphoric acid and a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone:isopropylacetate, acetone: cyclohexane, acetone:isobutyl acetate, acetonitrile:n-butanol, and acetone:n-butanol, forming a mixture, and obtaining Sitagliptin phosphate Form II.

10. The process of claim 9, wherein the first organic solvent and the second organic solvent ratio is about 1:1 to about 1:15.

11. The process of claim 6, wherein the wet Sitagliptin phosphate Form II comprises a solvent selected from the group consisting of methyl isobutyl ketone, dimethyl carbonate, tetrahydrofuran, acetonitrile, propylene glycol methyl ether, methanol, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, butyl acetate, isopropanol, dimethyl carbonate, n-hexane, acetone, cyclohexane, isobutyl acetate, and mixtures thereof.

12. The process of claim 1, wherein the mixture of Sitagliptin phosphate Form II and the crystalline form characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta are heated to a temperature of about 40° C. to about 100° C. under reduced pressure.

13. The process of claim 12, wherein the mixture is heated at a temperature of about 40° C. to about 60° C.

14. The process of claim 12, wherein said mixture is heated for about 10 to about 24 hours.

15. The process of claim 1, wherein the mixture of Sitagliptin phosphate Form II and the crystalline form, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is dried in a fluidized bed dryer at a temperature of about 30° C. to about 60° C.

16. A process for preparing Sitagliptin phosphate Form II, the process selected from the group consisting of:

a. a process comprising providing a slurry of crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, and a solvent selected from the group consisting of acetonitrile, methanol, ethanol, 1-propanol, isopropanol, acetone, tetrahydrofuran, n-butanol, iso-butanol, toluene, propylene glycol, propylene glycol methyl ether, chloroform, diethyl carbonate, dimethylformamide, or mixtures of dimethylformamide with methyl isobutyl ketone, or n-butanol; heating the slurry; and obtaining Sitagliptin phosphate Form II;

b. a process comprising combining Sitagliptin base and phosphoric acid in an organic solvent selected from the group consisting of dimethyl carbonate, tetrahydrofuran, propylene glycol methyl ether, methyl ethyl ketone,

ethanol, methyl acetate, dimethylformamide, diethyl carbonate, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, isopropanol, a mixture of acetonitrile and n-butanol, acetonitrile, dimethyl carbonate, and a mixture of dimethyl carbonate and n-hexane, forming a slurry; and obtaining Sitagliptin phosphate Form II;

c. a process comprising combining Sitagliptin base and phosphoric acid and a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone:isopropylacetate, acetone:cyclohexane, acetone:isobutyl acetate, acetonitrile:n-butanol, and acetone:n-butanol, forming a mixture; and crystallizing Sitagliptin phosphate from the mixture, obtaining Sitagliptin phosphate Form II;

d. a process comprising dissolving Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, in dimethylsulfoxide; and adding an antisolvent selected from the group consisting of iso-butanol, acetonitrile, diethyl ether, diethyl carbonate, and tert-butyl ether;

e. a process comprising granulating a crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta in the presence of isopropanol; and

f. a process comprising exposing crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta to a C_1-C_4 alcohol, preferably the alcohol is selected from the group consisting of ethanol, methanol, and isopropanol.

17. The process of claim **16**, wherein the slurry is heated to about 50° C. to about 80° C.

18. The process of claim **16**, wherein the Sitagliptin base and phosphoric acid are combined in an organic solvent selected from the group consisting of dimethyl carbonate, tetrahydrofuran, propylene glycol methyl ether, methyl ethyl ketone, ethanol, methyl acetate, dimethylformamide, diethyl carbonate, n-butanol, 1-propanol, toluene, isobutyl acetate, isopropyl acetate, isopropanol, a mixture of acetonitrile and n-butanol, acetonitrile, dimethyl carbonate, and a mixture of dimethyl carbonate and n-hexane, forming a slurry.

19. The process of claim **16**, wherein the Sitagliptin base and phosphoric acid and a mixture of a first organic solvent and a second organic solvent selected from the group consisting of acetone:isopropylacetate, acetone:cyclohexane, acetone:isobutyl acetate, acetonitrile:n-butanol, and acetone:n-butanol are combined forming a mixture; and Sitagliptin phosphate is crystallized from the mixture.

20. The process of claim **19**, wherein the first organic solvent and the second organic solvent ratio is about 1:1 to about 1:15.

21. The process of claim **16**, wherein the Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is dissolved in dimethylsulfoxide and an antisolvent selected from the group consisting of iso-butanol, acetonitrile, diethyl ether, diethyl carbonate, and tert-butyl ether is added.

22. The process of claim **16**, wherein crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is granulated in the presence of isopropanol.

23. The process of claim **22**, wherein the solvent/antisolvent ration is about 1:1 to about 1:20.

24. The process of claim **22**, wherein the solvent/antisolvent ration is about 3:10.

25. The process of claim **16**, wherein crystalline Sitagliptin phosphate, characterized by a powder XRD pattern with peaks at about 4.7, 13.5, 17.7, 18.3, and 23.7±0.2 degrees two theta, is exposed to a C_1-C_4 alcohol.

26. The process of claim **25**, wherein the alcohol is selected from the group consisting of ethanol, methanol, and isopropanol.

* * * * *

EXHIBIT G

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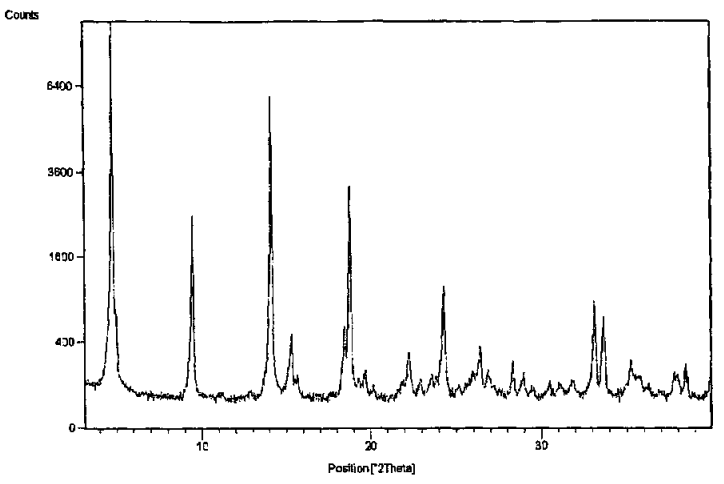
(54) Title: NOVEL CRYSTALLINE FORMS OF A PHOSPHORIC ACID SALT OF A DIPEPTIDYL PEPTIDASE-IV INHIBITOR



(57) Abstract: The present invention relates to crystalline anhydrate polymorphs of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as well as a process for their preparation, pharmaceutical compositions containing these novel forms, and methods of use of the novel forms and pharmaceutical compositions for the treatment of diabetes, obesity, and high blood pressure. The invention also concerns novel crystalline solvates of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as well as a crystalline desolvated polymorph and their use for the preparation of the anhydrate polymorphs of the present invention.

WO 2005/020920 A2

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TITLE OF THE INVENTION

NOVEL CRYSTALLINE FORMS OF A PHOSPHORIC ACID SALT OF A DIPEPTIDYL PEPTIDASE-IV INHIBITOR

FIELD OF THE INVENTION

The present invention relates to novel crystalline forms of a dihydrogenphosphate salt of a dipeptidyl peptidase-IV inhibitor. More particularly, the invention relates to novel crystalline solvates and anhydrates of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, which is a potent inhibitor of dipeptidyl peptidase-IV (DPP-IV). These novel crystalline forms of the DPP-IV inhibitor are useful for the preparation of pharmaceutical compositions containing the inhibitor which are useful for the treatment and prevention of diseases and conditions for which an inhibitor of dipeptidyl peptidase-IV is indicated, in particular Type 2 diabetes, hyperglycemia, insulin resistance, obesity, and high blood pressure. The invention further concerns pharmaceutical compositions comprising the novel crystalline dihydrogenphosphate salt anhydrate polymorphic forms of the present invention; processes for preparing the dihydrogenphosphate salt solvates and anhydrates and their pharmaceutical compositions; and methods of treating conditions for which a DPP-IV inhibitor is indicated comprising administering a composition of the present invention.

BACKGROUND OF THE INVENTION

Inhibition of dipeptidyl peptidase-IV (DPP-IV), an enzyme that inactivates both glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1), represents a novel approach to the treatment and prevention of Type 2 diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM). The therapeutic potential of DPP-IV inhibitors for the treatment of Type 2 diabetes has been reviewed: C. F. Deacon and J.J. Holst, "Dipeptidyl peptidase IV inhibition as an approach to the treatment and prevention of Type 2 diabetes: a historical perspective," Biochem. Biophys. Res. Commun., 294: 1-4 (2000); K. Augustyns, et al., "Dipeptidyl peptidase IV inhibitors as new therapeutic agents for the treatment of Type 2 diabetes," Exp. Opin. Ther. Patents, 13: 499-510 (2003); and D.J. Drucker, "Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of Type 2 diabetes," Exp. Opin. Investig. Drugs, 12: 87-100 (2003).

WO 03/004498 (published 16 January 2003) and U.S. Patent No. 6,699,871 (issued March 2, 2004), both assigned to Merck & Co., describe a class of beta-amino tetrahydrotriazolo[4,3-*a*]pyrazines, which are potent inhibitors of DPP-IV and therefore useful for the treatment of Type 2 diabetes. Specifically disclosed in WO 03/004498 is (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

However, there is no disclosure in the above references of the newly discovered crystalline solvates and anhydrates of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I below (hereinafter referred to as Compound I).

SUMMARY OF THE INVENTION

The present invention is concerned with novel crystalline solvates and anhydrates of the dihydrogenphosphate salt of the dipeptidyl peptidase-IV (DPP-IV) inhibitor (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I (Compound I). The crystalline solvates and anhydrates of the present invention have advantages in the preparation of pharmaceutical compositions of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, such as ease of processing, handling, and dosing. In particular, they exhibit improved physicochemical properties, such as solubility, stability to stress, and rate of dissolution, rendering them particularly suitable for the manufacture of various pharmaceutical dosage forms. The invention also concerns pharmaceutical compositions containing the novel anhydrate polymorphs; processes for the preparation of these solvates and anhydrates and their pharmaceutical compositions; and methods for using them for the prevention or treatment of Type 2 diabetes, hyperglycemia, insulin resistance, obesity, and high blood pressure.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a characteristic X-ray diffraction pattern of the crystalline anhydrate Form I of Compound I.

FIG. 2 is a carbon-13 cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectrum of the crystalline anhydrate Form I of Compound I.

FIG. 3 is a fluorine-19 magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectrum of the crystalline anhydrate Form I of Compound I.

FIG. 4 is a typical DSC curve of the crystalline anhydrate Form I of Compound I.

FIG. 5 is a typical thermogravimetric (TG) curve of the crystalline anhydrate Form I of Compound I.

FIG. 6 is a characteristic X-ray diffraction pattern of the crystalline desolvated anhydrate Form II of Compound I.

FIG. 7 is a carbon-13 cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectrum of the crystalline desolvated anhydrate Form II of Compound I.

FIG. 8 is a fluorine-19 magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectrum of the crystalline desolvated anhydrate Form II of Compound I.

FIG. 9 is a typical DSC curve of the crystalline desolvated anhydrate Form II of Compound I.

FIG. 10 is a typical TG curve of the crystalline desolvated anhydrate Form II of Compound I.

FIG. 11 is a characteristic X-ray diffraction pattern of the crystalline anhydrate Form III of Compound I.

FIG. 12 is a carbon-13 cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectrum of the crystalline anhydrate Form III of Compound I.

FIG. 13 is a fluorine-19 magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectrum of the crystalline anhydrate Form III of Compound I.

FIG. 14 is a typical DSC curve of the crystalline anhydrate Form III of Compound I.

FIG. 15 is a typical TG curve of the crystalline anhydrate Form III of Compound I.

FIG. 16 is a characteristic X-ray diffraction pattern of the crystalline ethanol solvate of Compound I.

FIG. 17 is a carbon-13 cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectrum of the crystalline ethanol solvate of Compound I.

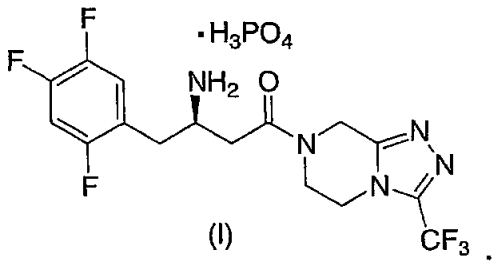
FIG. 18 is a fluorine-19 magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectrum of the crystalline ethanol solvate of Compound I.

FIG. 19 is a typical DSC curve of the crystalline ethanol solvate of Compound I.

FIG. 20 is a typical TG curve of the crystalline ethanol solvate of Compound I.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides novel crystalline solvates and anhydrates of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I (Compound I):



(I)

In one embodiment the solvate is a C_{1-4} alkanolate of Compound I. In a class of this embodiment the C_{1-4} alkanolate is a methanolate, ethanolate, 1-propanolate, or 2-propanolate. In another embodiment the solvate comprises an organic solvent such as acetone or acetonitrile. The crystalline solvates are useful for the preparation of the crystalline desolvated anhydrate Form II which converts spontaneously into crystalline anhydrate Form I or Form III or a mixture thereof, the composition of the mixture being dependent upon the conditions of treatment or storage. Anhydrate Forms I and III represent stable desolvated anhydrates of Compound I.

The present invention also provides a novel crystalline desolvated anhydrate Form II of Compound I which is obtained from the crystalline solvates of Compound I of the present invention.

The present invention also provides novel crystalline anhydrate Forms I and III of Compound I and mixtures thereof.

A further embodiment of the present invention provides the Compound I drug substance that comprises the crystalline anhydrate Form I or III or a mixture thereof in a detectable amount. By "drug substance" is meant the active pharmaceutical ingredient (API). The amount of crystalline anhydrate Form I or III or mixture thereof in the drug substance can be quantified by the use of physical methods such as X-ray powder diffraction (XRPD), solid-state fluorine-19 magic-angle spinning (MAS) nuclear magnetic resonance spectroscopy, solid-state carbon-13 cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance spectroscopy, solid state Fourier-transform infrared spectroscopy, and Raman spectroscopy. In a class of this embodiment, about 5% to about 100% by weight of the crystalline anhydrate Form I or III or mixture thereof is present in the drug substance. In a second class of this embodiment, about 10% to about 100% by weight of the crystalline anhydrate Form I or III or mixture thereof is present in the drug substance. In a third class of this embodiment, about 25% to about 100% by weight of the crystalline anhydrate Form I or III or mixture thereof is present in the drug substance. In a fourth class of this embodiment, about 50% to about 100% by weight of the crystalline anhydrate Form I or III or mixture thereof is present in the drug substance. In a fifth class of this embodiment, about 75% to about 100% by weight of the crystalline anhydrate Form I or III or mixture thereof is present in the drug substance. In a sixth class of this embodiment, substantially all of the Compound I drug substance is the crystalline anhydrate Form I or III or mixture thereof, i.e., the Compound I drug substance is substantially phase pure anhydrate Form I or III or a mixture thereof.

The crystalline solvates of the present invention are useful for the preparation of the crystalline anhydrate Forms I and III and mixtures thereof. The crystalline solvates are desolvated to afford the intermediate desolvated anhydrate Form II which converts into anhydrate Form I or Form III or a mixture thereof upon heating at 45°C for about 2 h.

Another aspect of the present invention provides a method for the prevention or treatment of clinical conditions for which an inhibitor of DPP-IV is indicated, which method comprises

administering to a patient in need of such prevention or treatment a prophylactically or therapeutically effective amount of the crystalline anhydrate Form I or III or a mixture thereof of Compound I. Such clinical conditions include diabetes, in particular Type 2 diabetes, hyperglycemia, insulin resistance, obesity, and high blood pressure.

The present invention also provides for the use of the crystalline anhydrate Form I or III or a mixture thereof of the present invention in the manufacture of a medicament for the prevention or treatment of clinical conditions for which an inhibitor of DPP-IV is indicated, in particular, Type 2 diabetes, hyperglycemia, insulin resistance, obesity, and high blood pressure. In one embodiment the clinical condition is Type 2 diabetes.

Another aspect of the present invention provides the crystalline anhydrate Form I or Form III or a mixture thereof for use in the treatment of clinical conditions for which an inhibitor of DPP-IV is indicated, in particular, Type 2 diabetes, hyperglycemia, insulin resistance, obesity, and high blood pressure. In one embodiment of this aspect the clinical condition is Type 2 diabetes.

The present invention also provides pharmaceutical compositions comprising the crystalline anhydrate Form I or III or a mixture thereof, in association with one or more pharmaceutically acceptable carriers or excipients. In one embodiment the pharmaceutical composition comprises a prophylactically or therapeutically effective amount of the active pharmaceutical ingredient (API) in admixture with pharmaceutically acceptable excipients wherein the API comprises a detectable amount of the crystalline anhydrate Form I or III or a mixture thereof of the present invention. In a second embodiment the pharmaceutical composition comprises a prophylactically or therapeutically effective amount of the API in admixture with pharmaceutically acceptable excipients wherein the API comprises about 5% to about 100% by weight of the crystalline anhydrate Form I or III or a mixture thereof of the present invention. In a class of this second embodiment, the API in such compositions comprises about 10% to about 100% by weight of the crystalline anhydrate Form I or III or a mixture thereof. In a second class of this embodiment, the API in such compositions comprises about 25% to about 100% by weight of the crystalline anhydrate Form I or III or a mixture thereof. In a third class of this embodiment, the API in such compositions comprises about 50% to about 100% by weight of the crystalline anhydrate Form I or III or a mixture thereof. In a fourth class of this embodiment, the API in such compositions comprises about 75% to about 100% by weight of the crystalline anhydrate Form I or III or a mixture thereof. In a fifth class of this embodiment, substantially all of the API is the crystalline anhydrate Form I or III or a mixture thereof of Compound I, i.e., the API is substantially phase pure Compound I anhydrate Form I or III or a mixture thereof.

The compositions in accordance with the invention are suitably in unit dosage forms such as tablets, pills, capsules, powders, granules, sterile solutions or suspensions, metered aerosol or liquid sprays, drops, ampoules, auto-injector devices or suppositories. The compositions are intended for

oral, parenteral, intranasal, sublingual, or rectal administration, or for administration by inhalation or insufflation. Formulation of the compositions according to the invention can conveniently be effected by methods known from the art, for example, as described in Remington's Pharmaceutical Sciences, 17th ed., 1995.

The dosage regimen is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; and the renal and hepatic function of the patient. An ordinarily skilled physician, veterinarian, or clinician can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

Oral dosages of the present invention, when used for the indicated effects, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day, preferably 0.01 to 10 mg/kg/day, and most preferably 0.1 to 5.0 mg/kg/day. For oral administration, the compositions are preferably provided in the form of tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100 and 500 milligrams of the API for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the API, preferably, from about 1 mg to about 200 mg of API. Intravenously, the most preferred doses will range from about 0.1 to about 10 mg/kg/minute during a constant rate infusion. Advantageously, the crystalline anhydrate forms of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, the crystalline anhydrate forms of the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

In the methods of the present invention, the Compound I anhydrate Forms I and III or a mixture thereof herein described in detail can form the API, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as 'carrier' materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active pharmaceutical ingredient can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral API can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants,

disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

The crystalline anhydrate Forms I and III or mixtures thereof of Compound I have been found to possess a high solubility in water, rendering them especially amenable to the preparation of formulations, in particular intranasal and intravenous formulations, which require relatively concentrated aqueous solutions of the API. The solubility of the crystalline Compound I anhydrate Form I or Form III or mixture thereof in water is greater than 120 mg/mL.

In a still further aspect, the present invention provides a method for the treatment and/or prevention of clinical conditions for which a DPP-IV inhibitor is indicated, which method comprises administering to a patient in need of such prevention or treatment a prophylactically or therapeutically effective amount of anhydrate Form I or III or a mixture thereof of the present invention or a pharmaceutical composition containing a prophylactically or therapeutically effective amount of anhydrate Form I or III or a mixture thereof.

The following non-limiting Examples are intended to illustrate the present invention and should not be construed as being limitations on the scope or spirit of the instant invention.

Compounds described herein may exist as tautomers such as keto-enol tautomers. The individual tautomers as well as mixtures thereof are encompassed with compounds of structural formula I.

The term "% enantiomeric excess" (abbreviated "ee") shall mean the % major enantiomer less the % minor enantiomer. Thus, a 70% enantiomeric excess corresponds to formation of 85% of one enantiomer and 15% of the other. The term "enantiomeric excess" is synonymous with the term "optical purity."

GENERAL METHODS FOR PREPARING SOLVATES OF COMPOUND I AND THE DESOLVATED ANHYDRATE FORM II AND FOR PREPARING AND INTERCONVERTING BETWEEN ANHYDRATE FORMS I AND III:

Compound I forms non-stoichiometric, isomorphous solvates with several organic solvents, such as methanol, ethanol, 1-propanol, 2-propanol, acetone, and acetonitrile. The various solvates of the present invention are isomorphic and exhibit similar X-ray powder diffraction patterns, F-19 solid-state NMR spectra, and DSC curves.

Solvates are prepared by contacting anhydrate Form I, II, or III, or mixtures thereof, with the solvating agent for about 5 min at about room temperature. Solvates will also result from the process of preparing the dihydrogenphosphate salt from free base in the presence of a solvating agent where the water activity is such that the solvate has a lower solubility than any of the other anhydrates or monohydrate. For example, the ethanol solvate can be formed by treating the free base with aqueous phosphoric acid in ethanol.

The ethanol solvate can be converted to desolvated anhydrate Form II by (a) drying with nitrogen flow over the sample for about 5 h at about 25 °C or (b) drying in vacuum for about 5 h at about 25 °C.

Desolvated anhydrate Form II is metastable and converts to anhydrate Form I or Form III or mixtures thereof in about 2 h at about 45 °C.

Anhydrate Form I can be converted into anhydrate Form III by (a) drying with physical agitation, (b) compaction, or (c) grinding. Anhydrate Form III can be converted into anhydrate Form I by heating at about 110 °C for about 30 min.

Mixtures of varying composition of anhydrate Forms I and III form upon grinding or compaction of Form I or mixtures thereof at room temperature, which results in the increased proportion of Form III in the mixture.

The anhydrate polymorphic Form I and Form III have an enantiotropic relationship, that is, one form is more stable at a lower temperature range, while the other is more stable at a higher temperature with a transition temperature of about 34 °C. Anhydrate Form III is the low temperature stable form and is stable below about 34 °C. Anhydrate Form I is the high temperature stable form and is stable above about 34 °C.

The anhydrate Forms I and III can be directly crystallized from a solvent that Compound I does not solvate with, such as isoamyl alcohol, at a water activity where the hydrate is not stable. Form III can be preferentially crystallized below about 34 °C, and Form I can be preferentially crystallized above about 34 °C.

GENERAL CONDITIONS FOR PREFERENTIALLY CRYSTALLIZING ANHYDRATE FORM I:

In isoamyl alcohol (IAA)/water system at 40 °C:

(1) crystallization from a mixture of compound I in IAA and water, such that the water concentration is below 3.4 weight percent;

(2) recovering the resultant solid phase; and

(3) removing the solvent therefrom.

In IAA/water system at 60°C:

(1) crystallization from a mixture of compound I in IAA and water, such that the water concentration is below 4.5 weight percent;

(2) recovering the resultant solid phase; and

(3) removing the solvent therefrom.

GENERAL CONDITIONS FOR PREFERENTIALLY CRYSTALLIZING ANHYDRATE FORM III:

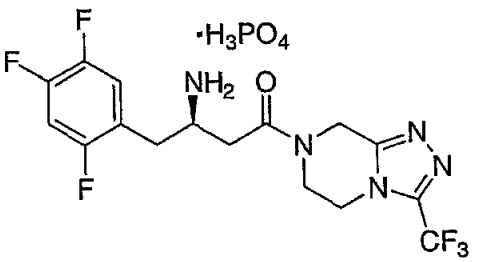
In isoamyl alcohol (IAA)/water system at 25°C:

(1) crystallization from a mixture of compound I in IAA and water, such that the water concentration is below 2.7 weight percent;

(2) recovering the resultant solid phase; and

(3) removing the solvent therefrom.

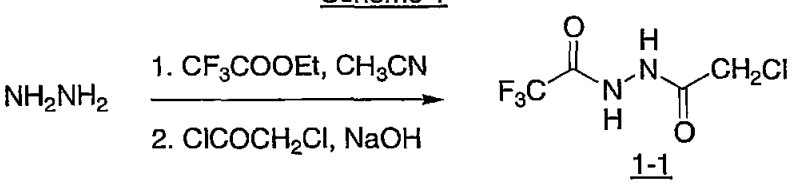
EXAMPLE 1



(2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dihydrogenphosphate anhydrate Form I and Form III mixture

Preparation of 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazine hydrochloride (1-4)

Scheme 1



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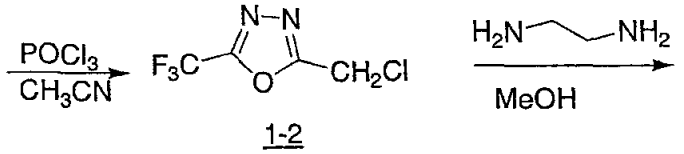

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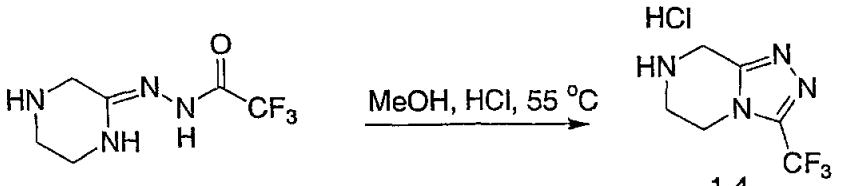


1-3 1-4

Step A: Preparation of bishydrazide (1-1)

Hydrazine (20.1 g, 35 wt% in water, 0.22 mol) was mixed with 310 mL of acetonitrile. 31.5 g of ethyl trifluoroacetate (0.22 mol) was added over 60 min. The internal temperature was increased to 25 °C from 14 °C. The resulting solution was aged at 22 - 25 °C for 60 min. The solution was cooled to 7 °C. 17.9 g of 50 wt% aqueous NaOH (0.22 mol) and 25.3 g of chloroacetyl chloride (0.22 mol) were added simultaneously over 130 min at a temperature below 16 °C. When the reaction was complete, the mixture was vacuum distilled to remove water and ethanol at 27 ~ 30 °C and under 26 ~ 27 in Hg vacuum. During the distillation, 720 mL of acetonitrile was added slowly to maintain constant volume (approximately 500 mL). The slurry was filtered to remove sodium chloride. The cake was rinsed with about 100 mL of acetonitrile. Removal of the solvent afforded bis-hydrazide 1-1 (43.2 g, 96.5% yield, 94.4 area% pure by HPLC assay).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.2 (s, 2H), 10.7 (s, 1H), and 11.6 (s, 1H) ppm.

^{13}C-NMR (100 MHz, DMSO-d_6): δ 41.0, 116.1 (q, J = 362 Hz), 155.8 (q, J = 50 Hz), and 165.4 ppm.

Step B: Preparation of 5-(trifluoromethyl)-2-(chloromethyl)-1,3,4-oxadiazole (1-2)

Bishydrazide 1-1 from Step A (43.2 g, 0.21 mol) in ACN (82 mL) was cooled to 5 °C. Phosphorus oxychloride (32.2 g, 0.21 mol) was added, maintaining the temperature below 10 °C. The mixture was heated to 80 °C and aged at this temperature for 24 h until HPLC showed less than 2 area% of 1-1. In a separate vessel, 260 mL of IPAc and 250 mL of water were mixed and cooled to 0 °C. The reaction slurry was charged to the quench keeping the internal temperature below 10 °C. After the addition, the mixture was agitated vigorously for 30 min, the temperature was increased to room temperature and the aqueous layer was cut. The organic layer was then washed with 215 mL of water, 215 mL of 5 wt% aqueous sodium bicarbonate and finally 215 mL of 20 wt% aqueous brine solution. HPLC assay yield after work up was 86-92%. Volatiles were removed by distillation at 75-80 mm Hg,

55 °C to afford an oil which could be used directly in Step C without further purification. Otherwise the product can be purified by distillation to afford 1-2 in 70-80% yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.8 (s, 2H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ 32.1, 115.8 (q, J = 337 Hz), 156.2 (q, J = 50 Hz), and 164.4 ppm.

Step C: Preparation of N-[(2Z)-piperazin-2-ylidene]trifluoroacetohydrazide (1-3)

To a solution of ethylenediamine (33.1 g, 0.55 mol) in methanol (150 mL) cooled at –20 °C was added distilled oxadiazole 1-2 from Step B (29.8 g, 0.16 mol) while keeping the internal temperature at –20 °C. After the addition was complete, the resulting slurry was aged at –20 °C for 1 h. Ethanol (225 mL) was then charged and the slurry slowly warmed to –5 °C. After 60 min at –5 °C, the slurry was filtered and washed with ethanol (60 mL) at –5 °C. Amidine 1-3 was obtained as a white solid in 72% yield (24.4 g, 99.5 area wt% pure by HPLC).

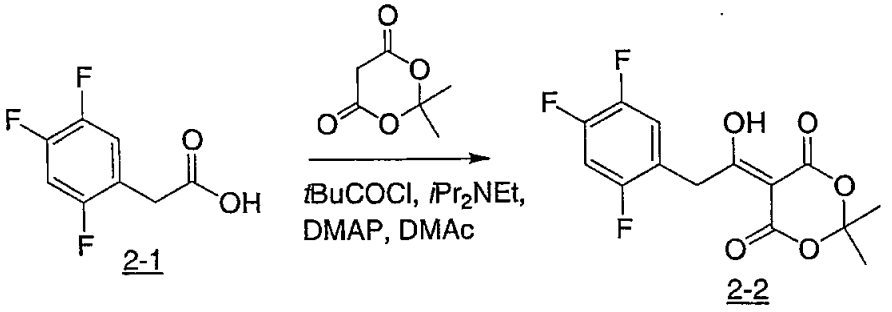
^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.9 (t, 2H), 3.2 (t, 2H), 3.6 (s, 2H), and 8.3 (b, 1H) ppm. ^{13}C-NMR (100 MHz, DMSO-d_6): δ 40.8, 42.0, 43.3, 119.3 (q, J = 350 Hz), 154.2, and 156.2 (q, J = 38 Hz) ppm.

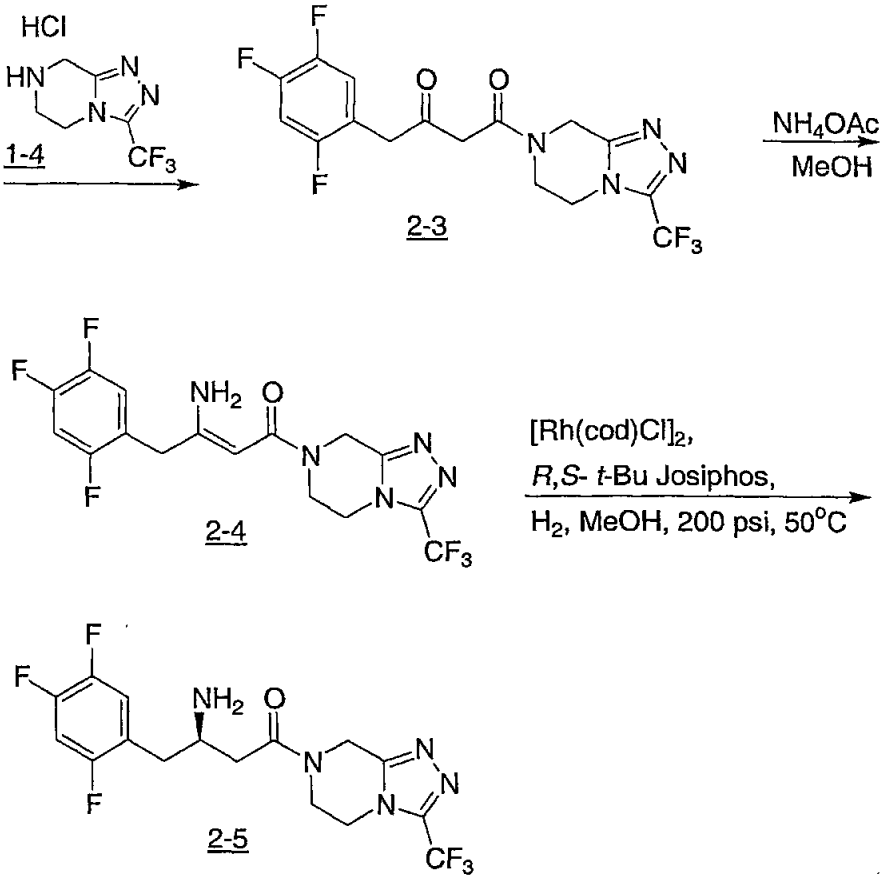
Step D: Preparation of 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazine hydrochloride (1-4)

A suspension of amidine 1-3 (27.3 g, 0.13 mol) in 110 mL of methanol was warmed to 55 °C. 37% Hydrochloric acid (11.2 mL, 0.14 mol) was added over 15 min at this temperature. During the addition, all solids dissolved resulting in a clear solution. The reaction was aged for 30 min. The solution was cooled down to 20 °C and aged at this temperature until a seed bed formed (10 min to 1 h). 300 mL of MTBE was charged at 20 °C over 1 h. The resulting slurry was cooled to 2 °C, aged for 30 min and filtered. Solids were washed with 50 mL of ethanol:MTBE (1:3) and dried under vacuum at 45 °C. Yield of triazole 1-4 was 26.7 g (99.5 area wt% pure by HPLC).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.6 (t, 2H), 4.4 (t, 2H), 4.6 (s, 2H), and 10.6 (b, 2H) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6): δ: 39.4, 39.6, 41.0, 118.6 (q, J = 325 Hz), 142.9 (q, J = 50 Hz), and 148.8 ppm.

Scheme 2





Step A: Preparation of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one (2-3)

2,4,5-Trifluorophenylacetic acid (2-1) (150 g, 0.789 mol), Meldrum's acid (125 g, 0.868 mol), and 4-(dimethylamino)pyridine (DMAP) (7.7 g, 0063 mol) were charged into a 5 L three-neck flask. *N,N*-Dimethylacetamide (DMAc) (525 mL) was added in one portion at room temperature to dissolve the solids. *N,N*-diisopropylethylamine (282 mL, 1.62 mol) was added in one portion at room temperature while maintaining the temperature below 40 °C. Pivaloyl chloride (107 mL, 0.868 mol) was added dropwise over 1 to 2 h while maintaining the temperature between 0 and 5 °C. The reaction mixture was aged at 5 °C for 1 h. Triazole hydrochloride 1-4 (180 g, 0.789 mol) was added in one portion at 40-50 °C. The reaction solution was aged at 70 °C for several h. 5% Aqueous sodium hydrogencarbonate solution (625 mL) was then added dropwise at 20 – 45 °C. The batch was seeded and aged at 20 – 30 °C for 1-2 h. Then an additional 525 mL of 5% aqueous sodium hydrogencarbonate solution was added dropwise over 2-3 h. After aging several h at room temperature, the slurry was cooled to 0 – 5 °C and aged 1 h before filtering the solid. The wet cake was displacement-washed with

20% aqueous DMAc (300 mL), followed by an additional two batches of 20% aqueous DMAc (400 mL), and finally water (400 mL). The cake was suction-dried at room temperature. The isolated yield of final product 2-3 was 89%.

Step B: Preparation of (2*Z*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)but-2-en-2-amine (2-4)

A 5 L round-bottom flask was charged with methanol (100 mL), the ketoamide 2-3 (200 g), and ammonium acetate (110.4 g). Methanol (180 mL) and 28% aqueous ammonium hydroxide (58.6 mL) were then added keeping the temperature below 30 °C during the addition. Additional methanol (100 mL) was added to the reaction mixture. The mixture was heated at reflux temperature and aged for 2 h. The reaction was cooled to room temperature and then to about 5 °C in an ice-bath. After 30 min, the solid was filtered and dried to afford 2-4 as a solid (180 g); m.p. 271.2 °C.

Step C: Preparation of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2-5)

Into a 500 ml flask were charged chloro(1,5-cyclooctadiene)rhodium(I) dimer {[Rh(cod)Cl]$_2$}(292 mg, 1.18 mmol) and (*R,S*) *t*-butyl Josiphos (708 mg, 1.3 mmol) under a nitrogen atmosphere. Degassed MeOH was then added (200 mL) and the mixture was stirred at room temperature for 1 h. Into a 4 L hydrogenator was charged the enamine amide 2-4 (118 g, 0.29 mol) along with MeOH (1 L). The slurry was degassed. The catalyst solution was then transferred to the hydrogenator under nitrogen. After degassing three times, the enamine amide was hydrogenated under 200 psi hydrogen gas at 50 °C for 13 h. Assay yield was determined by HPLC to be 93% and optical purity to be 94% ee.

The optical purity was further enhanced in the following manner. The methanol solution from the hydrogenation reaction (18 g in 180 mL MeOH) was concentrated and switched to methyl *t*-butyl ether (MTBE) (45 mL). Into this solution was added aqueous H_3PO_4 solution (0.5 M, 95 mL). After separation of the layers, 3*N* NaOH (35 mL) was added to the water layer, which was then extracted with MTBE (180 mL + 100 mL). The MTBE solution was concentrated and solvent switched to hot toluene (180 mL, about 75 °C). The hot toluene solution was then allowed to cool to 0 °C slowly (5 – 10 h). The crystals were isolated by filtration (13 g, yield 72%, 98 – 99% ee); m.p. 114.1 – 115.7 °C.

^{1}H NMR (300 MHz, CD_3CN): δ 7.26 (m), 7.08 (m), 4.90 (s), 4.89 (s), 4.14 (m), 3.95 (m), 3.40 (m), 2.68 (m), 2.49 (m), 1.40 (bs).

Compound 2-5 exists as amide bond rotamers. Unless indicated, the major and minor rotamers are grouped together since the carbon-13 signals are not well resolved:

^{13}C NMR (CD_3CN): δ 171.8, 157.4 (ddd , J_{CF} = 242.4, 9.2, 2.5 Hz), 152.2 (major), 151.8 (minor), 149.3 (ddd; J_{CF} = 246.7, 14.2, 12.9 Hz), 147.4 (ddd, J_{CF} = 241.2, 12.3, 3.7 Hz), 144.2 (q, J_{CF} = 38.8 Hz), 124.6

(ddd , J_{CF} = 18.5, 5.9, 4.0 Hz), 120.4 (dd , J_{CF} = 19.1, 6.2 Hz), 119.8 (q, J_{CF} = 268.9 Hz), 106.2 (dd , J_{CF} = 29.5, 20.9 Hz), 50.1, 44.8, 44.3 (minor), 43.2 (minor), 42.4, 41.6 (minor), 41.4, 39.6, 38.5 (minor), 36.9.

The crystalline free base 2-5 can also be isolated as follows:

(a) The reaction mixture upon completion of the hydrogenation step is charged with 25 wt% of Ecosorb C-941. The mixture is stirred under nitrogen for one h and then filtered. The cake is washed with 2L/kg of methanol. Recovery of free base is about 95% and optical purity about 95% ee.

(b) The freebase solution in methanol is concentrated to 3.5-4.0 L/kg volume (based on free base charge) and then solvent-switched into isopropanol (IPA) to final volume of 3.0 L/kg IPA.

(c) The slurry is heated to 40 °C and aged 1 h at 40°C and then cooled to 25 °C over 2 h.

(d) Heptane (7L/kg) is charged over 7 h and the slurry stirred for 12 h at 22-25°C. The supernatant concentration before filtering is 10-12 mg/g.

(e) The slurry is filtered and the solid washed with 30% IPA/heptane (2L/kg).

(f) The solid is dried in a vacuum oven at 40 °C.

(g) The optical purity of the free base is about 99% ee.

The following high-performance liquid chromatographic (HPLC) conditions were used to determine percent conversion to product:

Column:	Waters Symmetry C18, 250 mm x 4.6 mm
Eluent:	Solvent A: 0.1 vol% $HClO_4/H_2O$
	Solvent B: acetonitrile
Gradient:	0 min 75% A : 25% B
	10 min 25% A : 75% B
	12.5 min 25% A : 75% B
	15 min 75% A : 25% B
Flow rate:	1 mL/min
Injection Vol.:	10 µL
UV detection:	210 nm
Column temp.:	40 °C
Retention times:	compound 2-4: 9.1 min
	compound 2-5: 5.4 min
	*t*Bu Josiphos: 8.7 min

The following high-performance liquid chromatographic (HPLC) conditions were used to determine optical purity:

Column: Chirapak, AD-H, 250 mm x 4.6 mm
Eluent: Solvent A: 0.2 vol.% diethylamine in heptane
Solvent B: 0.1 vol% diethylamine in ethanol
Isochratic Run Time: 18 min
Flow rate: 0.7 mL/min
Injection Vol.: 7 µL
UV detection: 268 nm
Column temp.: 35 °C
Retention times: (*R*)-amine 2-5: 13.8 min
(*S*)-amine 2-5: 11.2 min

Preparation of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dihydrogenphosphate anhydrate Form I and III mixture

A 250 mL round bottom flask equipped with an overhead stirrer, heating mantle and thermocouple, was charged with 60 mL of ethanol, 19 mL water, 15.0 g (36.9 mmol) of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine freebase, and 4.25 g (36.9 mmol) of 85% aqueous phosphoric acid. The mixture was heated to 75 to 78 °C. A thick white precipitate formed at lower temperatures but dissolved upon reaching 75 °C. The solution was cooled to 68 °C and then held at that temperature for 4-8 h. A slurry bed of solids of ethanol solvate formed during this age time. The slurry was then cooled at a rate of 4 °C/h to 21 °C and then held overnight. 70 mL of ethanol was then added to the slurry of ethanol solvate. After 1 h the slurry of ethanol solvate was filtered and washed with 45 mL ethanol. The solids were dried in a vacuum oven at 40 °C for 18 h. 17.1 g of solids that were a mixture of Form I and Form III were recovered. The solids were found to greater than 99.8% pure by HPLC area percentage (HPLC conditions same as those given above). The crystal form of the solids was shown to be a mixture of anhydrate Forms I and III by X-ray powder diffraction and solid state NMR spectroscopy, with Form I predominating.

EXAMPLE 2

(2*R*)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine freebase 2-5 in isoamyl alcohol solution (~200 mg/g) was added to the crystallizer. A seed was then added, followed by isoamyl alcohol and water to constitute a 96% isoamyl alcohol and 4% water mixture. The mixture was first aged, and then heated up to about 50 °C. About 1 equivalent of phosphoric acid in 96% isoamyl alcohol and 4% water (to achieve a final batch concentration of 85 mg/g) was then added to the slurry to crystallize the anhydrate Form I. The slurry

was aged and then cooled to room temperature. The solids were filtered and washed with isoamyl alcohol. The wet solids were dried at 75-80 °C. The crystal form of the solids was shown to be a mixture of anhydrate Forms I and III by X-ray powder diffraction and solid state NMR spectroscopy, with Form I predominating.

X-ray powder diffraction studies are widely used to characterize molecular structures, crystallinity, and polymorphism. The X-ray powder diffraction patterns of the crystalline polymorphs of the present invention were generated on a Philips Analytical X'Pert PRO X-ray Diffraction System with PW3040/60 console. A PW3373/00 ceramic Cu LEF X-ray tube K-Alpha radiation was used as the source.

FIG. 1 shows the X-ray diffraction pattern for the crystalline anhydrate Form I. The anhydrate Form I exhibited characteristic reflections corresponding to d-spacings of 18.42, 9.35, and 6.26 angstroms. The anhydrate Form I was further characterized by reflections corresponding to d-spacings of 5.78, 4.71, and 3.67 angstroms. The anhydrate Form I was even further characterized by reflections corresponding to d-spacings of 3.99, 2.71, and 2.66 angstroms.

FIG. 11 shows the X-ray diffraction pattern for the crystalline anhydrate Form III. The anhydrate Form III exhibited characteristic reflections corresponding to d-spacings of 17.88, 6.06, and 4.26 angstroms. The anhydrate Form III was further characterized by reflections corresponding to d-spacings of 9.06, 5.71, and 4.55 angstroms. The anhydrate Form III was even further characterized by reflections corresponding to d-spacings of 13.69, 6.50, and 3.04 angstroms.

FIG. 6 shows the X-ray diffraction pattern for the crystalline desolvated anhydrate Form II. The desolvated anhydrate Form II exhibited characteristic reflections corresponding to d-spacings of 7.09, 5.27, and 4.30 angstroms. The desolvated anhydrate Form II was further characterized by reflections corresponding to d-spacings of 18.56, 9.43 and 4.19 angstroms. The desolvated anhydrate Form II was even further characterized by reflections corresponding to d-spacings of 6.32, 5.82, and 3.69 angstroms.

FIG.16 shows the X-ray diffraction pattern for the crystalline ethanol solvate. The crystalline ethanol solvate exhibited the same XRPD pattern as desolvated anhydrate Form II with characteristic reflections corresponding to d-spacings of 7.09, 5.27, and 4.30 angstroms. The crystalline ethanol solvate was further characterized by reflections corresponding to d-spacings of 18.56, 9.43 and 4.19 angstroms. The crystalline ethanol solvate was even further characterized by reflections corresponding to d-spacings of 6.32, 5.82, and 3.69 angstroms.

In addition to the X-ray powder diffraction patterns described above, the crystalline polymorphic forms of Compound I of the present invention were further characterized by their solid-state carbon-13 and fluorine-19 nuclear magnetic resonance (NMR) spectra. The solid-state carbon-13 NMR

spectrum was obtained on a Bruker DSX 400WB NMR system using a Bruker 4 mm double resonance CPMAS probe. The carbon-13 NMR spectrum utilized proton/carbon-13 cross-polarization magic-angle spinning with variable-amplitude cross polarization. The sample was spun at 15.0 kHz, and a total of 1024 scans were collected with a recycle delay of 5 seconds. A line broadening of 40 Hz was applied to the spectrum before FT was performed. Chemical shifts are reported on the TMS scale using the carbonyl carbon of glycine (176.03 p.p.m.) as a secondary reference.

The solid-state fluorine-19 NMR spectrum was obtained on a Bruker DSX 400WB NMR system using a Bruker 4mm CRAMPS probe. The NMR spectrum utilized a simple pulse-acquire pulse program. The samples were spun at 15.0 kHz, and a total of 128 scans were collected with a recycle delay of 5 seconds. A vespel endcap was utilized to minimize fluorine background. A line broadening of 100 Hz was applied to the spectrum before FT was performed. Chemical shifts are reported using poly(tetrafluoroethylene) (teflon) as an external secondary reference which was assigned a chemical shift of –122 ppm.

DSC data were acquired using TA Instruments DSC 2910 or equivalent instrumentation. Between 2 and 6 mg of sample were weighed into an open pan. This pan was then crimped and placed at the sample position in the calorimeter cell. An empty pan was placed at the reference position. The calorimeter cell was closed and a flow of nitrogen was passed through the cell. The heating program was set to heat the sample at a heating rate of 10 °C/min to a temperature of approximately 250 °C. The heating program was started. When the run was completed, the data were analyzed using the DSC analysis program contained in the system software. The melting endotherm was integrated between baseline temperature points that are above and below the temperature range over which the endotherm was observed. The data reported are the onset temperature, peak temperature and enthalpy.

FIG. 2 shows the solid-state carbon-13 CPMAS NMR spectrum for the crystalline anhydrate Form I of Compound I.

FIG. 3 shows the solid-state fluorine-19 MAS NMR spectrum for the crystalline anhydrate Form I of Compound I. Form I exhibited characteristic signals with chemical shift values of -65.3, -105.1, and -120.4 p.p.m. Further characteristic of Form I are the signals with chemical shift values of -80.6, -93.5, and -133.3 p.p.m.

FIG. 4 shows the differential calorimetry scan for the crystalline anhydrate Form I. Form I exhibited a melting endotherm with an onset temperature of 215 °C, a peak temperature of 217 °C, and an enthalpy of 221J/g.

FIG. 7 shows the solid-state carbon-13 CPMAS NMR spectrum for the crystalline desolvated anhydrate Form II of Compound I.

FIG. 8 shows the solid-state fluorine-19 MAS NMR spectrum for the crystalline desolvated anhydrate Form II of Compound I. Form II exhibited characteristic signals with chemical

shift values of -65.1, -104.9, and -120.1 p.p.m. Further characteristic of Form II are the signals with chemical shift values of -80.3, -94.5, -134.4, and -143.3 p.p.m.

FIG. 9 shows the differential calorimetry scan for crystalline desolvated anhydrate Form II. Form II exhibited a solid-solid transition exotherm to crystalline anhydrate Form I with an onset temperature of 114 °C, a peak temperature of 125 °C, and an enthalpy of 2.3J/g.

FIG. 12 shows the solid-state carbon-13 CPMAS NMR spectrum for the crystalline anhydrate Form III of Compound I.

FIG. 13 shows the solid-state fluorine-19 MAS NMR spectrum for the crystalline anhydrate Form III of Compound I. Form III exhibited characteristic signals with chemical shift values of -63.0, -103.1, and -120.2 p.p.m. Further characteristic of Form III are the signals with chemical shift values of -95.3, -98.7, -135.2, and -144.0 p.p.m.

FIG. 14 shows the differential calorimetry scan for crystalline anhydrate Form III. Form III exhibited a solid-solid transition endotherm to crystalline anhydrate Form I with an onset temperature of 80 °C, a peak temperature of 84 °C, and an enthalpy of 1.3J/g.

FIG. 17 shows the solid-state carbon-13 CPMAS NMR spectrum for the crystalline ethanol solvate of Compound I.

FIG. 18 shows the solid-state fluorine-19 MAS NMR spectrum for the crystalline ethanol solvate of Compound I. The ethanol solvate exhibited characteristic signals with chemical shift values of -64.7, -104.5, and -121.9 p.p.m. Further characteristic of ethanol solvate are the signals with chemical shift values of -94.3, -117.7, -131.2, and -142.6 p.p.m.

The crystalline Compound I anhydrate Form I or Form III or mixture thereof of the present invention has a phase purity of at least about 5% of Form I or Form III or mixture thereof with the above X-ray powder diffraction, fluorine-19 MAS NMR, carbon-13 CPMAS NMR, and DSC physical characteristics. In one embodiment the phase purity is at least about 10% of Form I or Form III or mixture thereof with the above solid-state physical characteristics. In a second embodiment the phase purity is at least about 25% of Form I or Form III or mixture thereof with the above solid-state physical characteristics. In a third embodiment the phase purity is at least about 50% of Form I or Form III or mixture thereof with the above solid-state physical characteristics. In a fourth embodiment the phase purity is at least about 75% of Form I or Form III or mixture thereof with the above solid-state physical characteristics. In a fifth embodiment the phase purity is at least about 90% of Form I or Form III or mixture thereof with the above solid-state physical characteristics. In a sixth embodiment the crystalline Compound I is the substantially phase pure Form I or Form III or mixture thereof with the above solid-state physical characteristics. By the term "phase purity" is meant the solid state purity of the Compound I anhydrate Form I or Form III or mixture thereof with regard to another particular crystalline or

amorphous form of Compound I as determined by the solid-state physical methods described in the present application.

EXAMPLES OF PHARMACEUTICAL COMPOSITIONS:

1) Direct compression process:

Compound I anhydrate Form I or Form III or a mixture thereof (API) was formulated into a tablet by a direct compression process. A 100 mg potency tablet is composed of 124 mg of the API, 130 mg microcrystalline cellulose, 130 mg of mannitol (or 130 mg of dicalcium phosphate), 8 mg of croscarmellose sodium, 8 mg of magnesium stearate and 16 mg of Opadry white (proprietary coating material made by Colorcon, West Point, PA). The API, microcrystalline cellulose, mannitol (or dicalcium phosphate), and croscarmellose sodium were first blended, and the mixture was then lubricated with magnesium stearate and pressed into tablets. The tablets were then film coated with Opadry White.

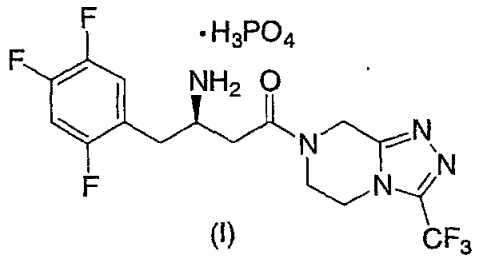
2) Roller compaction process:

Compound I anhydrate Form I or Form III or a mixture thereof was formulated into a tablet by a roller compaction process. A 100 mg potency tablet is composed of 124 mg of the API, 195 mg microcrystalline cellulose, 65 mg of mannitol, 8 mg of croscarmellose sodium, 8 mg of magnesium stearate and 16 mg of Opadry white (proprietary coating material made by Colorcon, West Point, PA). The API, microcrystalline cellulose, mannitol, and croscarmellose sodium were first blended, and the mixture was then lubricated with one third the total amount of magnesium stearate and roller compacted into ribbons. These ribbons were then milled and the resulting granules were lubricated with the remaining amount of the magnesium stearate and pressed into tablets. The tablets were then film coated with Opadry White.

3) An intravenous (i.v.) aqueous formulation is defined as the anhydrate Form I or Form III or a mixture thereof of Compound I in 10 mM sodium acetate/0.8% saline solution at pH 4.5 ± 0.2. For a formulation with a concentration of 4.0 mg/mL, 800 mg of NaCl is dissolved in 80 mL of water, then 57.5 µL of glacial acetic acid is added, followed by 496 mg of the anhydrate Form I or Form III or a mixture thereof. The pH is adjusted to 4.5 ± 0.2 with 0.1 N NaOH solution. The final volume is adjusted to 100 mL with water. A 2.0-mg/mL solution can be made by dilution of 50.0 mL of the 4.0-mg/mL solution to 100.0 mL with placebo. A 1.0-mg/mL solution can be made by dilution of 25.0 mL of the 4.0-mg/mL solution to 100.0 mL with placebo.

WHAT IS CLAIMED IS:

1. A dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I)

characterized as being a crystalline anhydrate Form I.

2. The crystalline anhydrate Form I of Claim 1 characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 18.42, 9.35, and 6.26 angstroms.

3. The crystalline anhydrate Form I of Claim 2 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 5.78, 4.71, and 3.67 angstroms.

4. The crystalline anhydrate Form I of Claim 3 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 3.99, 2.71, and 2.66 angstroms.

5. The crystalline anhydrate Form I of Claim 4 further characterized by the X-ray powder diffraction pattern of FIG. 1.

6. The crystalline anhydrate Form I of Claim 1 characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -65.3, -105.1, and -120.4 p.p.m.

7. The crystalline anhydrate Form I of Claim 6 further characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at

-80.6, -93.5, and -133.3 p.p.m.

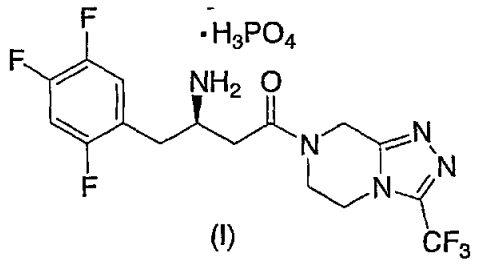
8. The crystalline anhydrate Form I of Claim 7 further characterized by the solid-state fluorine-19 MAS nuclear magnetic resonance spectrum of FIG. 3.

9. The crystalline anhydrate Form I of Claim 1 characterized by the solid-state carbon-13 CPMAS nuclear magnetic resonance spectrum of FIG. 2.

10. The crystalline anhydrate Form I of Claim 1 characterized by the thermogravimetric analysis curve of FIG. 5.

11. The crystalline anhydrate Form I of Claim 1 characterized by the differential scanning calorimetric (DSC) curve of FIG. 4.

12. A dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I)

characterized as being a crystalline anhydrate Form III.

13. The crystalline anhydrate Form III of Claim 12 characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 17.88, 6.06, and 4.26 angstroms.

14. The crystalline anhydrate Form III of Claim 13 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 9.06, 5.71, and 4.55 angstroms.

15. The crystalline anhydrate Form III of Claim 14 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 13.69, 6.50, and 3.04 angstroms.

16. The crystalline anhydrate Form III of Claim 15 further characterized by the X-ray powder diffraction pattern of FIG. 11.

17. The crystalline anhydrate Form III of Claim 12 characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -63.0, -103.1, and -120.2 p.p.m.

18. The crystalline anhydrate Form III of Claim 17 further characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -95.3, -98.7, -135.2, and -144.0 p.p.m.

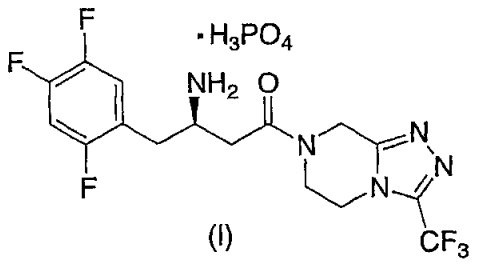
19. The crystalline anhydrate Form III of Claim 18 further characterized by the solid-state fluorine-19 MAS nuclear magnetic resonance spectrum of FIG. 13.

20. The crystalline anhydrate Form III of Claim 12 characterized by the solid-state carbon-13 CPMAS nuclear magnetic resonance spectrum of FIG. 12.

21. The crystalline anhydrate Form III of Claim 12 characterized by the thermogravimetric analysis curve of FIG. 15.

22. The crystalline anhydrate Form III of Claim 12 characterized by the differential scanning calorimetric (DSC) curve of FIG. 14.

23. A dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I)

characterized as being a crystalline desolvated anhydrate Form II.

24. The crystalline desolvated anhydrate Form II of Claim 23 characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 7.09, 5.27, and 4.30 angstroms.

25. The crystalline desolvated anhydrate Form II of Claim 24 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 18.56, 9.43, and 4.19 angstroms.

26. The crystalline desolvated anhydrate Form II of Claim 25 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 6.32, 5.82, and 3.69 angstroms.

27. The crystalline desolvated anhydrate Form II of Claim 26 further characterized by the X-ray powder diffraction pattern of FIG. 6.

28. The crystalline desolvated anhydrate Form II of Claim 23 characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -65.1, -104.9, and -120.1 p.p.m.

29. The crystalline desolvated anhydrate Form II of Claim 28 further characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -80.3, -94.5, -134.4, and -143.3 p.p.m.

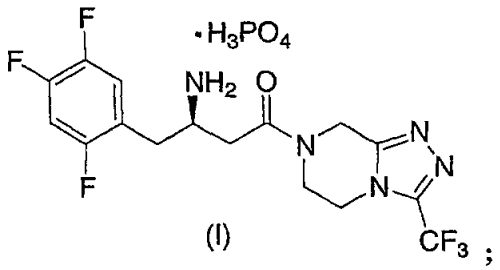
30. The crystalline desolvated anhydrate Form II of Claim 29 further characterized by the solid-state fluorine-19 MAS nuclear magnetic resonance spectrum of FIG. 8.

31. The crystalline desolvated anhydrate Form II of Claim 23 characterized by the solid-state carbon-13 CPMAS nuclear magnetic resonance spectrum of FIG. 7.

32. The crystalline desolvated anhydrate Form II of Claim 23 characterized by the thermogravimetric analysis curve of FIG. 10.

33. The crystalline desolvated anhydrate Form II of Claim 23 characterized by the differential scanning calorimetric (DSC) curve of FIG. 9.

34. A dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I) ;

characterized as being a crystalline solvate wherein the solvate is selected from the group consisting of acetone solvate, acetonitrile solvate, methanolate, ethanolate, 1-propanolate, and 2-propanolate.

35. The crystalline solvate of Claim 34 wherein said solvate is an ethanolate.

36. The crystalline ethanolate of Claim 35 characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 7.09, 5.27, and 4.30 angstroms.

37. The crystalline ethanolate of Claim 36 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 18.56, 9.43, and 4.19 angstroms.

38. The crystalline ethanolate of Claim 37 further characterized by characteristic reflections obtained from the X-ray powder diffraction pattern at spectral d-spacings of 6.32, 5.82, and 3.69 angstroms.

39. The crystalline ethanolate of Claim 38 further characterized by the X-ray powder diffraction pattern of FIG. 16.

40. The crystalline ethanolate of Claim 35 characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -64.7, -104.5, and -121.9 p.p.m.

41. The crystalline ethanolate of Claim 40 further characterized by a solid-state fluorine-19 MAS nuclear magnetic resonance spectrum showing signals at -94.3, -117.7, -131.2, and -142.6 p.p.m.

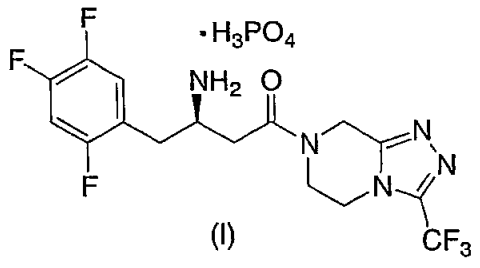
42. The crystalline ethanolate of Claim 41 further characterized by the solid-state fluorine-19 MAS nuclear magnetic resonance spectrum of FIG. 18.

43. The crystalline ethanolate of Claim 35 characterized by the solid-state carbon-13 CPMAS nuclear magnetic resonance spectrum of FIG. 17.

44. The crystalline ethanolate of Claim 35 characterized by the thermogravimetric analysis curve of FIG. 20.

45. The crystalline ethanolate of Claim 35 characterized by the differential scanning calorimetric (DSC) curve of FIG. 19.

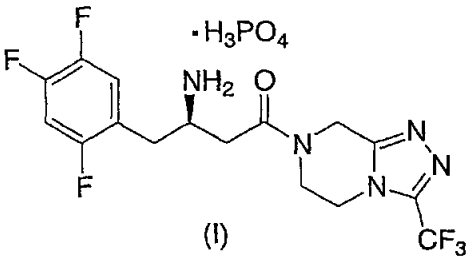
46. A drug substance which is the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I)

comprising a mixture of crystalline anhydrate Form I and crystalline anhydrate Form III.

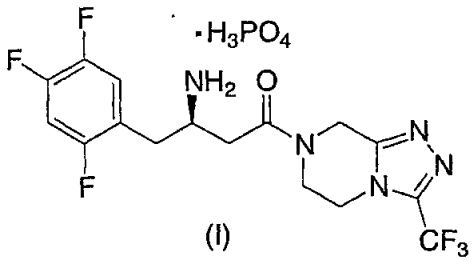
47. A dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I)

comprising a detectable amount of crystalline anhydrate Form I or crystalline anhydrate Form III or a mixture thereof.

48. A dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of structural formula I:



(I)

comprising substantially all by weight of crystalline anhydrate Form I or crystalline anhydrate Form III or a mixture thereof.

49. A pharmaceutical composition comprising a prophylactically or therapeutically effective amount of the salt of Claim 1 or Claim 12 or a mixture thereof in association with one or more pharmaceutically acceptable carriers or excipients.

50. A method of treating Type 2 diabetes comprising administering to a patient in need of such treatment a therapeutically effective amount of the salt according to Claim 1 or Claim 12 or a mixture thereof.

51. The salt of Claim 1 or Claim 12 or a mixture thereof for use in the treatment of Type 2 diabetes.

52. Use of the salt of Claim 1 or Claim 12 or a mixture thereof as active ingredient in the manufacture of a medicament for use in the treatment of Type 2 diabetes.

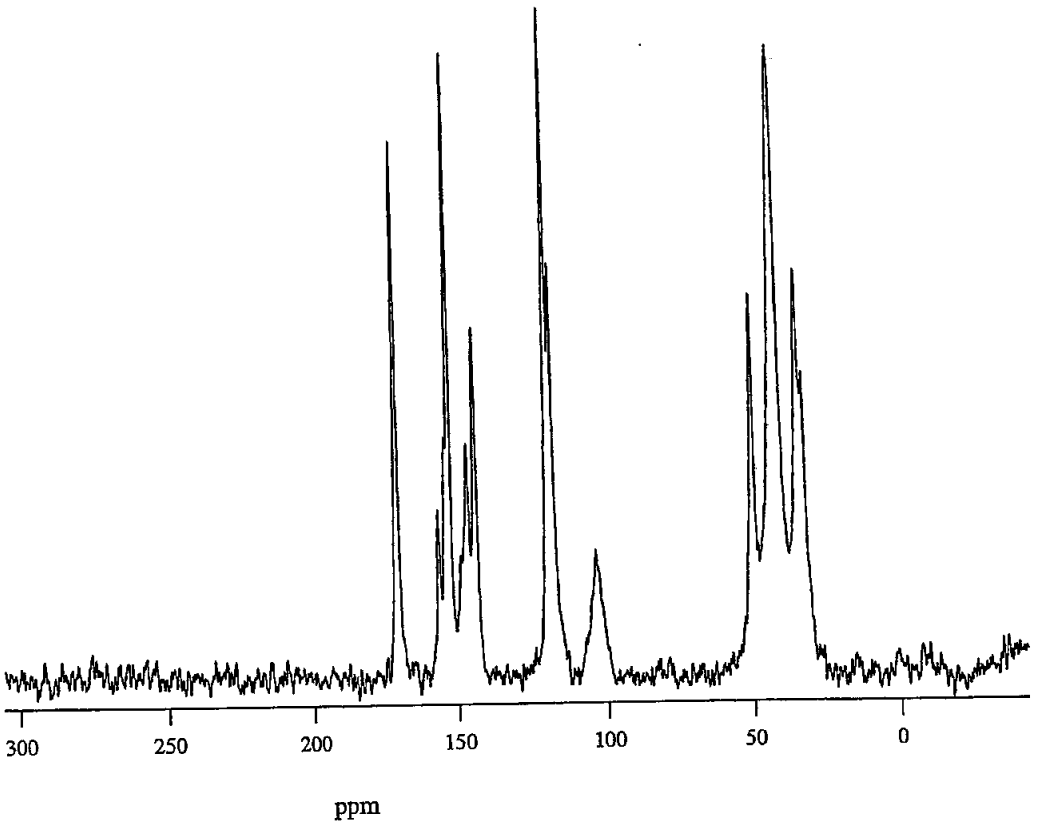


FIGURE 2

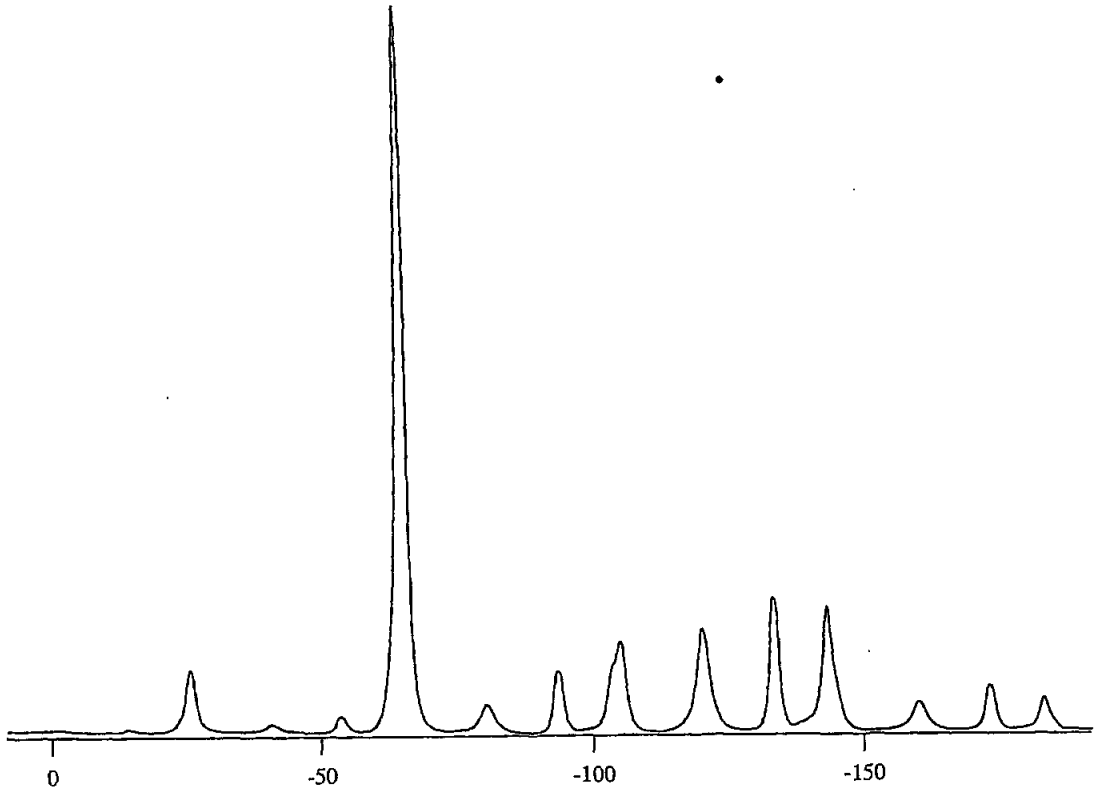


FIGURE 3

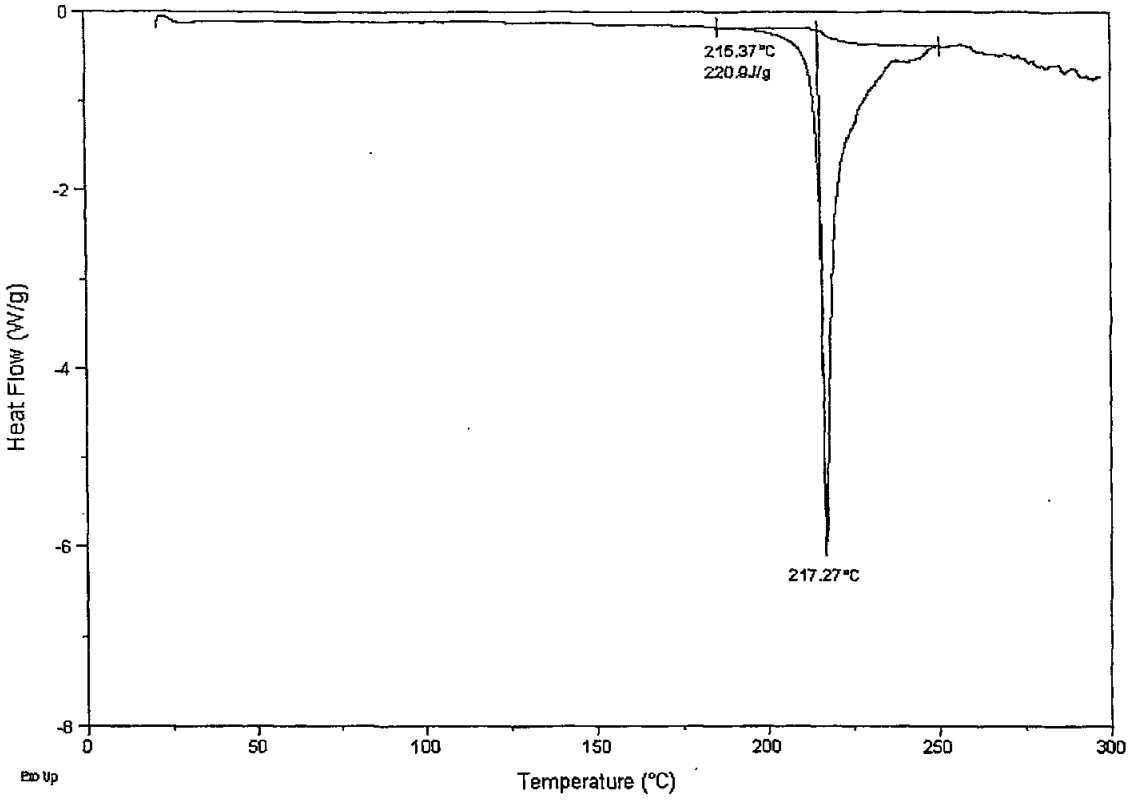

0
-2
-4
-6
-8
Heat Flow (W/g)
215.37°C
220.9J/g
217.27°C
0
50
100
150
200
250
300
Temperature (°C)
Exo Up

FIGURE 4

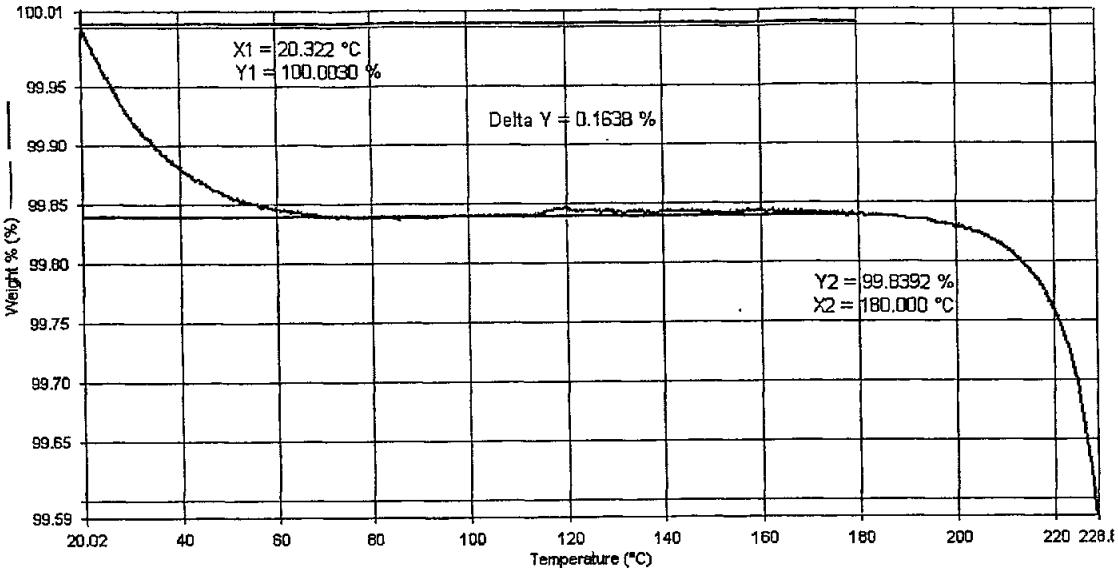

100.01
99.95
99.90
99.85
99.80
99.75
99.70
99.65
99.59
Weight % (%)
X1 = 20.322 °C
Y1 = 100.0030 %
Delta Y = 0.1638 %
Y2 = 99.8392 %
X2 = 180.000 °C
20.02
40
60
80
100
120
140
160
180
200
220
Temperature (°C)

FIGURE 5

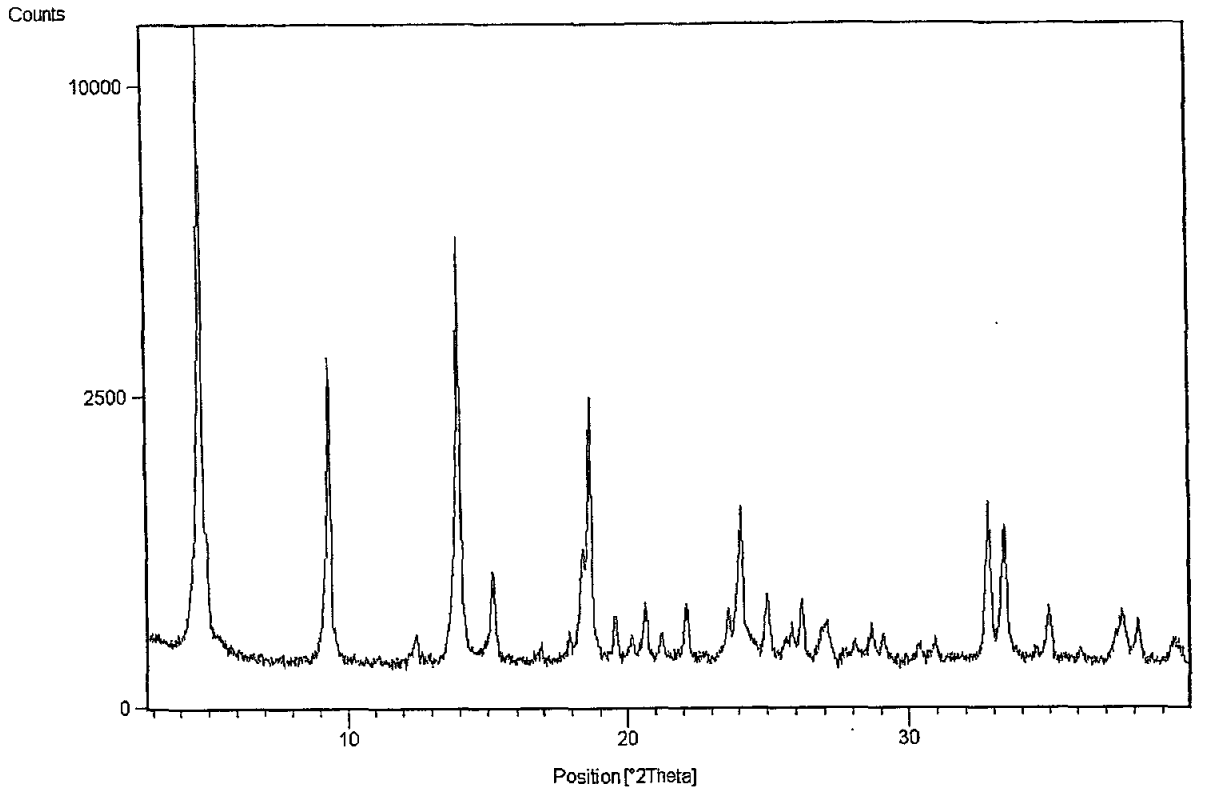


FIGURE 6

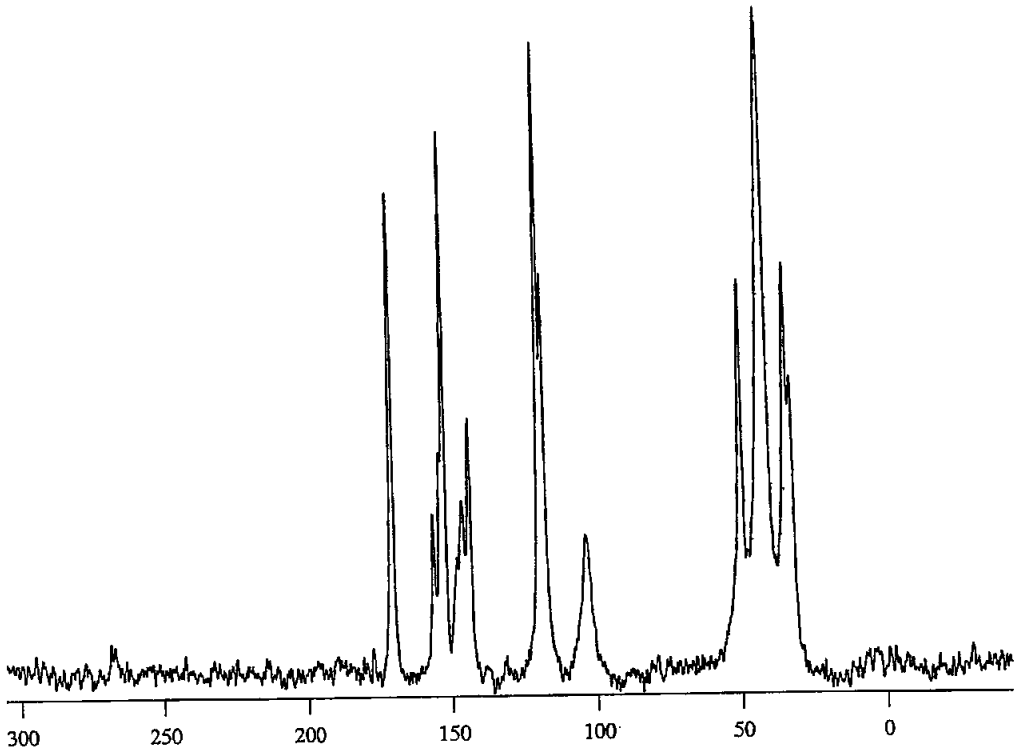


FIGURE 7

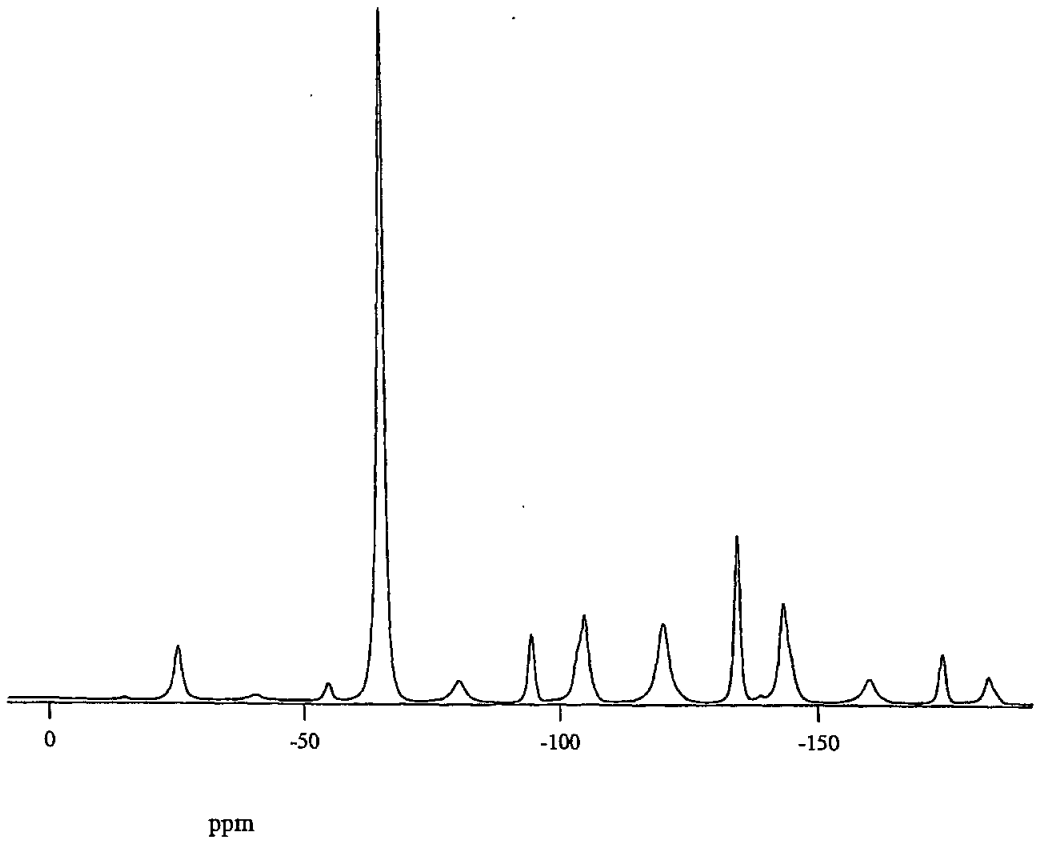


FIGURE 8

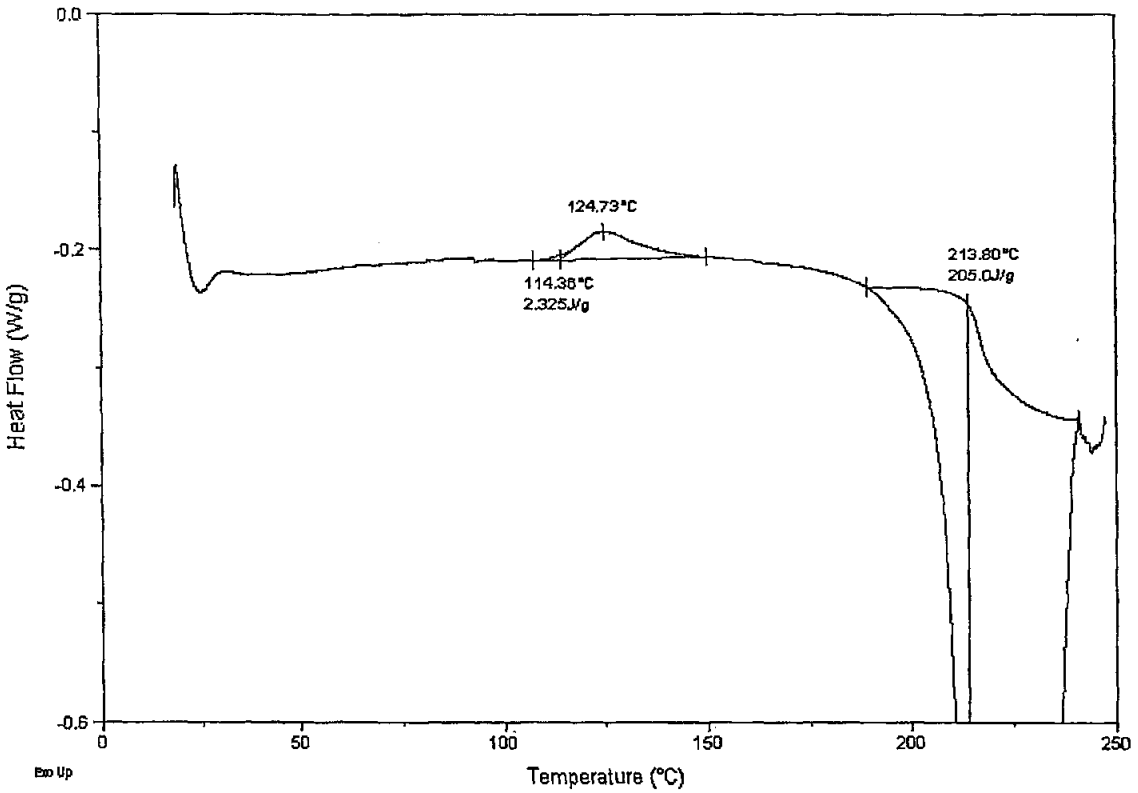

0.0
-0.2
-0.4
-0.6
Heat Flow (W/g)
Exo Up
124.73°C
114.36°C
2.325J/g
213.80°C
205.0J/g
0
50
100
150
200
250
Temperature (°C)

FIGURE 9

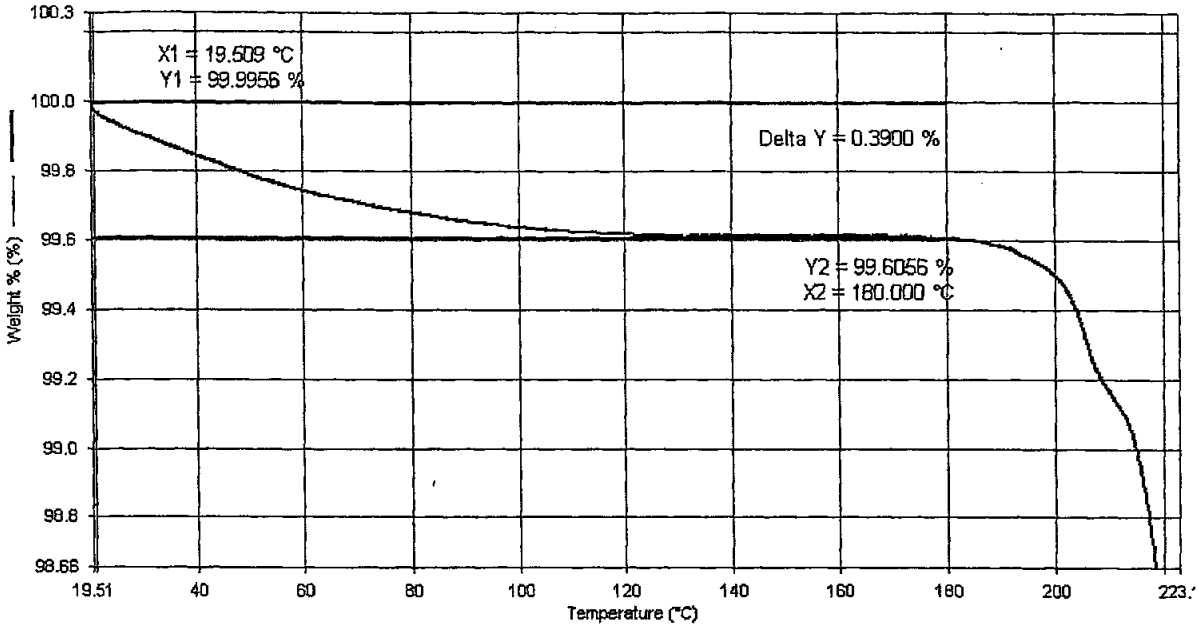

X1 = 19.509 °C
Y1 = 99.9956 %
Delta Y = 0.3900 %
Y2 = 99.6056 %
X2 = 180.000 °C
Weight % (%)
Temperature (°C)
100.3
100.0
99.8
99.6
99.4
99.2
99.0
98.8
98.66
19.51
40
60
80
100
120
140
160
180
200
223.

FIGURE 10

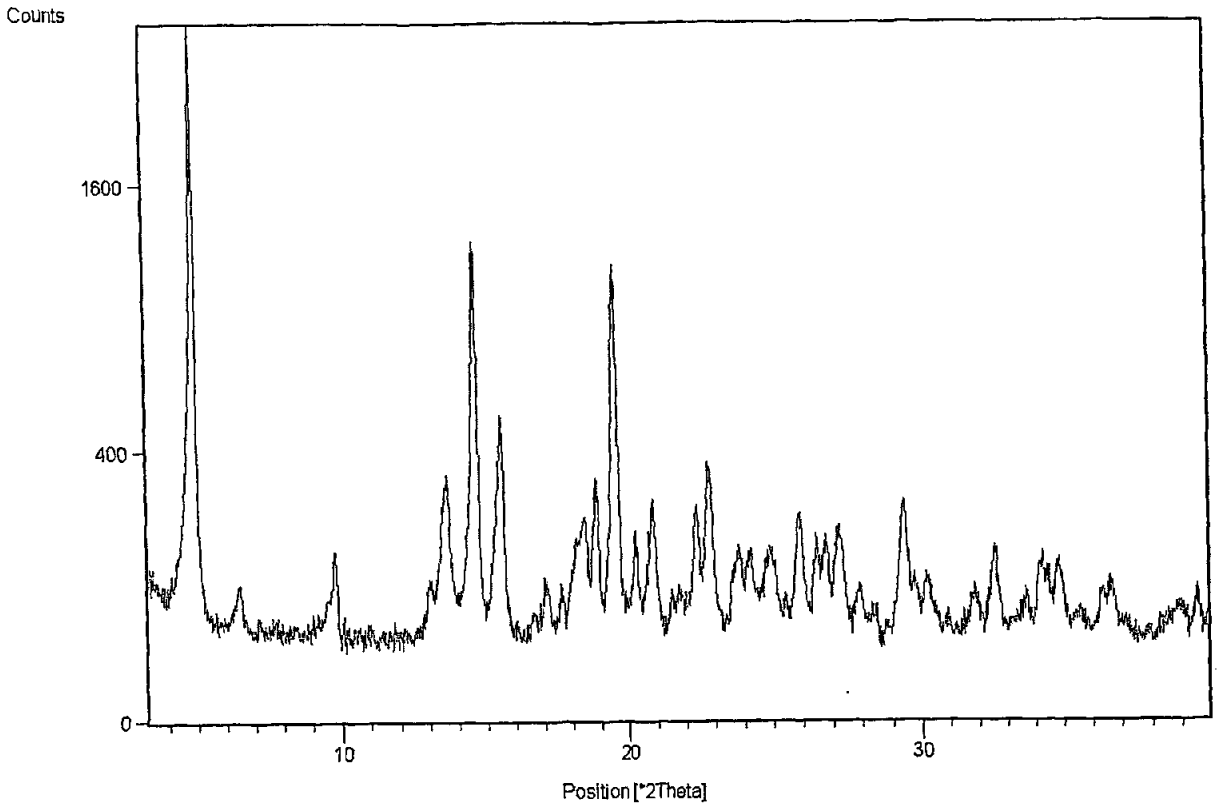


FIGURE 11

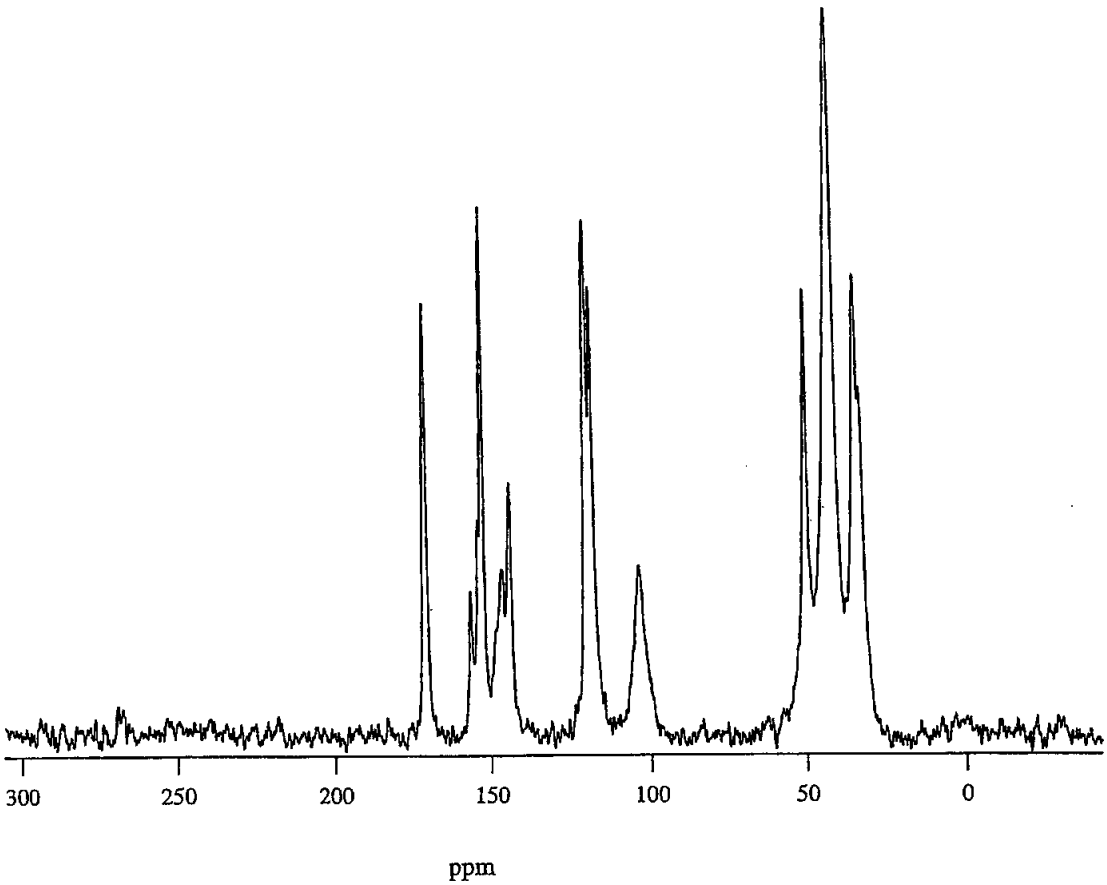


FIGURE 12

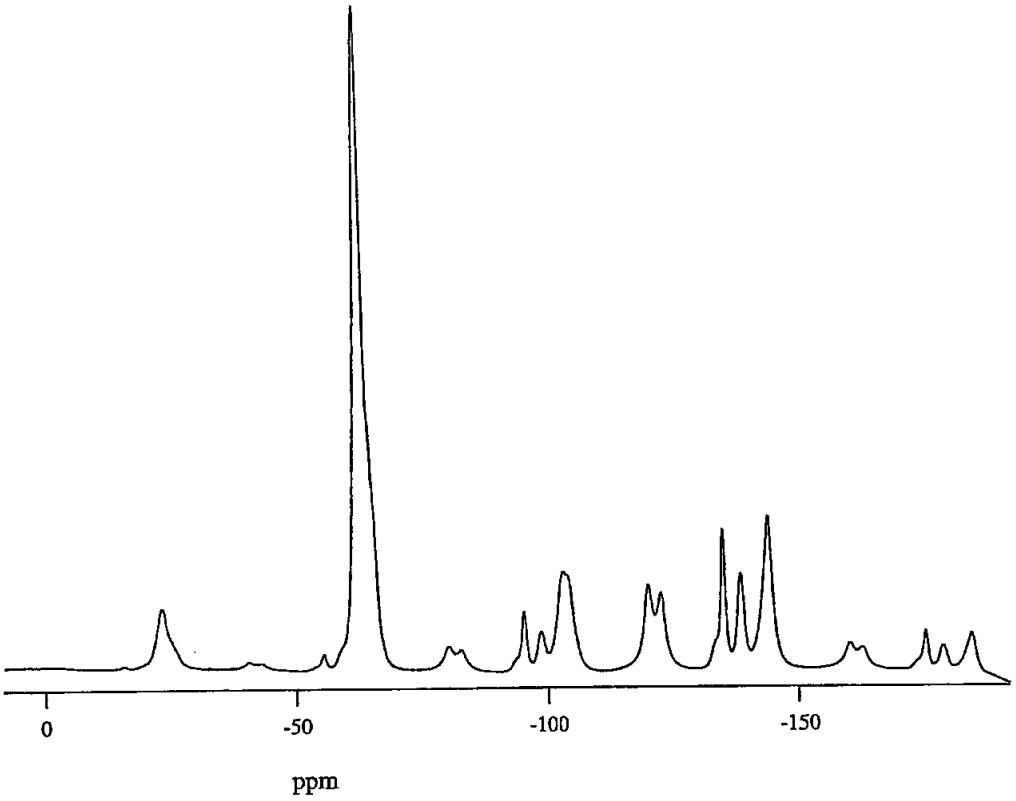


FIGURE 13

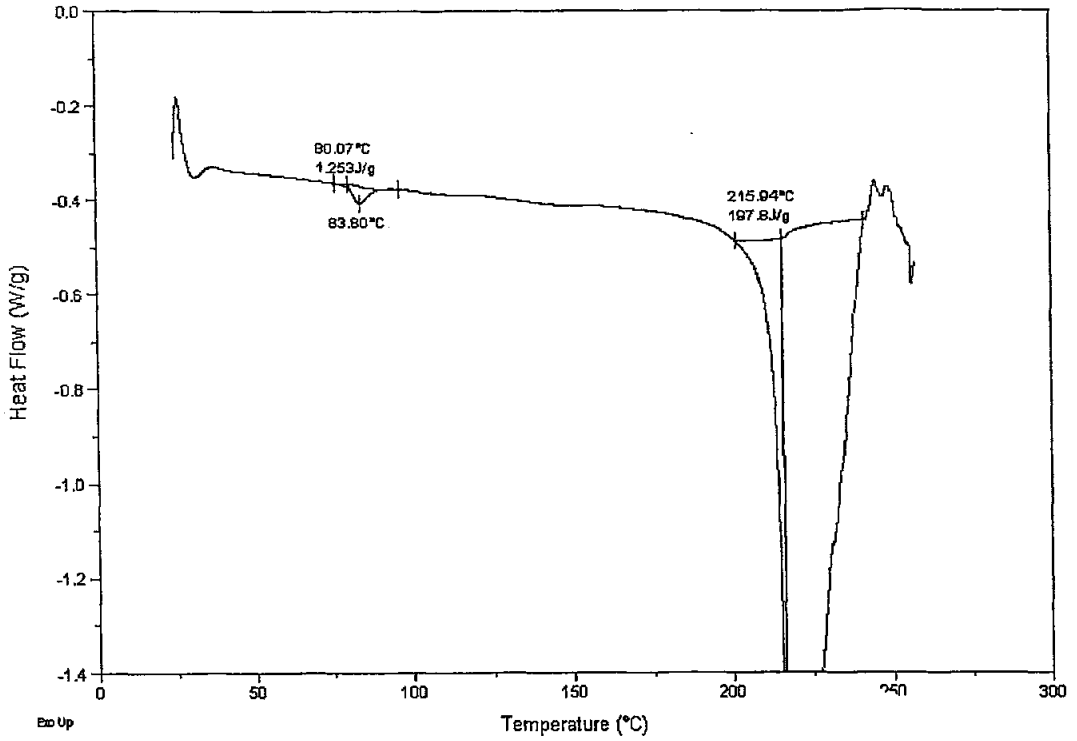

0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
Heat Flow (W/g)
80.07°C
1.253J/g
83.80°C
215.94°C
197.8J/g
0
50
100
150
200
300
Temperature (°C)
Exo Up

FIGURE 14

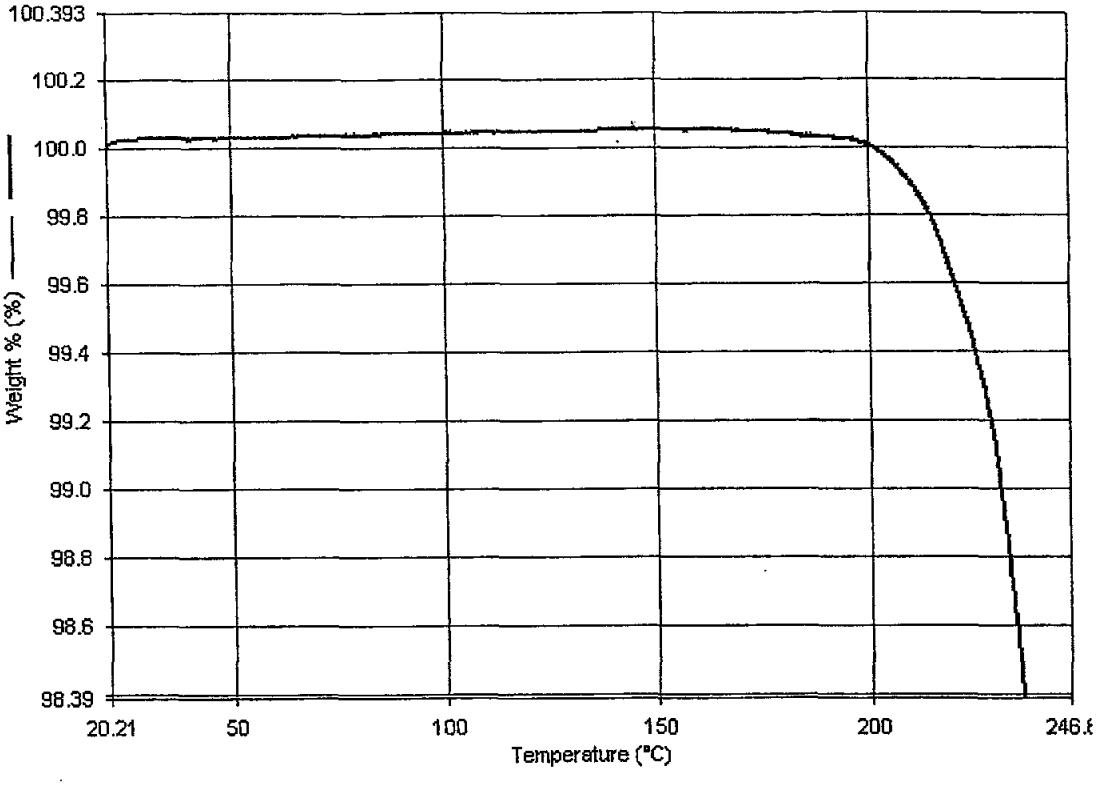

100.393
100.2
100.0
99.8
99.6
99.4
99.2
99.0
98.8
98.6
98.39
Weight % (%)
20.21
50
100
150
200
246.8
Temperature (°C)

FIGURE 15

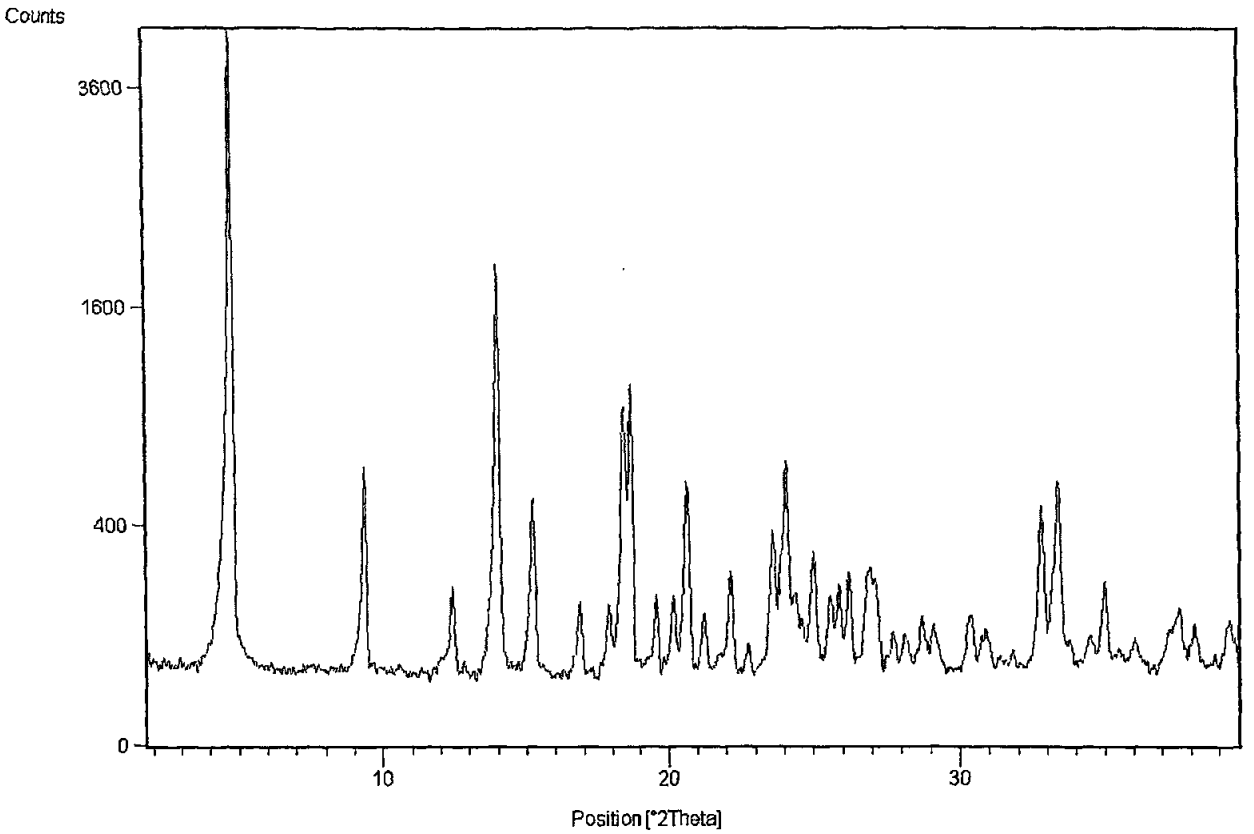


FIGURE 16

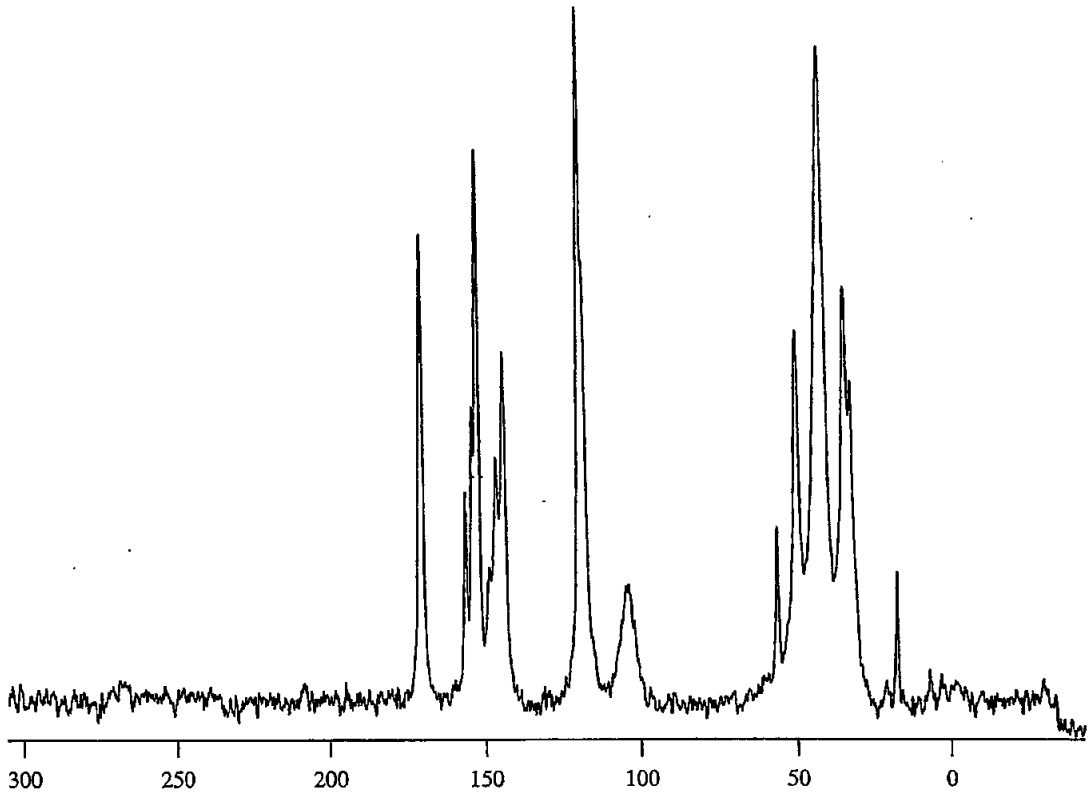


FIGURE 17

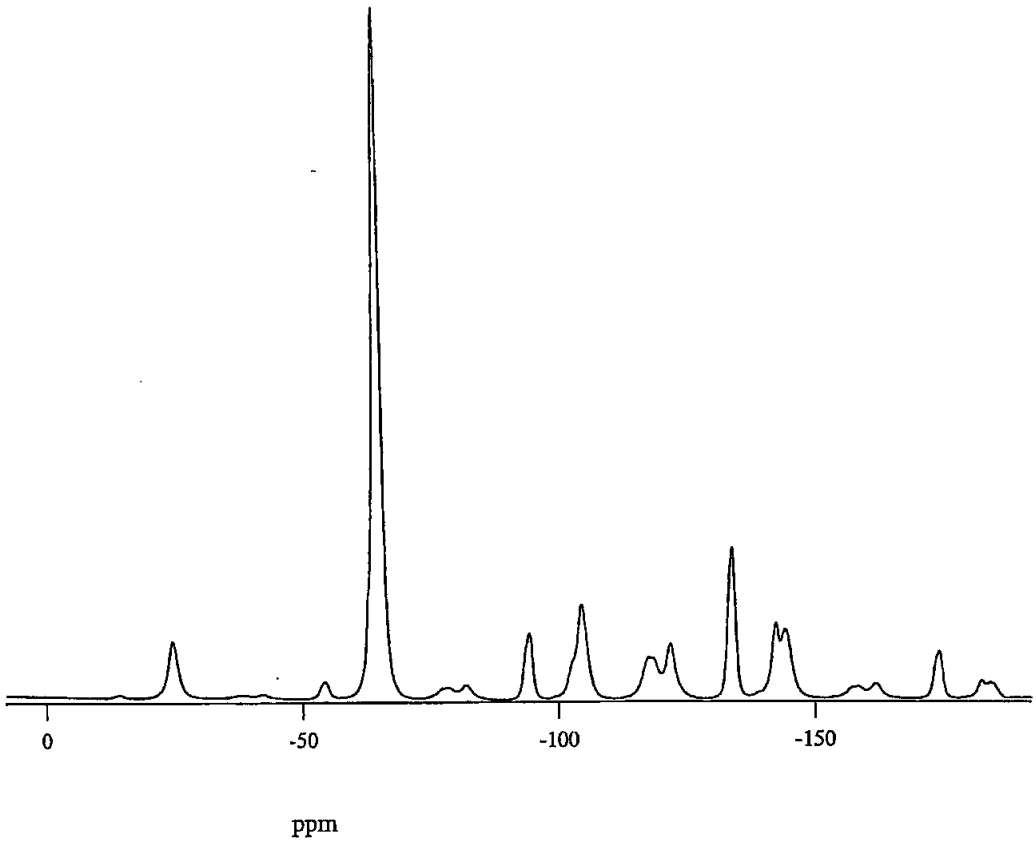


FIGURE 18

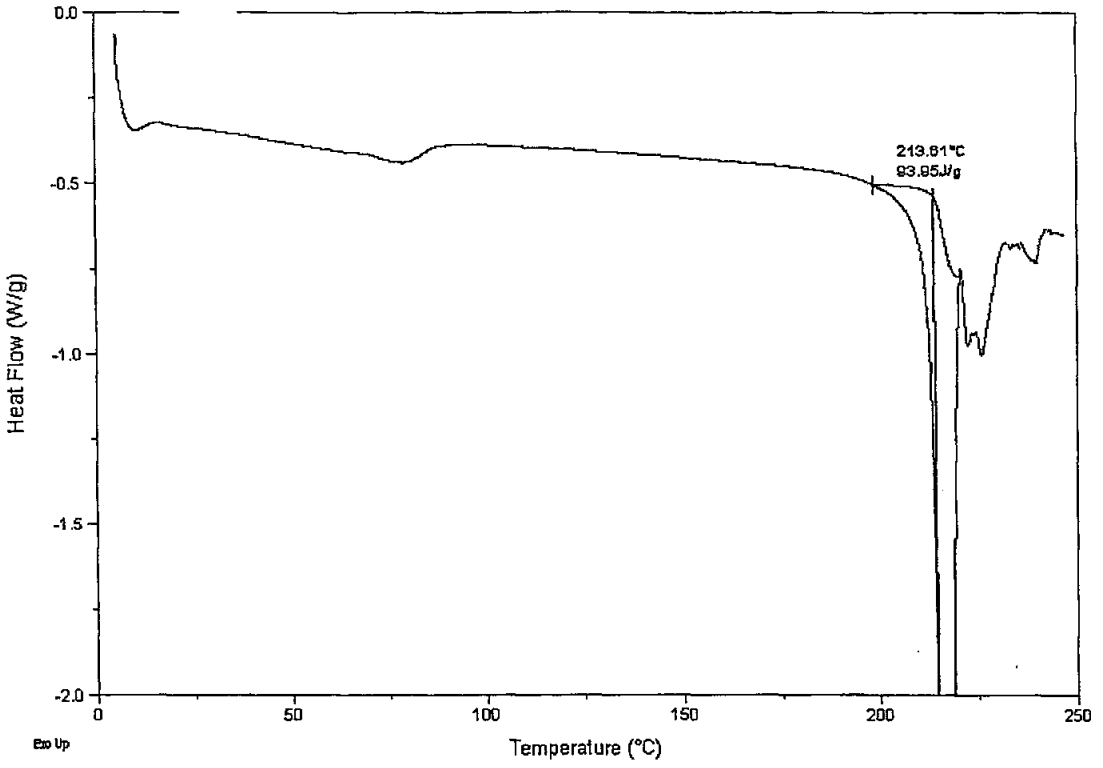

0.0
-0.5
-1.0
-1.5
-2.0
Heat Flow (W/g)
213.61°C
93.95J/g
0
50
100
150
200
250
Temperature (°C)
Exo Up

FIGURE 19

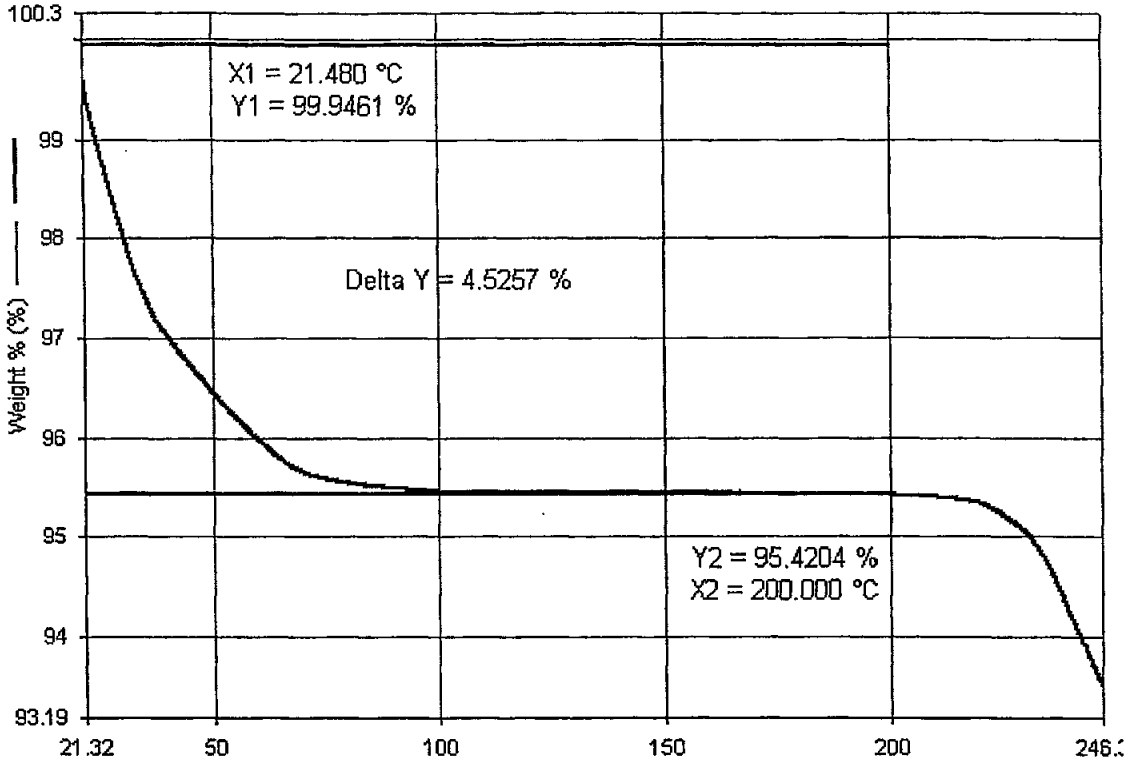

100.3
99
98
97
96
95
94
93.19
Weight % (%)
X1 = 21.480 °C
Y1 = 99.9461 %
Delta Y = 4.5257 %
Y2 = 95.4204 %
X2 = 200.000 °C
21.32
50
100
150
200

FIGURE 20

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International Bureau

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10 March 2005 (10.03.2005)

PCT

(10) International Publication Number
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(51) International Patent Classification[7]: A01N 43/58, 43/60, A61K 31/495, 31/50

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(25) Filing Language: English

(26) Publication Language: English

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60/499,629 2 September 2003 (02.09.2003) US

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WENSLOW, Robert, M.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **ARMSTRONG, Joseph, D., III** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CHEN, Alex, M.** [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CYPES, Stephen** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **FERLITA, Russell, R.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **HANSEN, Karl** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **LINDEMANN, Christopher, M.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SPARTALIS, Evangelia** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

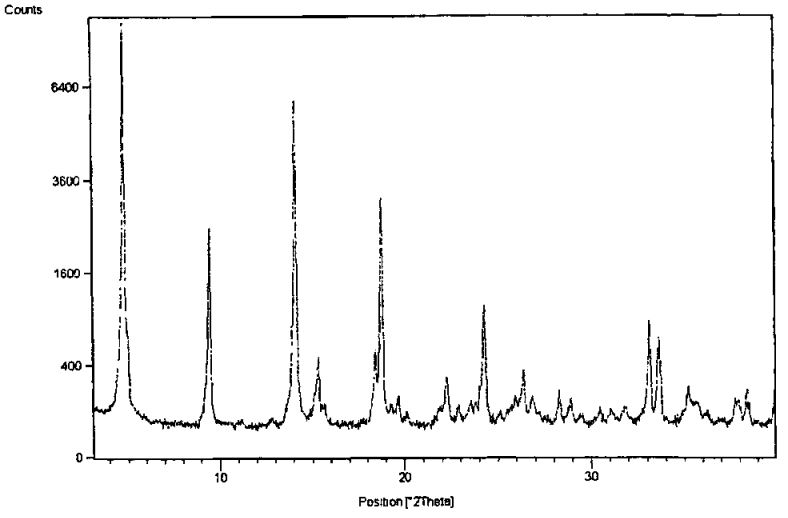
(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: NOVEL CRYSTALLINE FORMS OF A PHOSPHORIC ACID SALT OF A DIPEPTIDYL PEPTIDASE-IV INHIBITOR



(57) Abstract: The present invention relates to crystalline anhydrate polymorphs of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as well as a process for their preparation, pharmaceutical compositions containing these novel forms, and methods of use of the novel forms and pharmaceutical compositions for the treatment of diabetes, obesity, and high blood pressure. The invention also concerns novel crystalline solvates of the dihydrogenphosphate salt of (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as well as a crystalline desolvated polymorph and their use for the preparation of the anhydrate polymorphs of the present invention.

WO 2005/020920 A3

GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

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(88) Date of publication of the international search report:
28 April 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/27983

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A01N 43/58, 43/60; A61K 31/495, 31/50
US CL : 514/249
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/249

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: STRUCTURE SEARCH IN FILE REGISTRY, ANSWERS CROSSED IN FILE CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 6,699,871 B2 (EDMONDSON et al) 2 March 2004 (02.03.2004), column 32, example 7.	1-52

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 March 2005 (01.03.2005)

Date of mailing of the international search report
16 MAR 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Zachary C. Tucker

Telephone No. (571) 272-1600

Form PCT/ISA/210 (second sheet) (January 2004)

EXHIBIT H

Lot D-1895NN-13067/3
LIMS 231102
file 397467-1

Lot D-1895NN-13067/3
LIMS 231202
file 398647-1

EXHIBIT I

INEL XRG-3000
X-ray Tube: 1.54187000 Å Voltage: 40 (kV) Amperage: 30 (mA) Acquisition Time: 300 sec
Spinning capillary. Step size: approximately 0.03 °2θ.

398023_233140_4063-02-01, Compound 184 06-May-2010 07:51:04

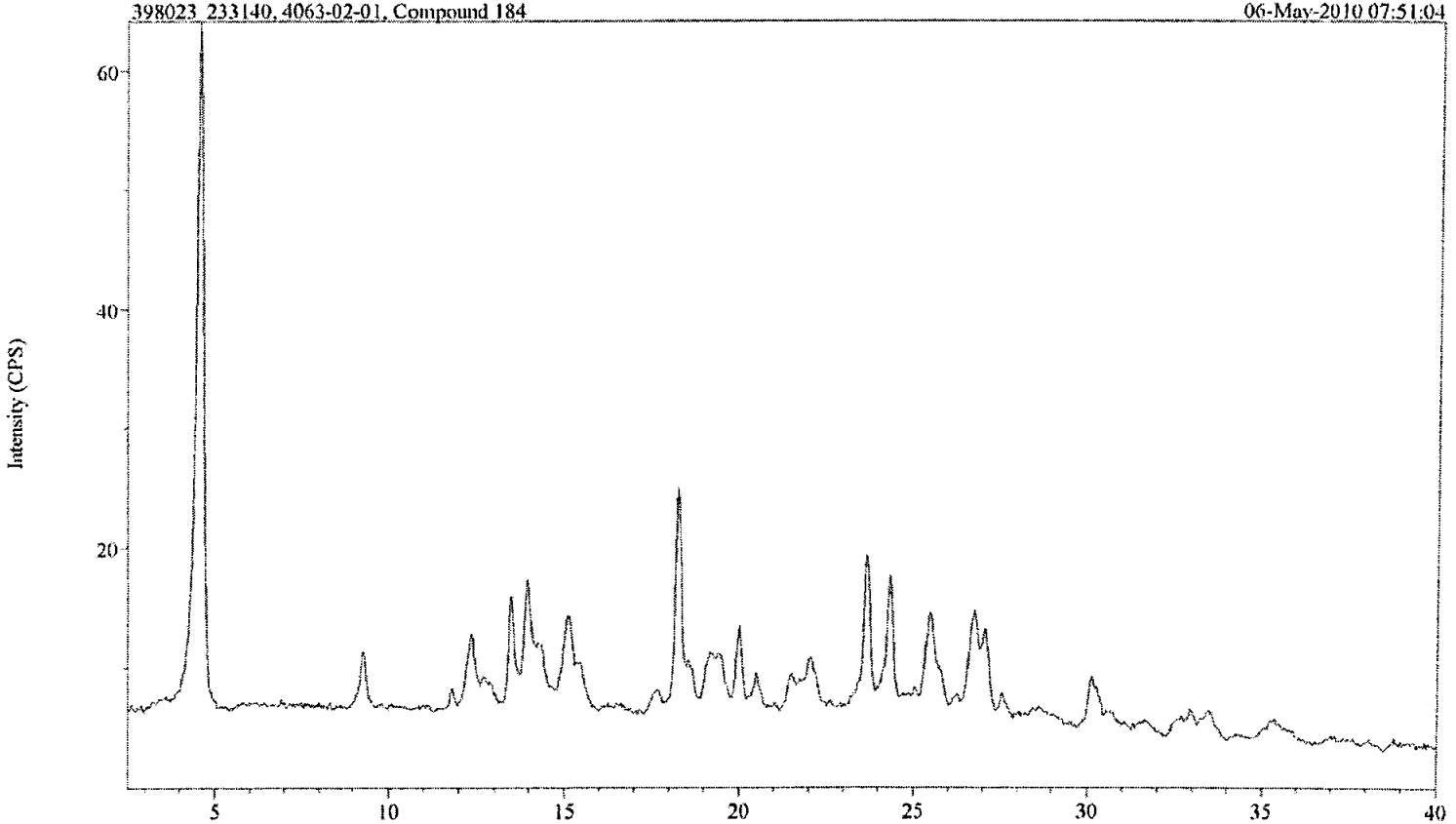

Image by File Monkey v3.2.3

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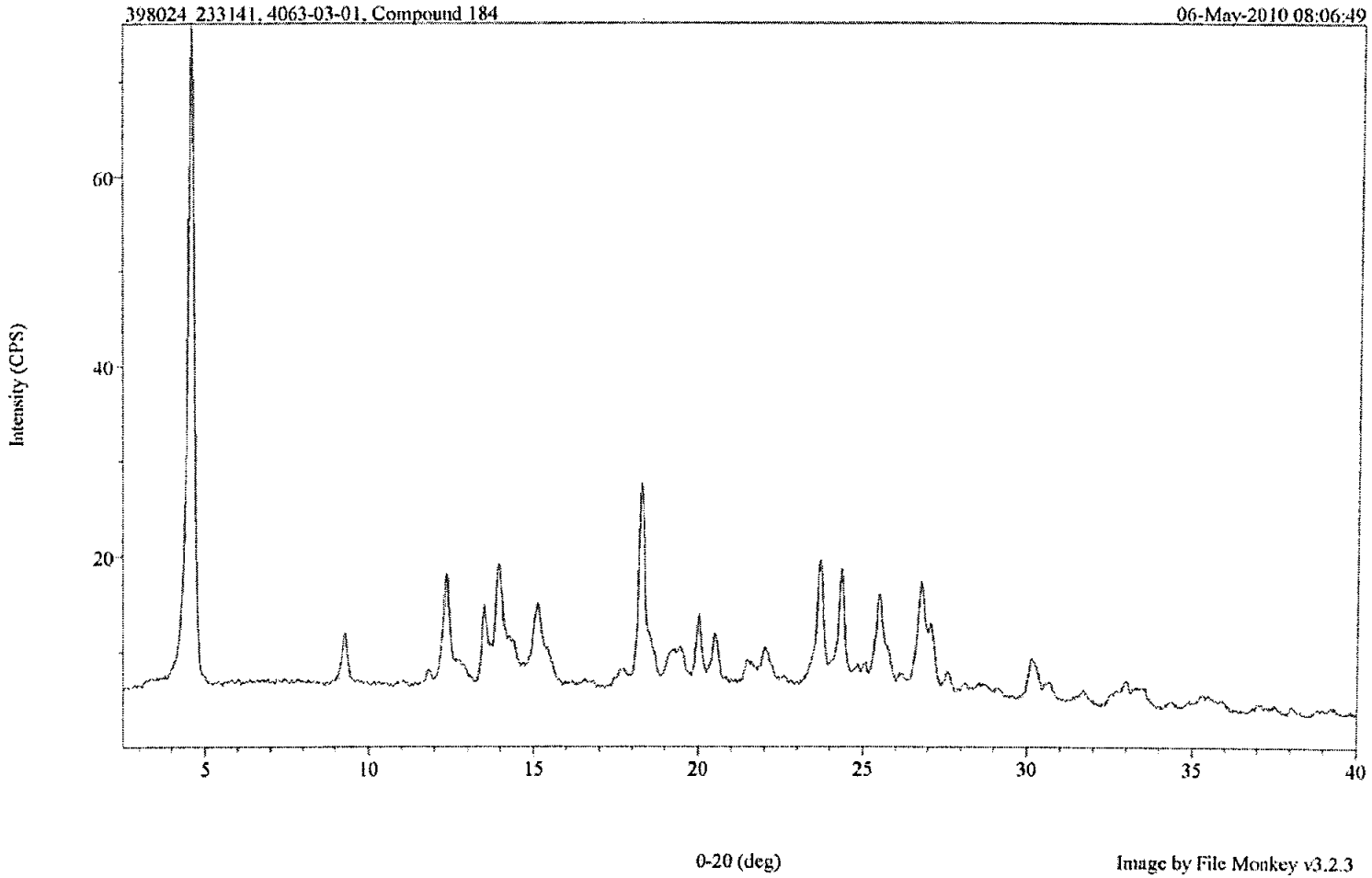
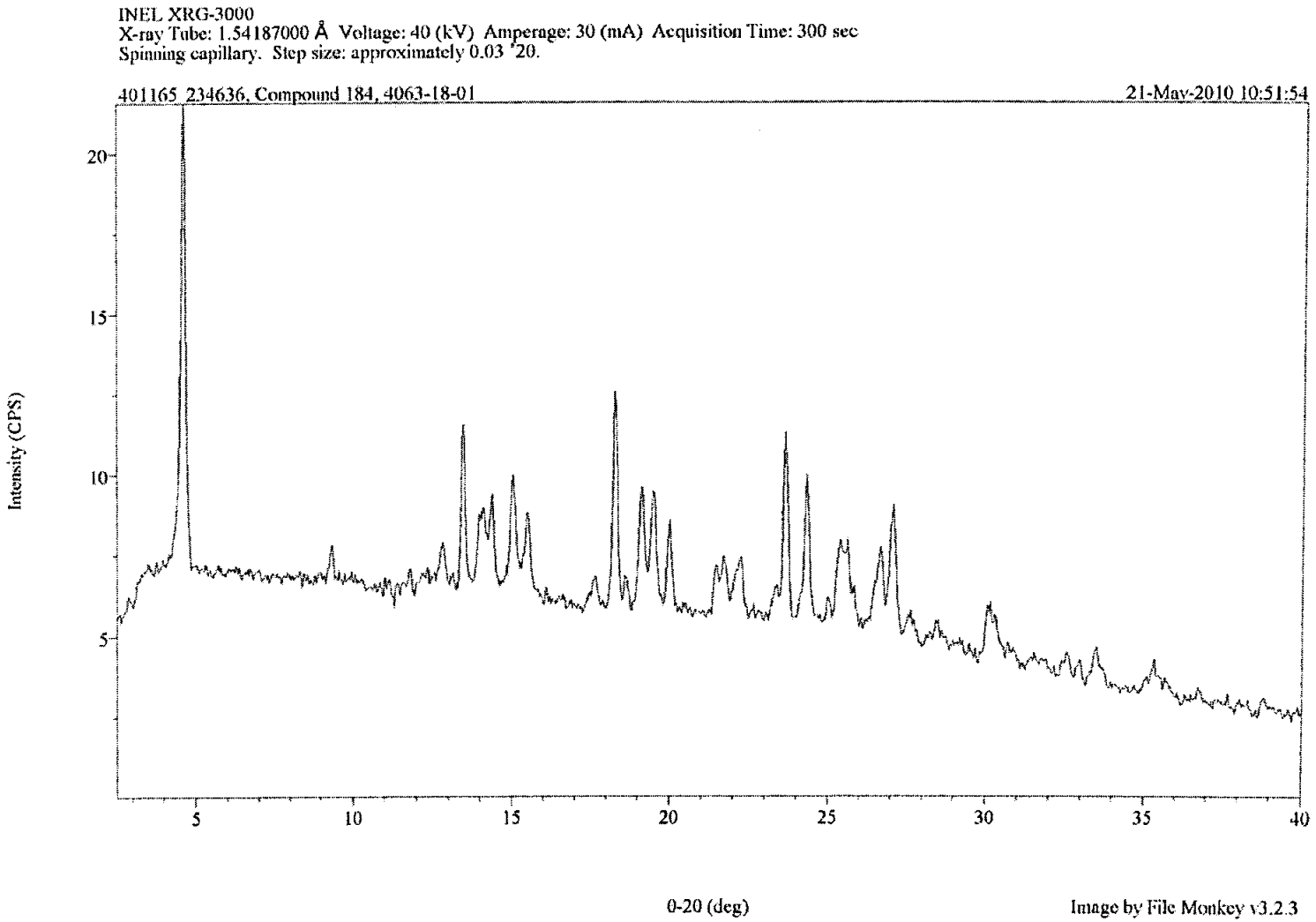
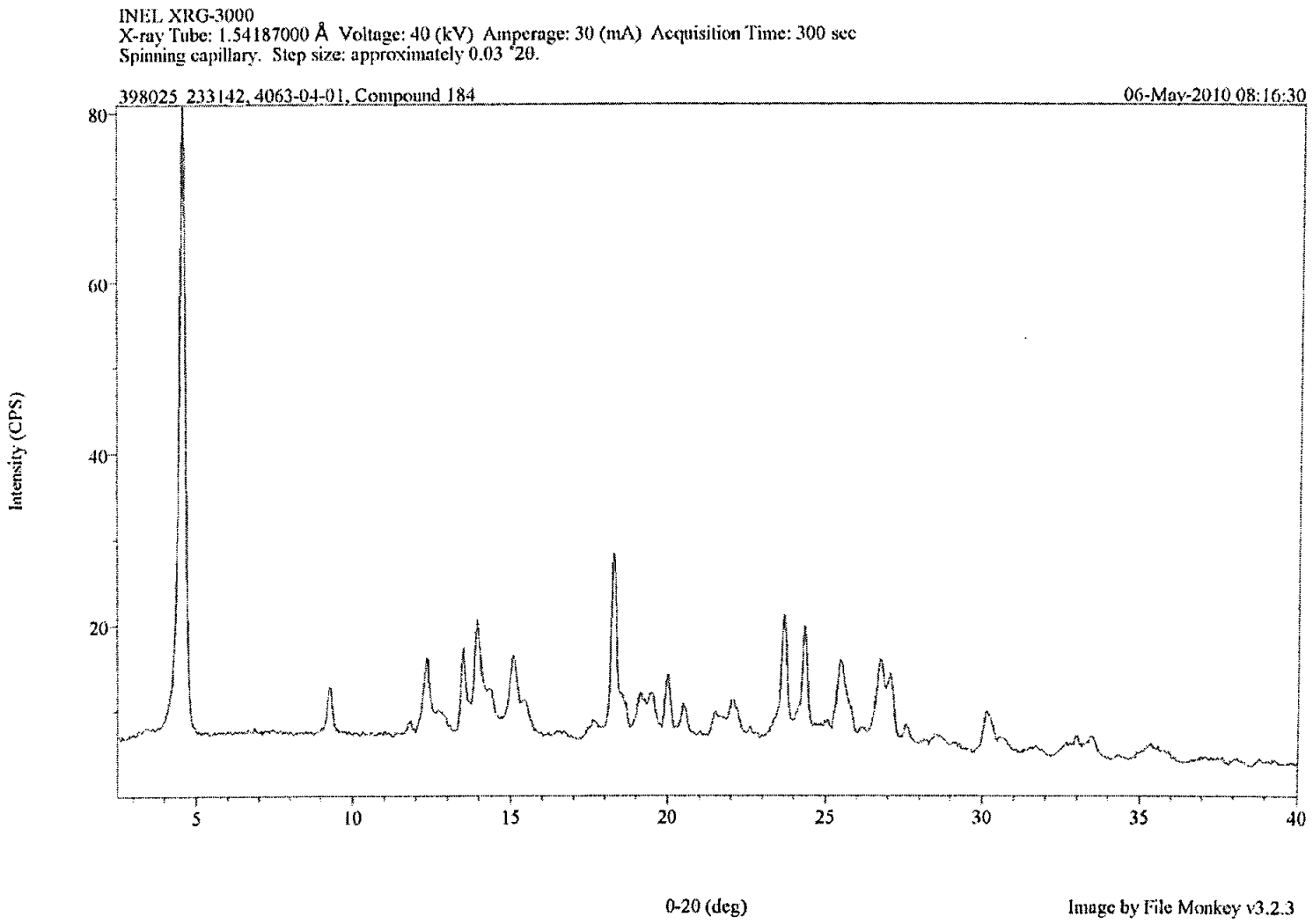
Spinning capillary. Step size: approximately 0.03 °2θ.







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X-ray Tube: 1.54187000 Å Voltage: 40 (kV) Amperage: 30 (mA) Acquisition Time: 300 sec
Spinning capillary. Step size: approximately 0.03 °2θ.

401062_234584_4063-19-01, Compound 184 20-May-2010 09:08:45

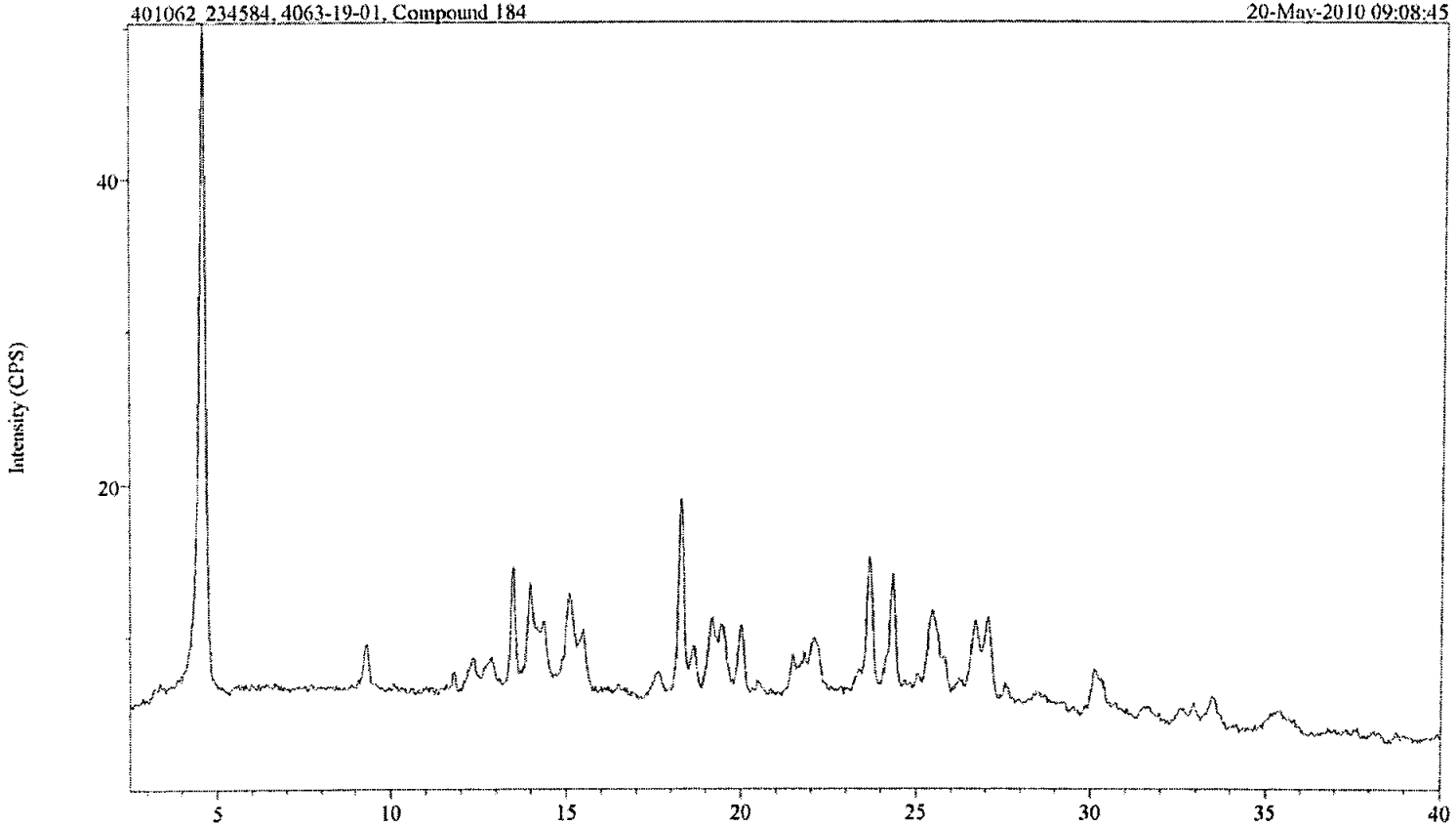
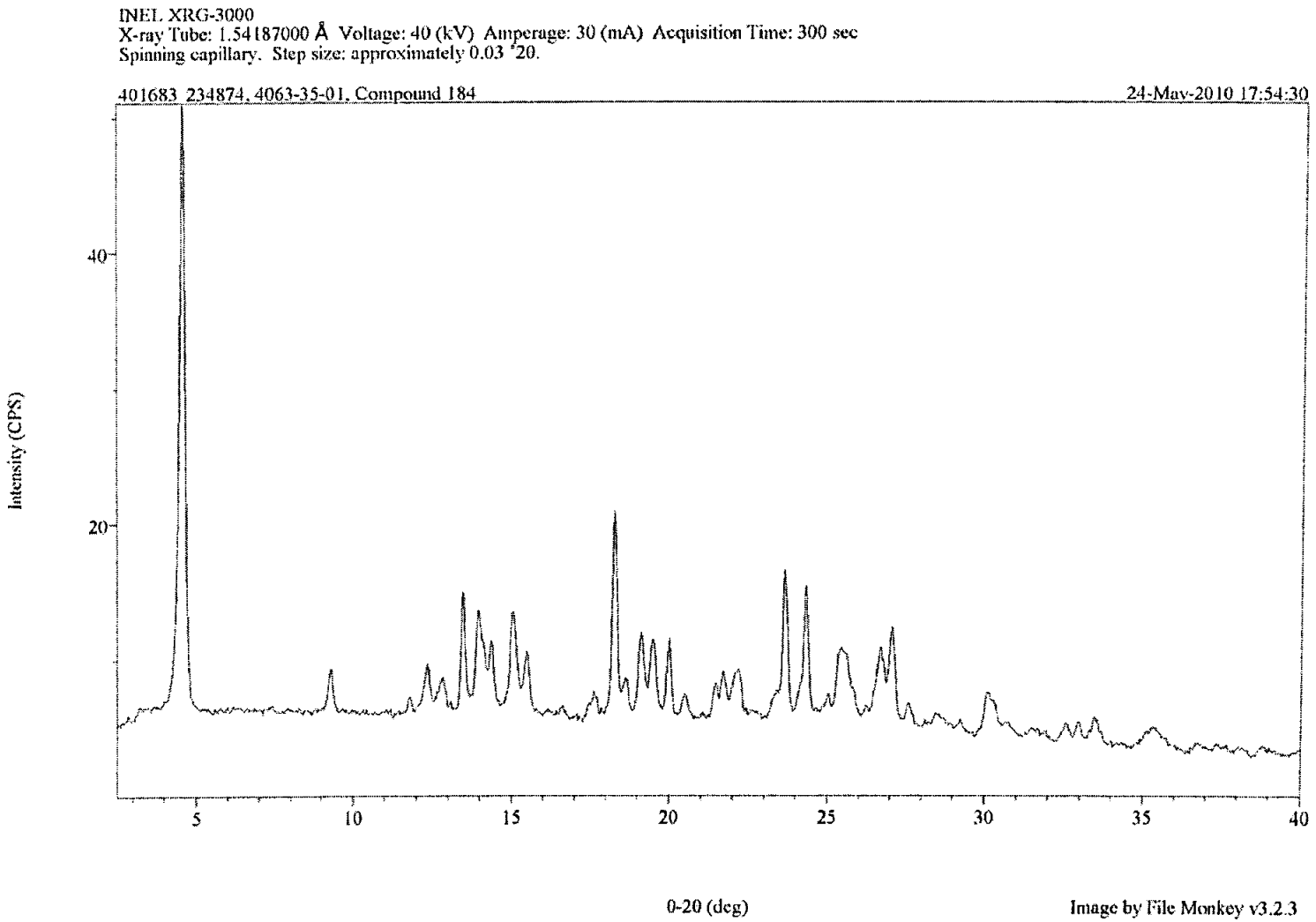
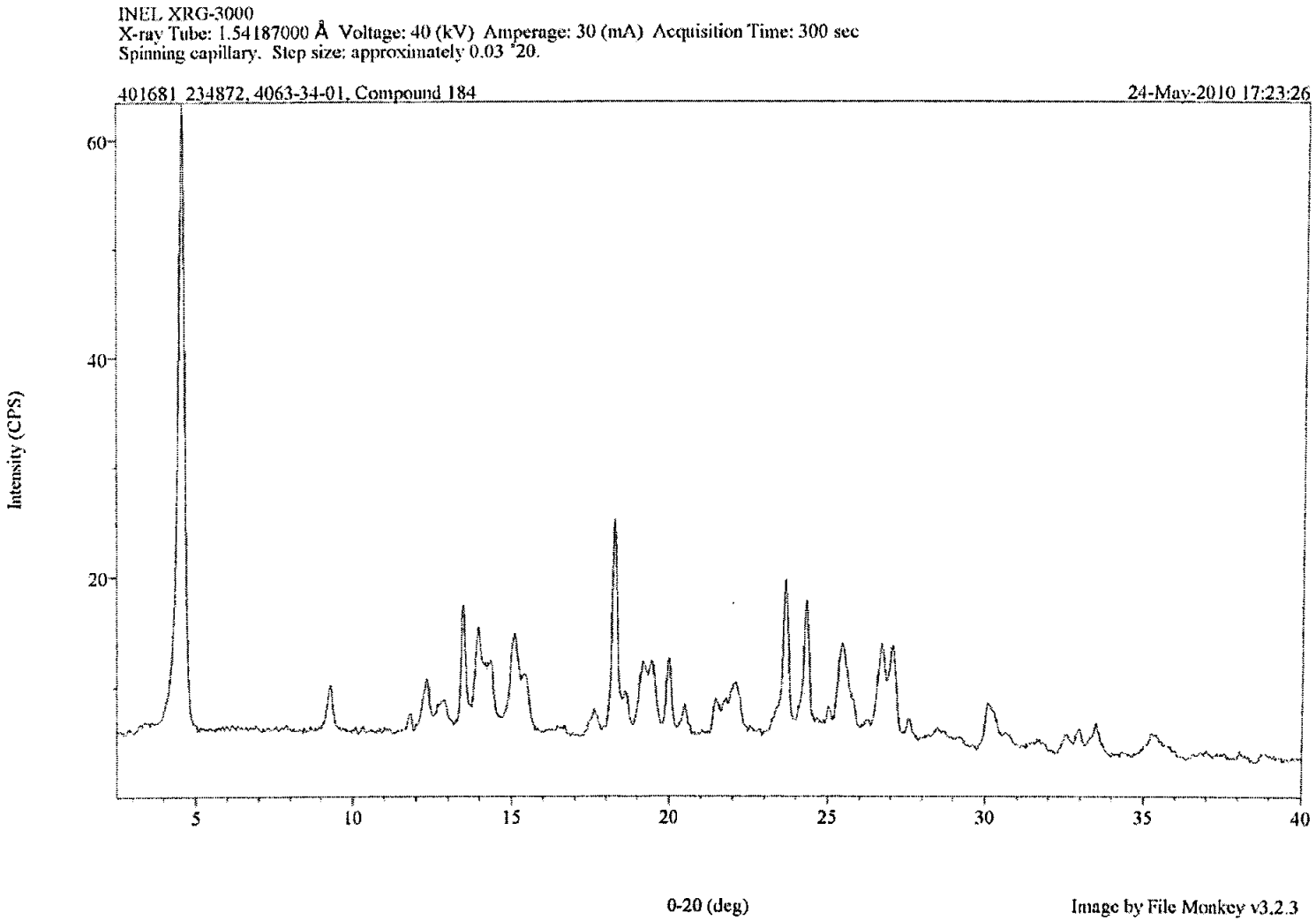


Image by File Monkey v3.2.3





INEL XRG-3000
X-ray Tube: 1.54187000 Å Voltage: 40 (kV) Amperage: 30 (mA) Acquisition Time: 300 sec
Spinning capillary. Step size: approximately 0.03 °2θ.

401682_234873, 4063-32-01, Compound 184 24-May-2010 17:41:49

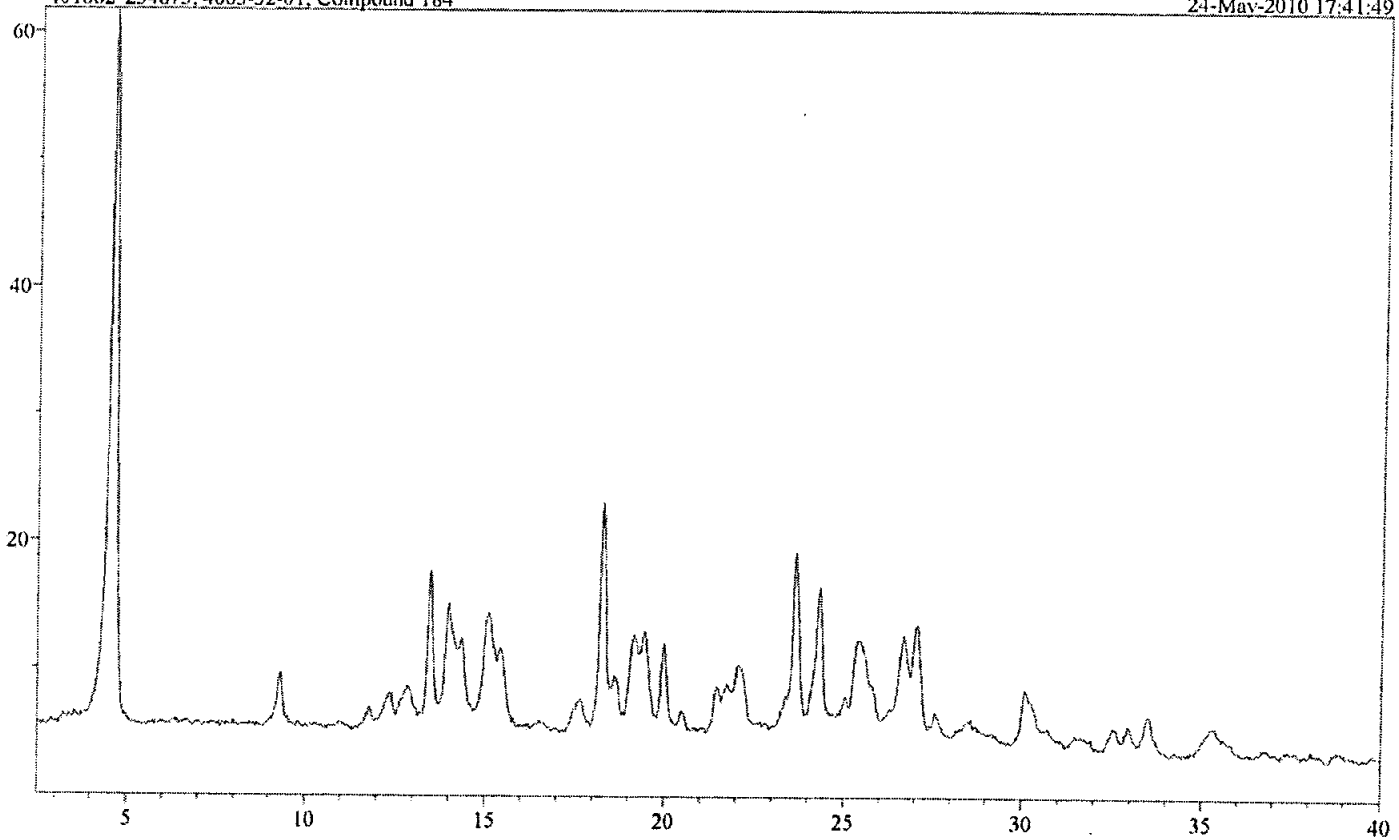


Image by File Monkey v3.2.3

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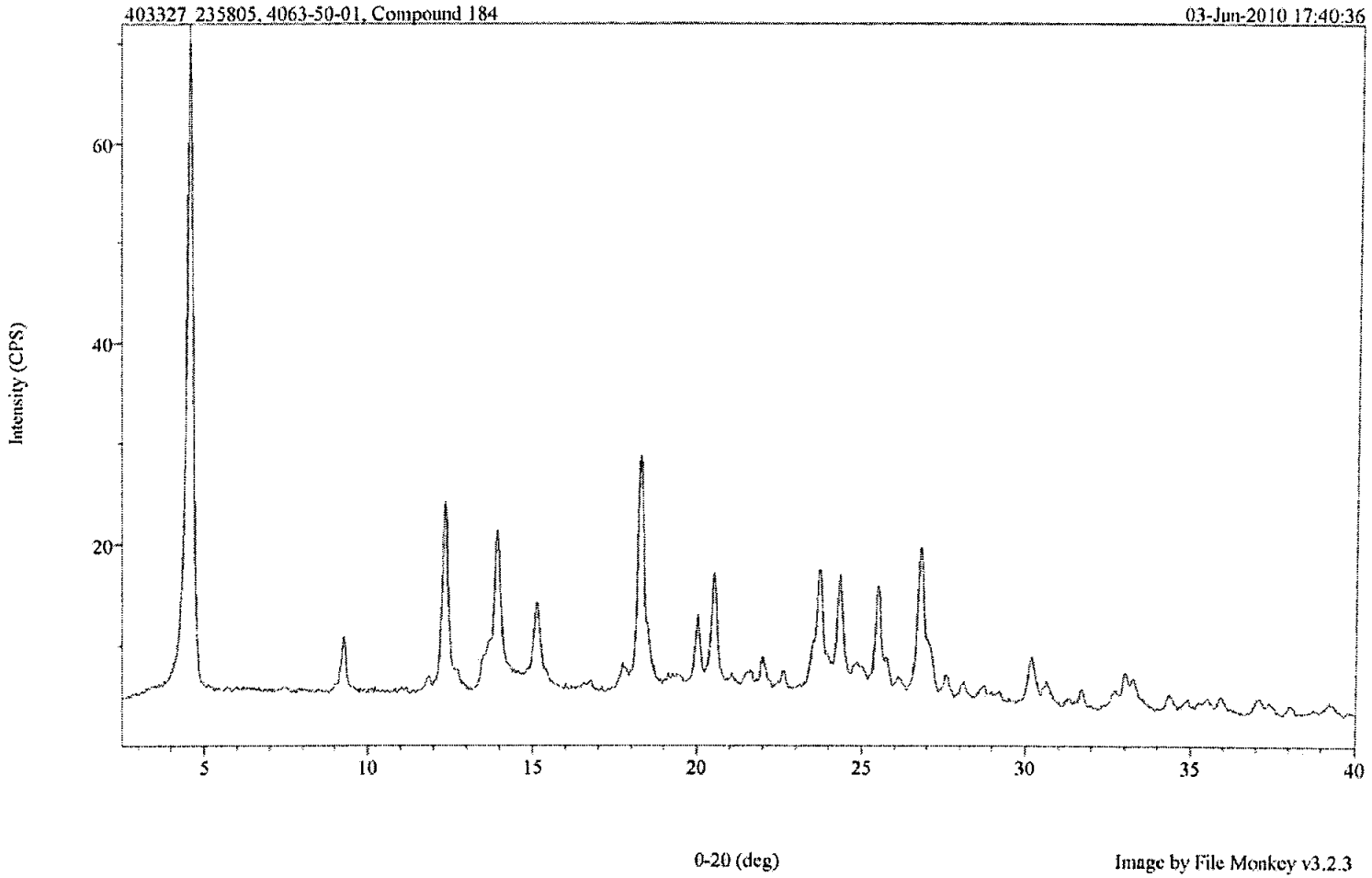
Spinning capillary. Step size: approximately 0.03 °2θ.



INEL XRG-3000
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Spinning capillary. Step size: approximately 0.03 °2θ.

403328_235806_4063-51-01, Compound 184 03-Jun-2010 17:51:57

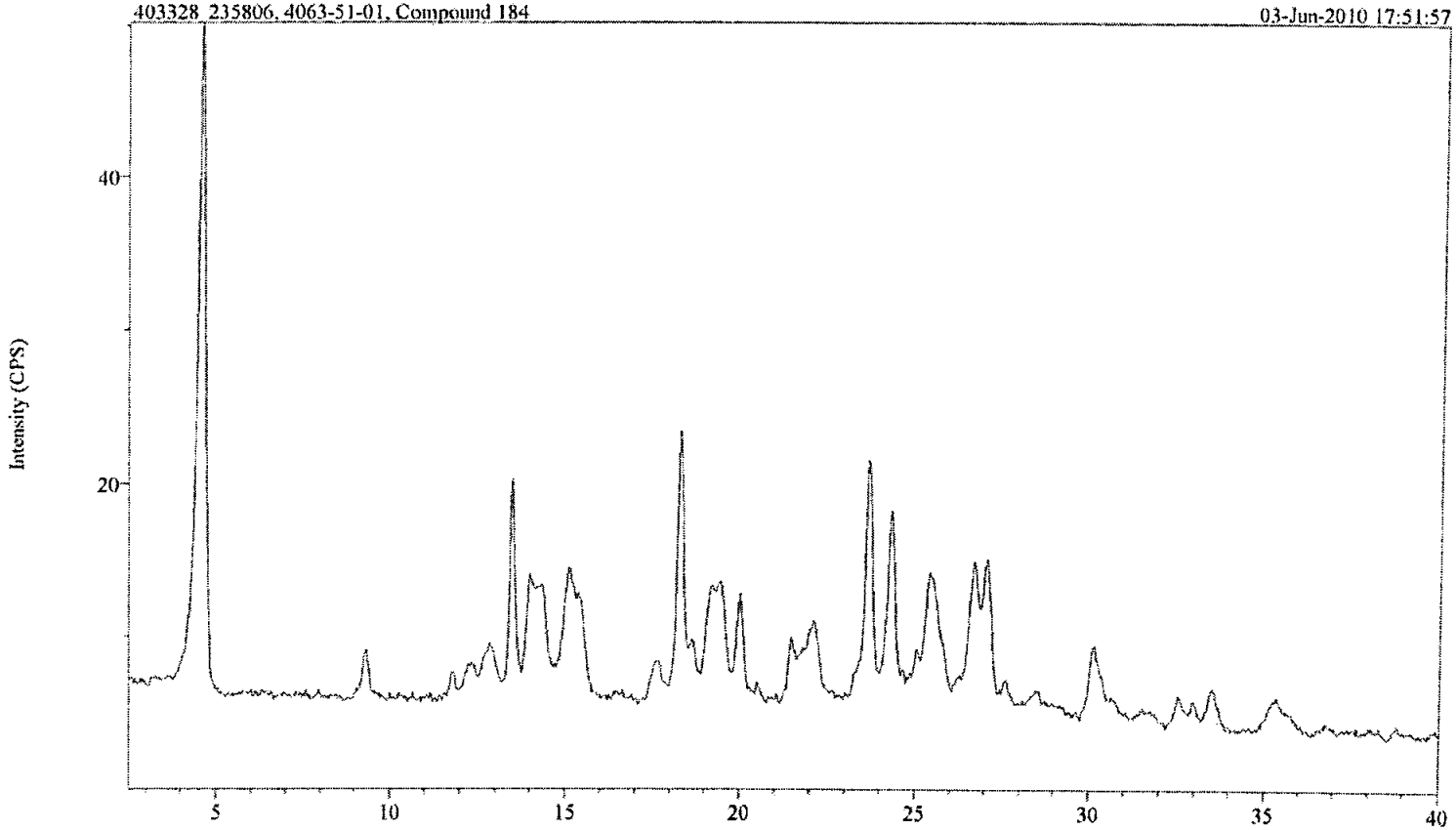
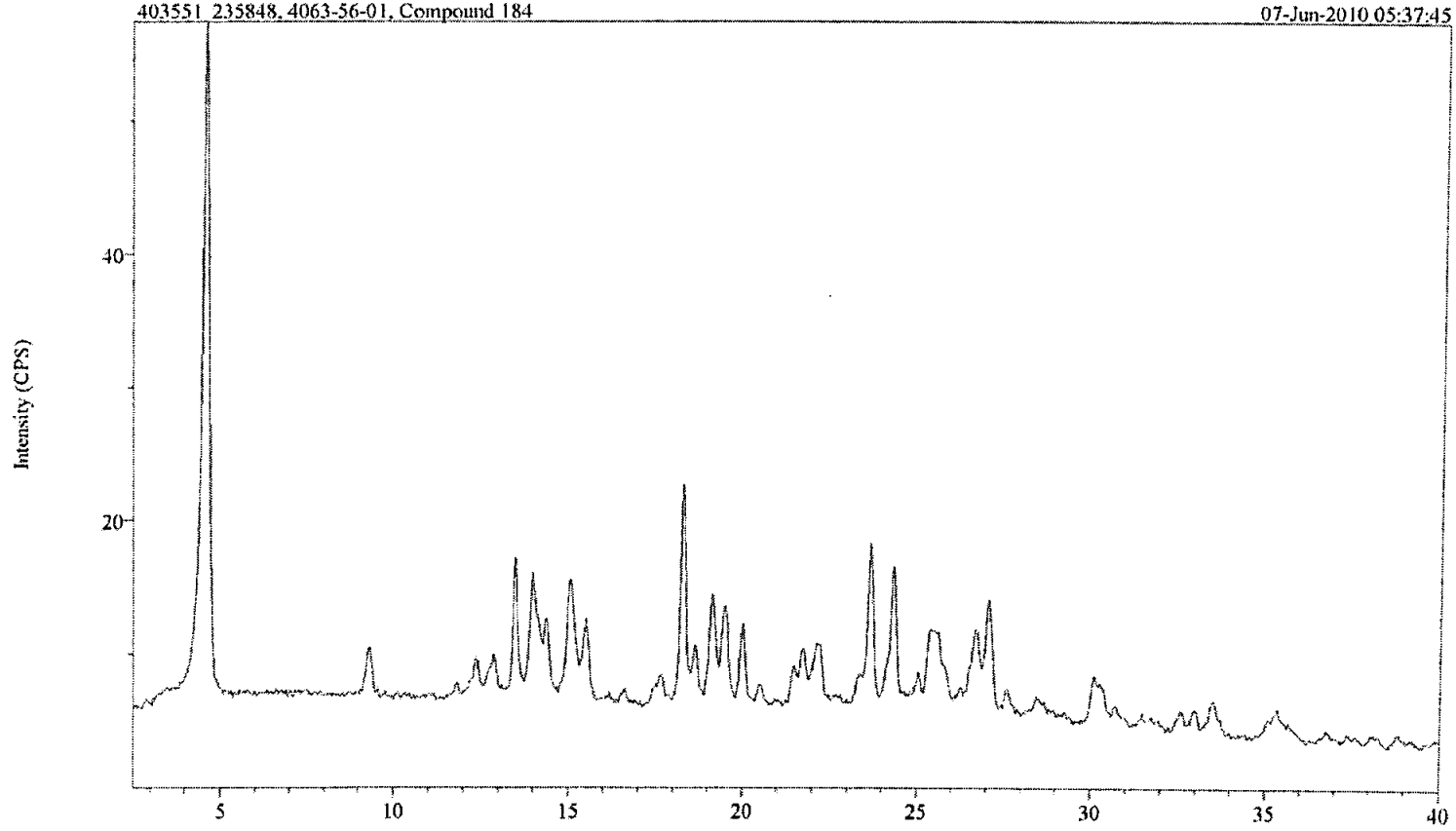


Image by File Monkey v3.2.3

INEL XRG-3000
X-ray Tube: 1.54187000 Å Voltage: 40 (kV) Amperage: 30 (mA) Acquisition Time: 300 sec
Spinning capillary. Step size: approximately 0.03 °2θ.

403551 235848, 4063-56-01, Compound 184 07-Jun-2010 05:37:45



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Spinning capillary. Step size: approximately 0.03 °2θ.

403552_235849, 4063-57-01, Compound 184 07-Jun-2010 05:49:07

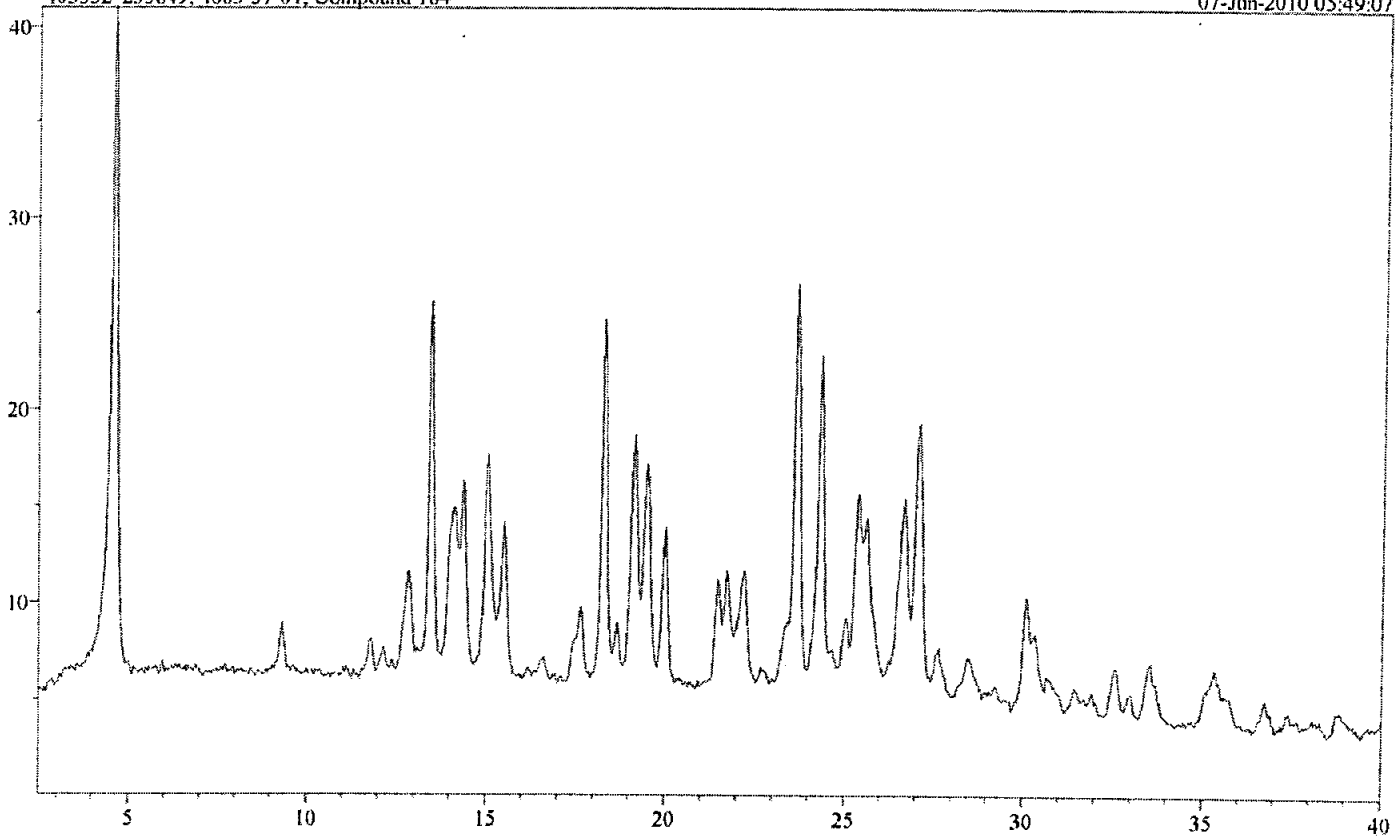


Image by File Monkey v3.2.3

EXHIBIT J

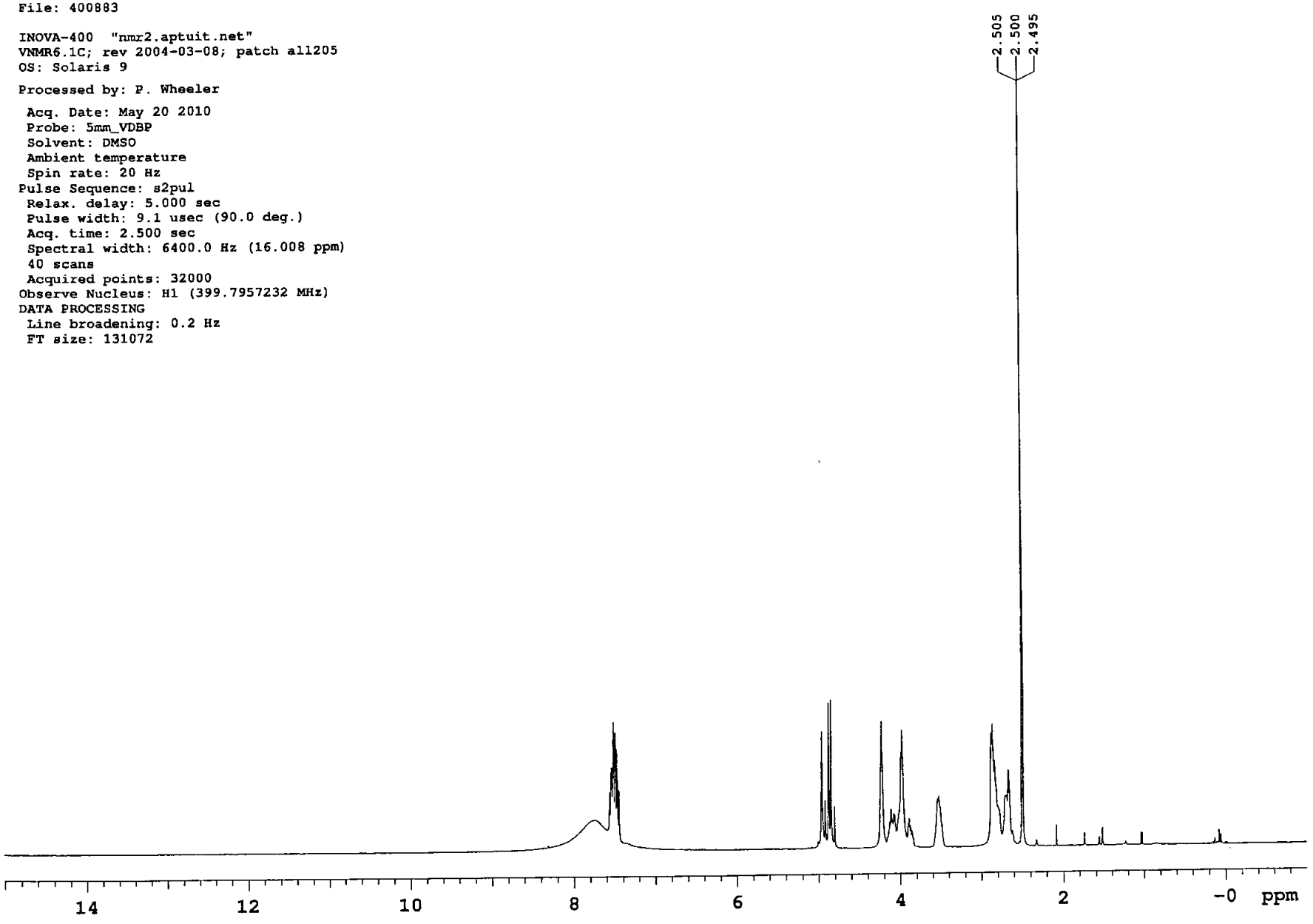
233140, 4063-02-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 400883

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

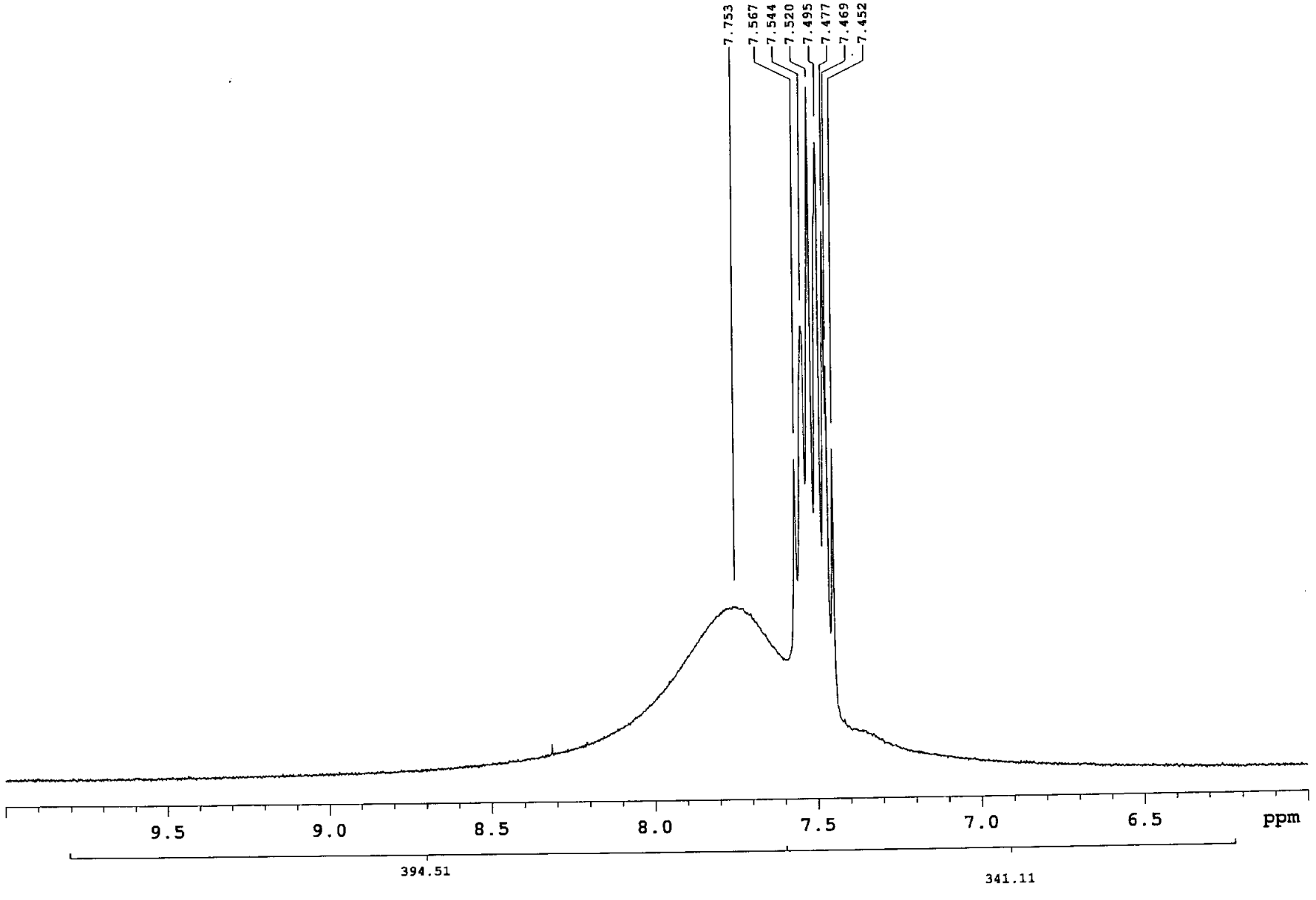
Acq. Date: May 20 2010
Probe: 5mm_VDBP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 9.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 400883-1

233140, 4063-02-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

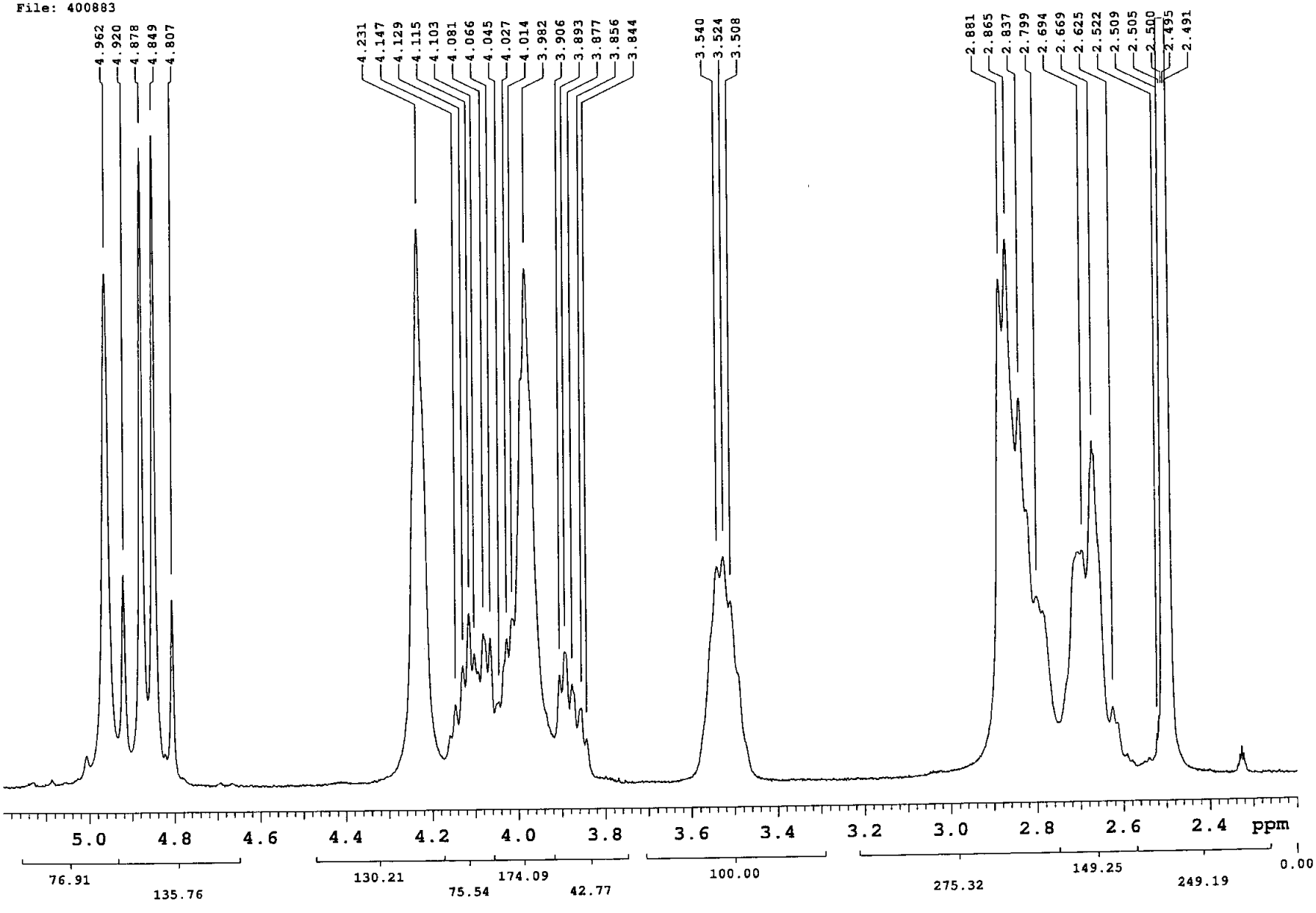
File: 400883



Plot file: 400883-2

233140, 4063-02-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

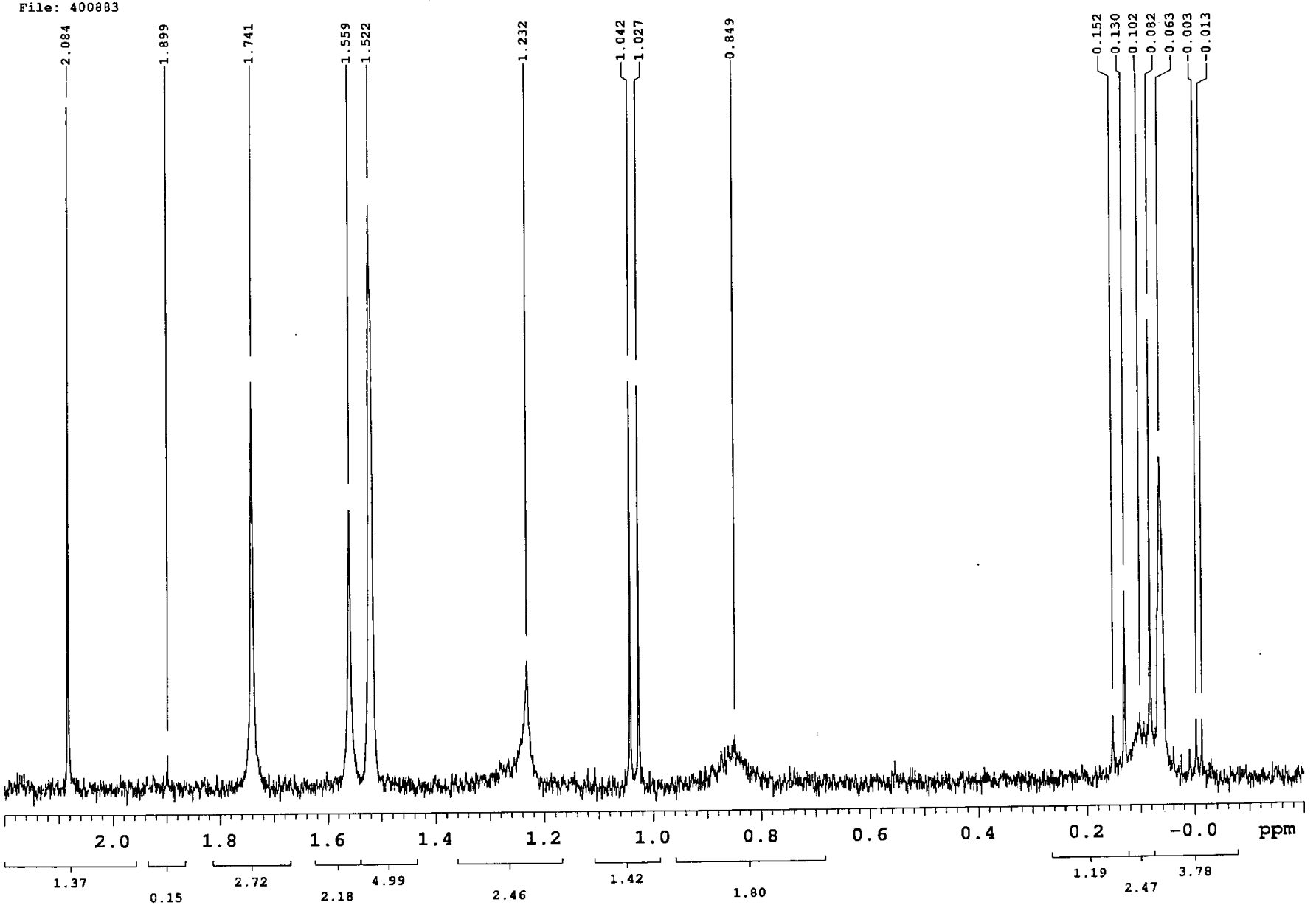
File: 400883



Plot file: 400883-3

233140, 4063-02-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 400883



Plot file: 400883-4

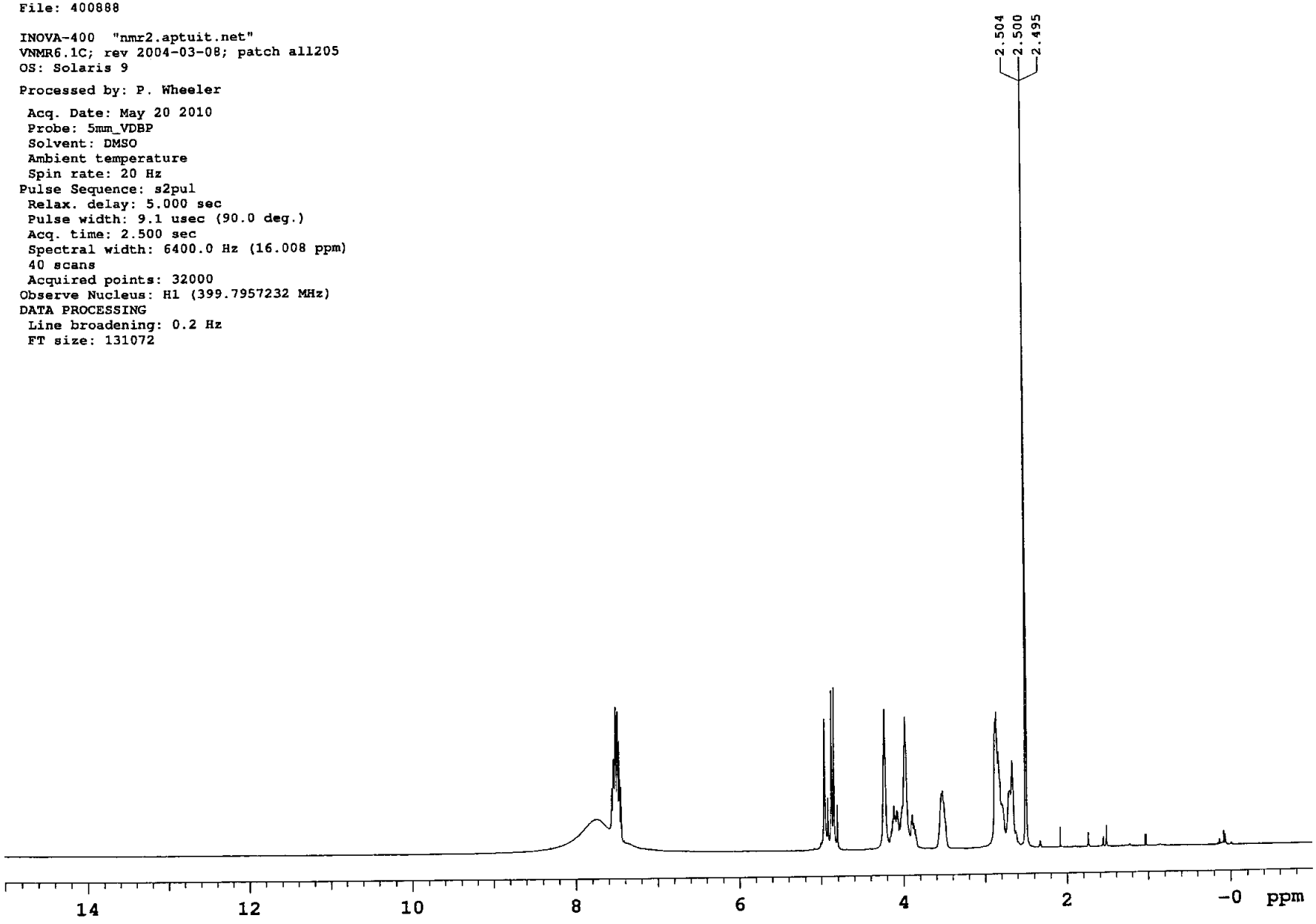
233141, 4063-03-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 400888

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

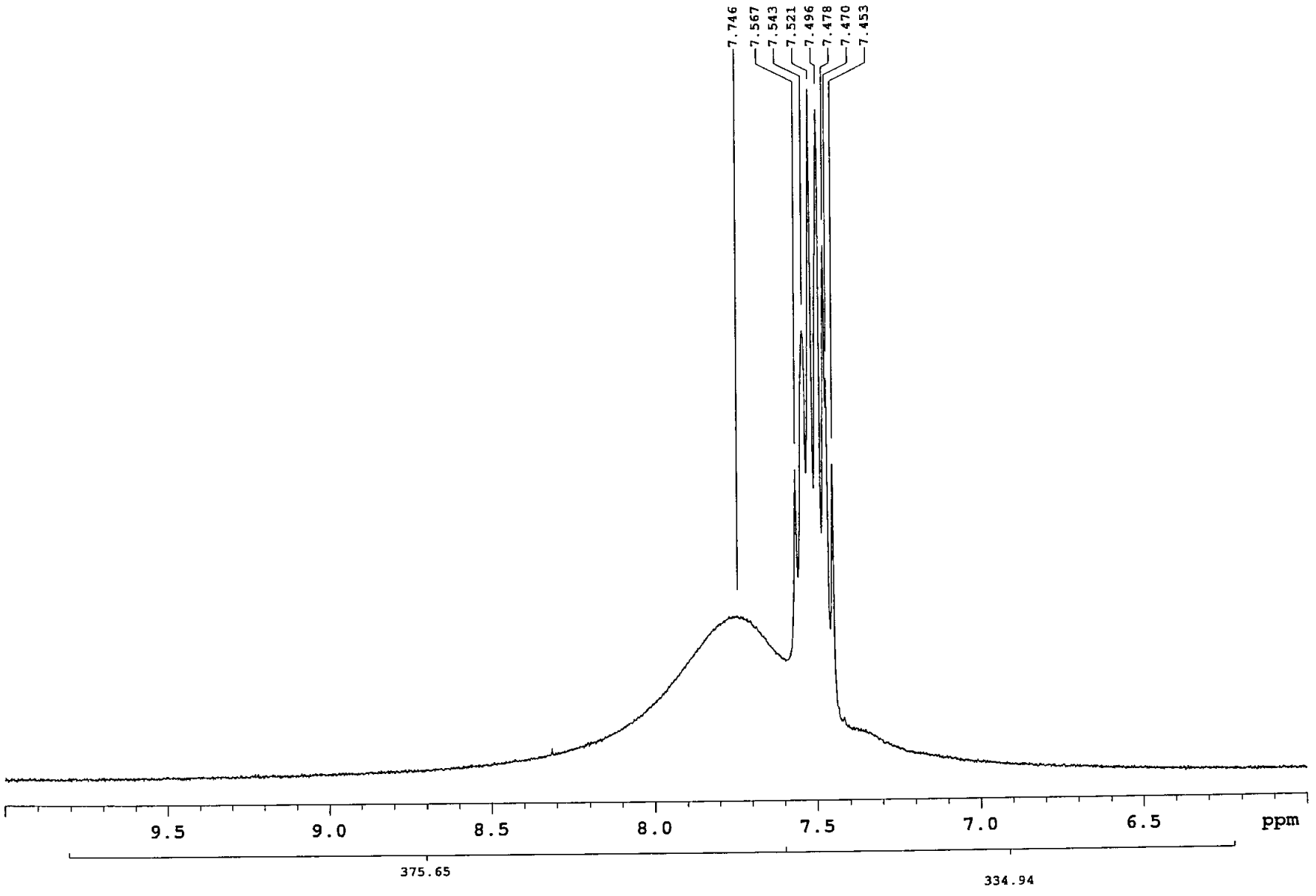
Acq. Date: May 20 2010
Probe: 5mm_VDBP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 9.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 400888-1

233141, 4063-03-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

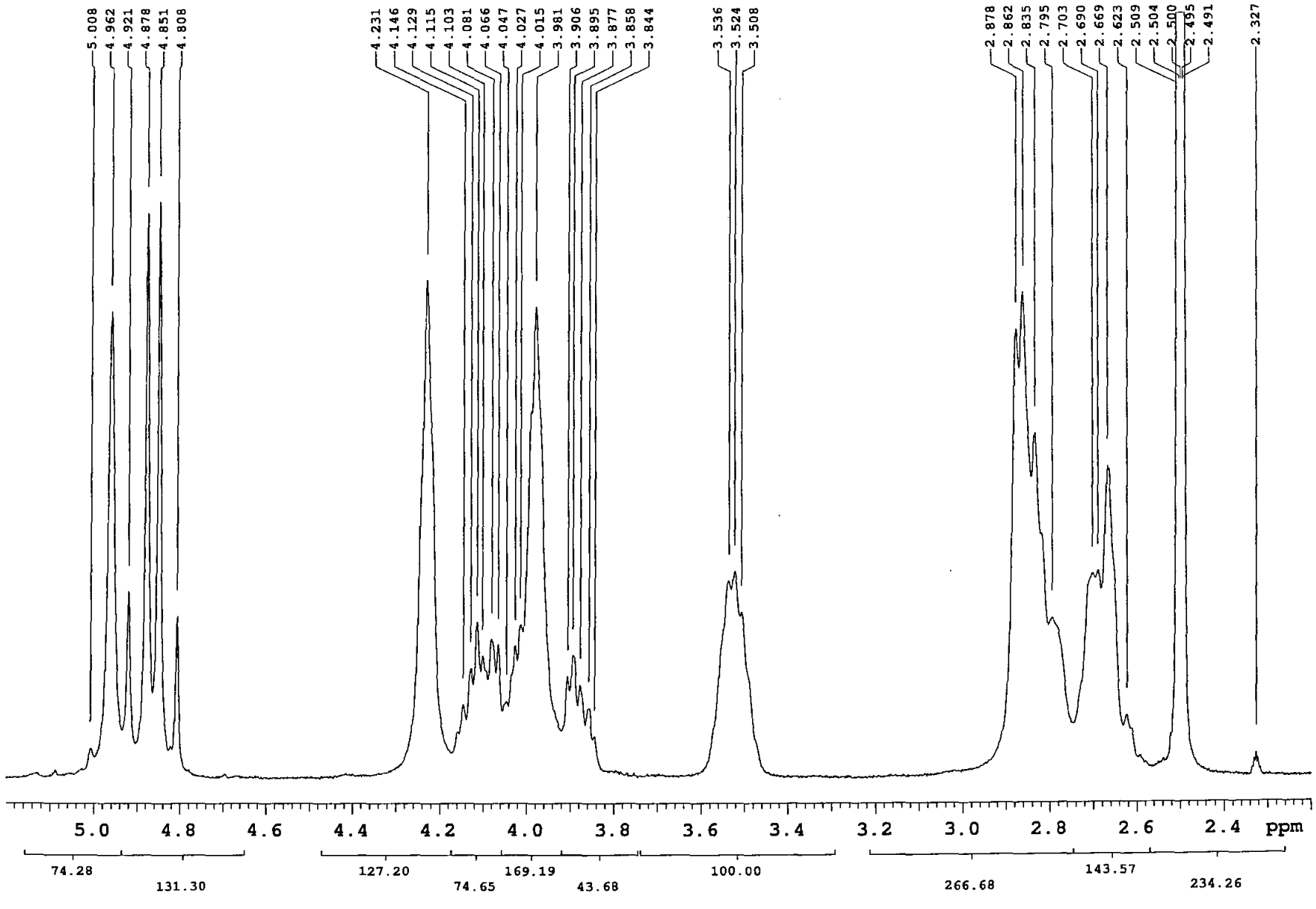
File: 400888



Plot file: 400888-2

233141, 4063-03-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

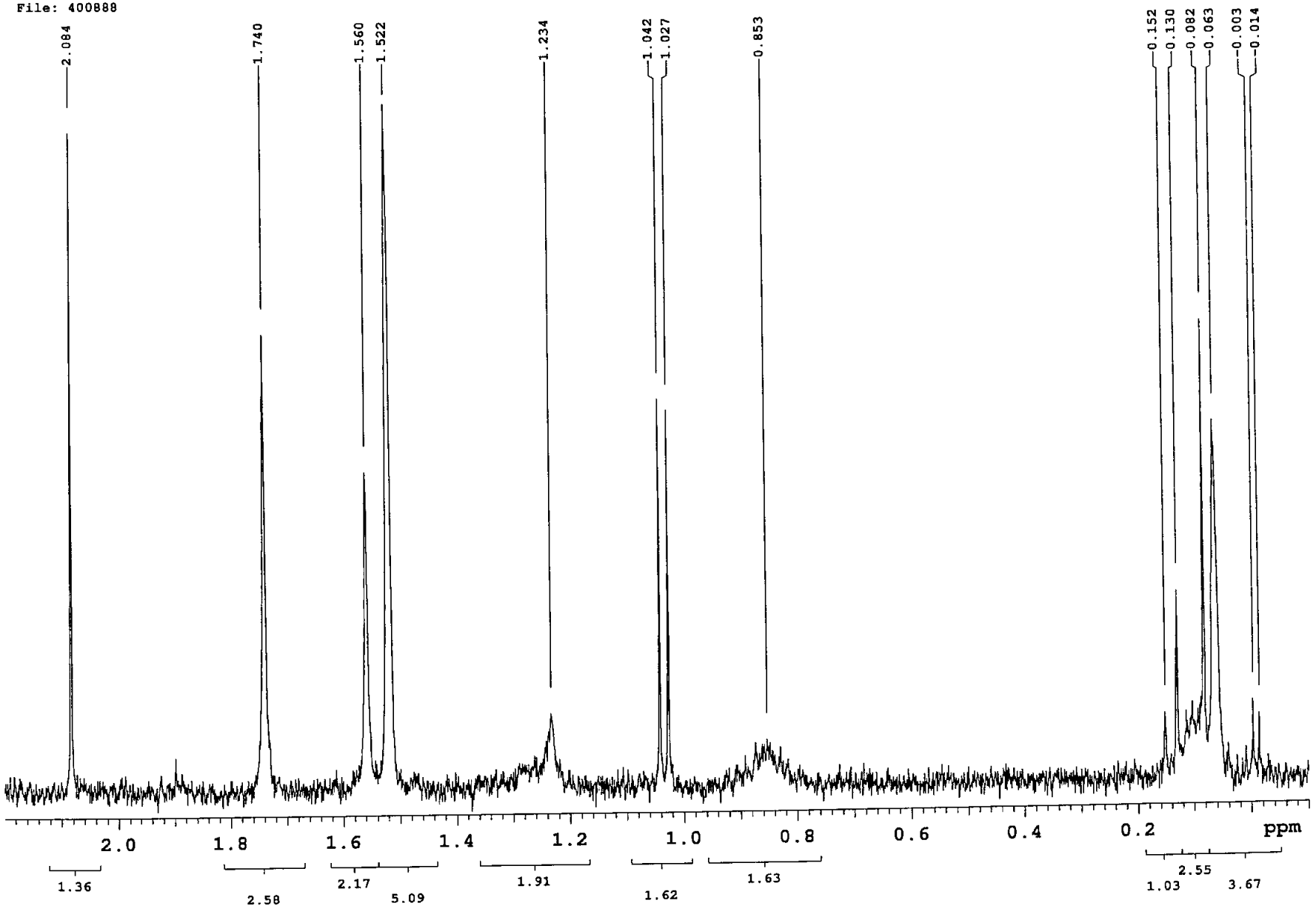
File: 400888



Plot file: 400888-3

233141, 4063-03-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 400888



Plot file: 400888-4

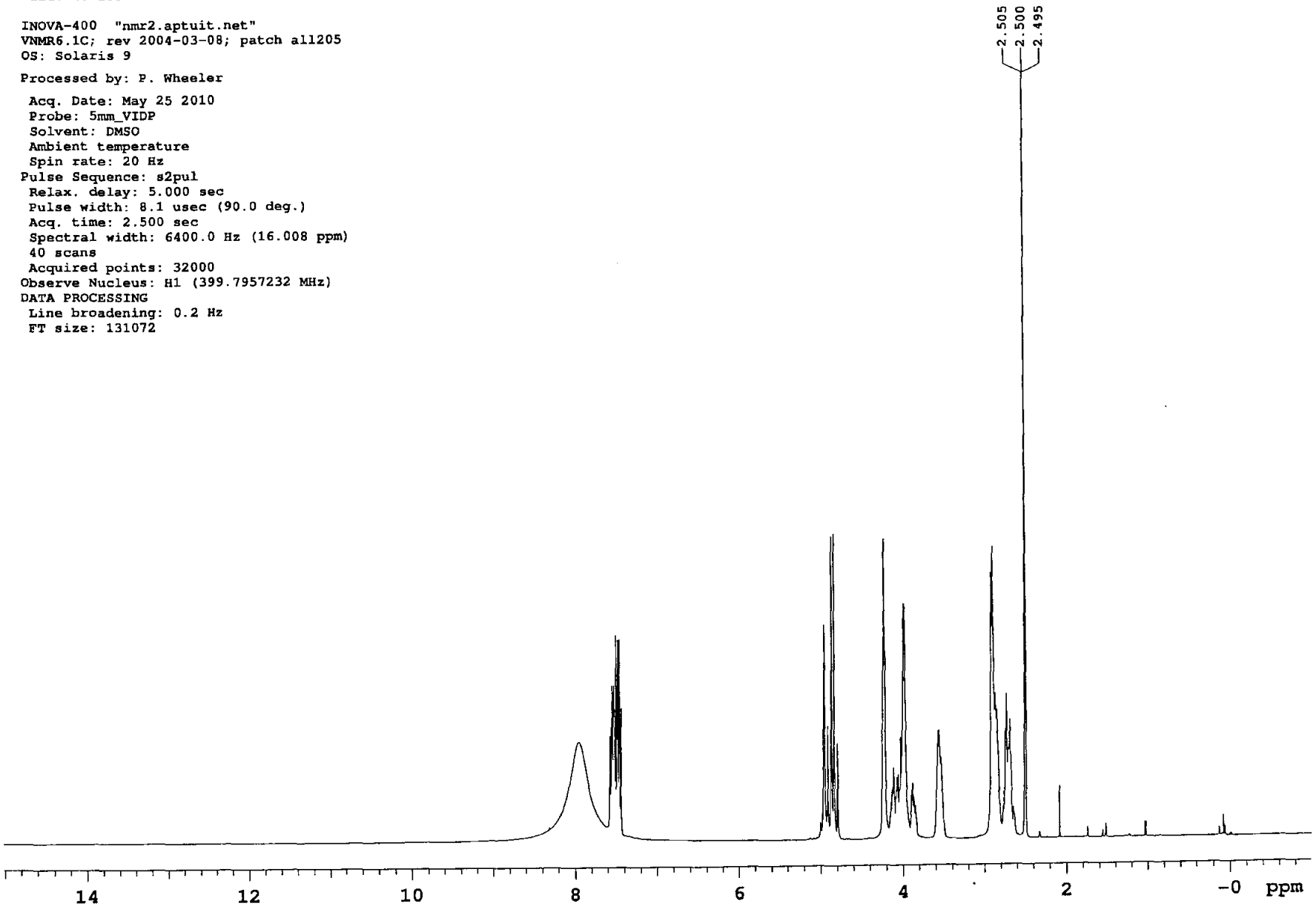
234636, 4063-18-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401158

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

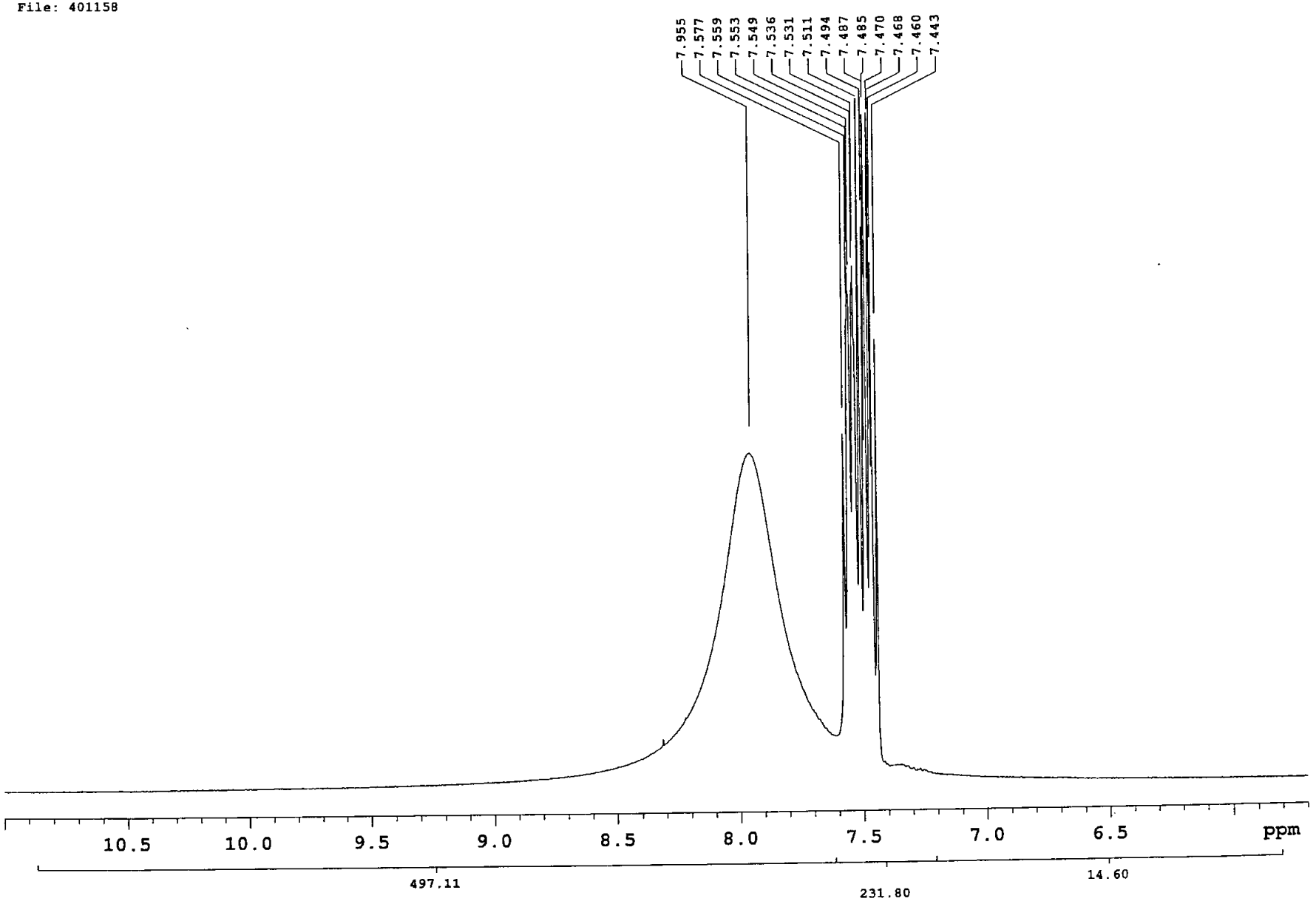
Acq. Date: May 25 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 401158-1

234636, 4063-18-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

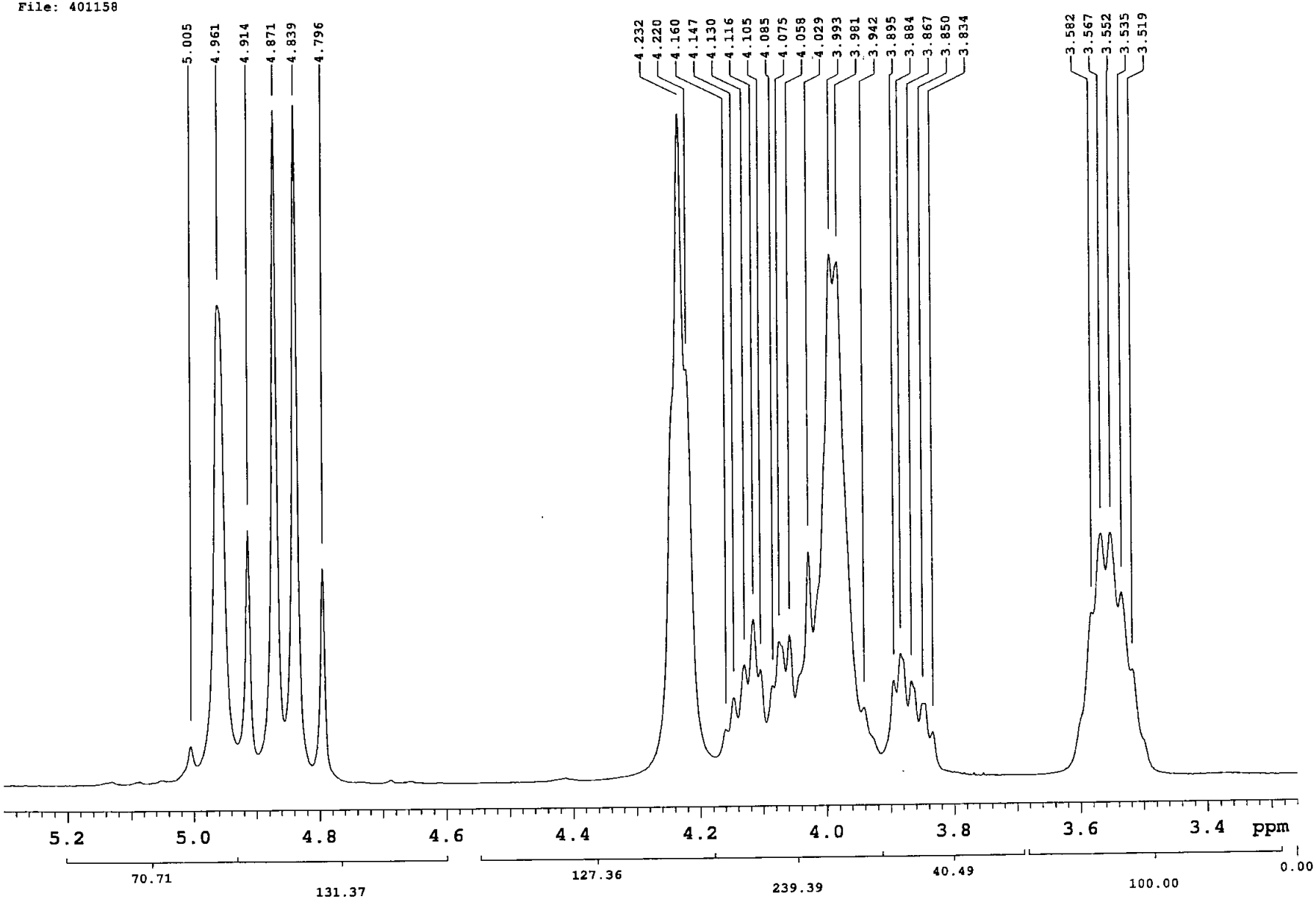
File: 401158



Plot file: 401158-2

234636, 4063-18-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

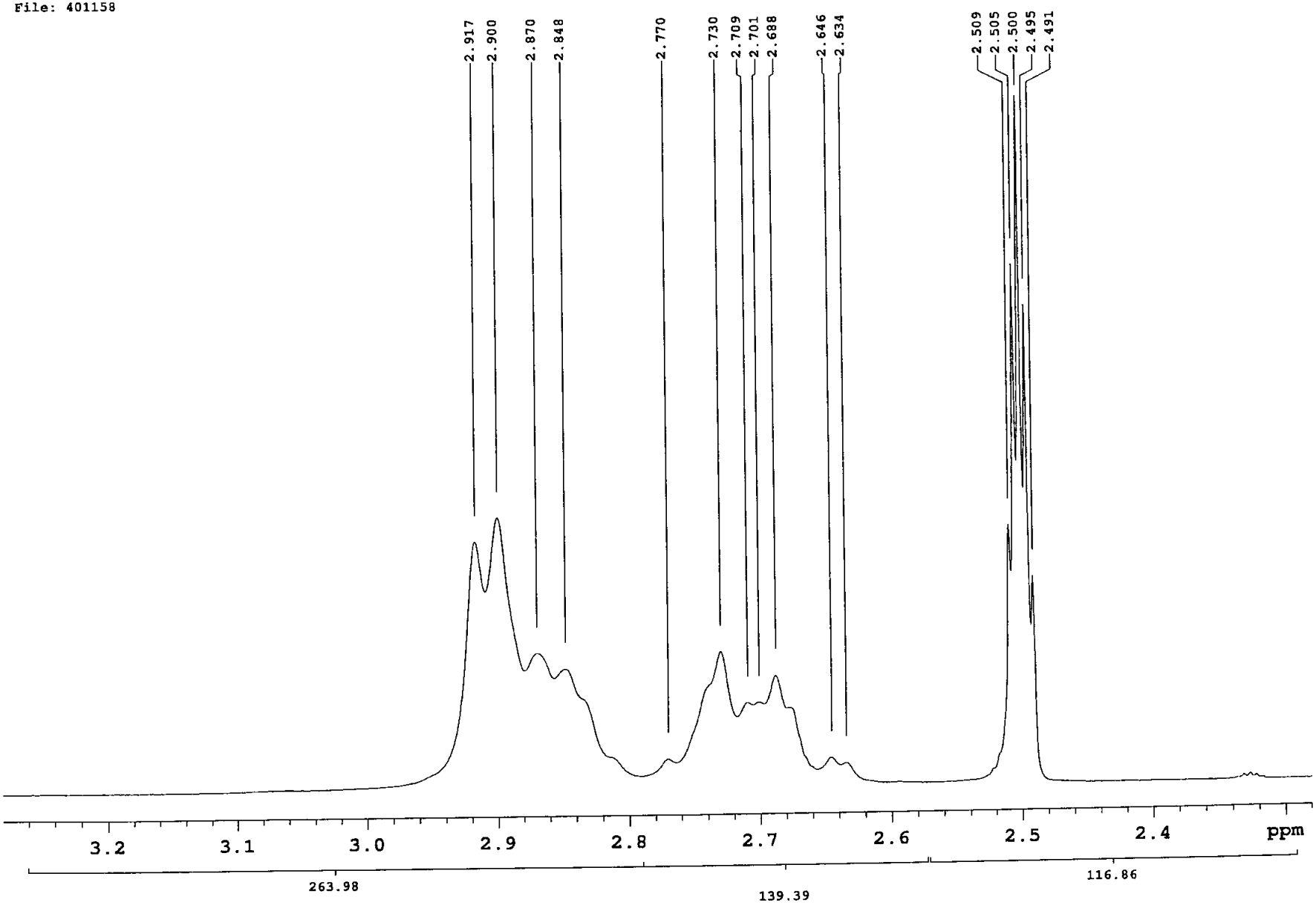
File: 401158



Plot file: 401158-3

234636, 4063-18-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

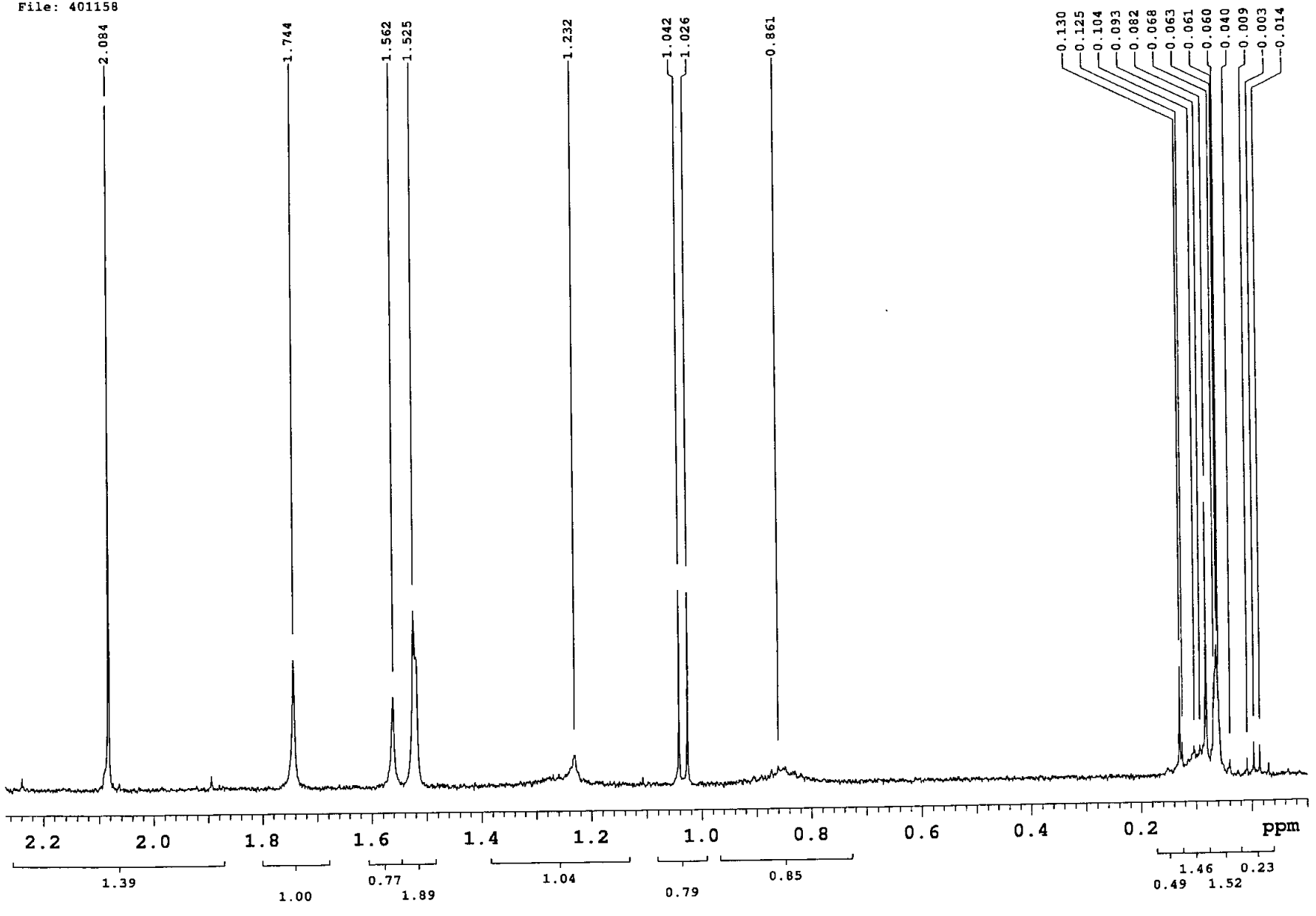
File: 401158



Plot file: 401158-4

234636, 4063-18-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401158



Plot file: 401158-5

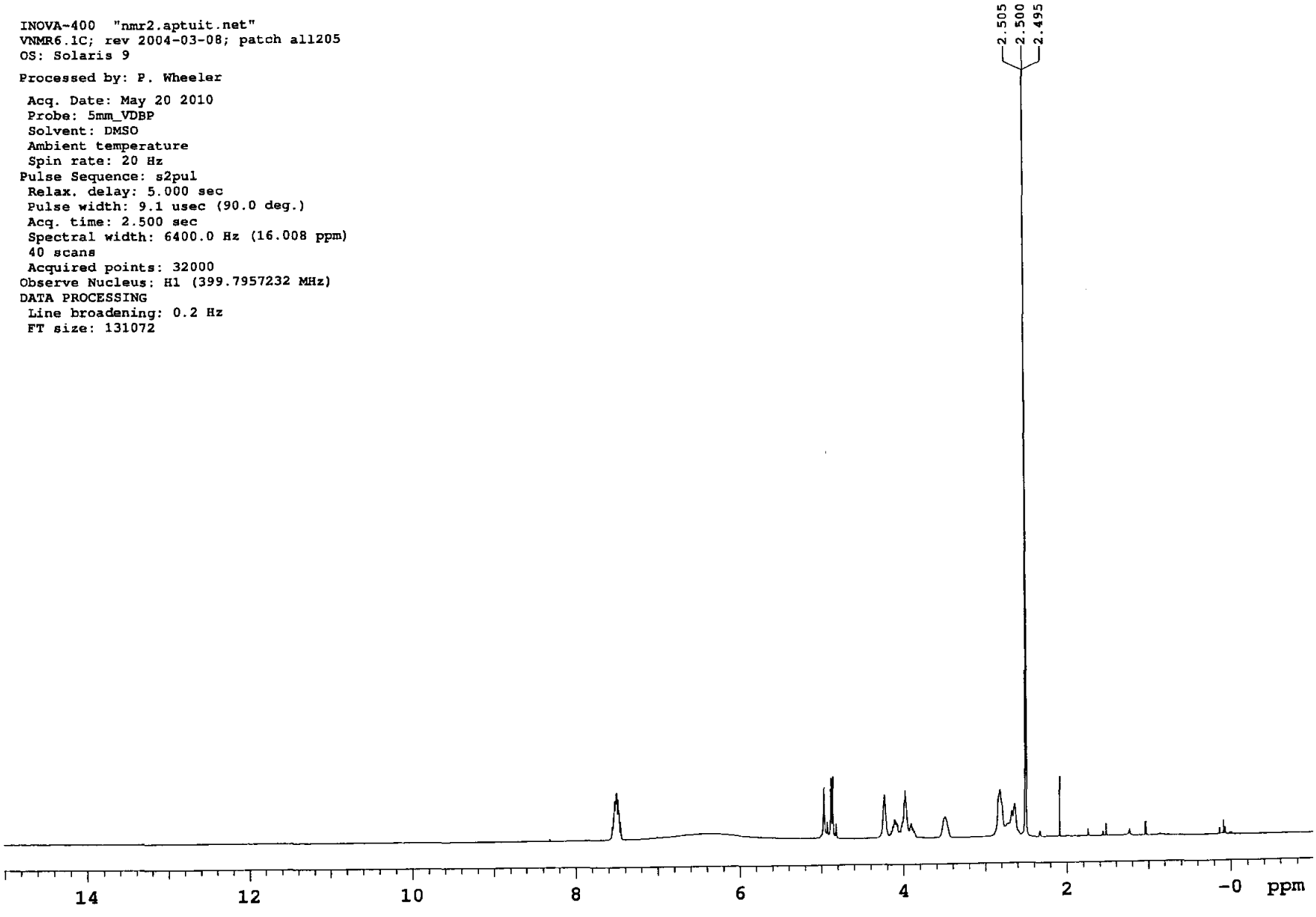
233142, 4063-04-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 400891

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

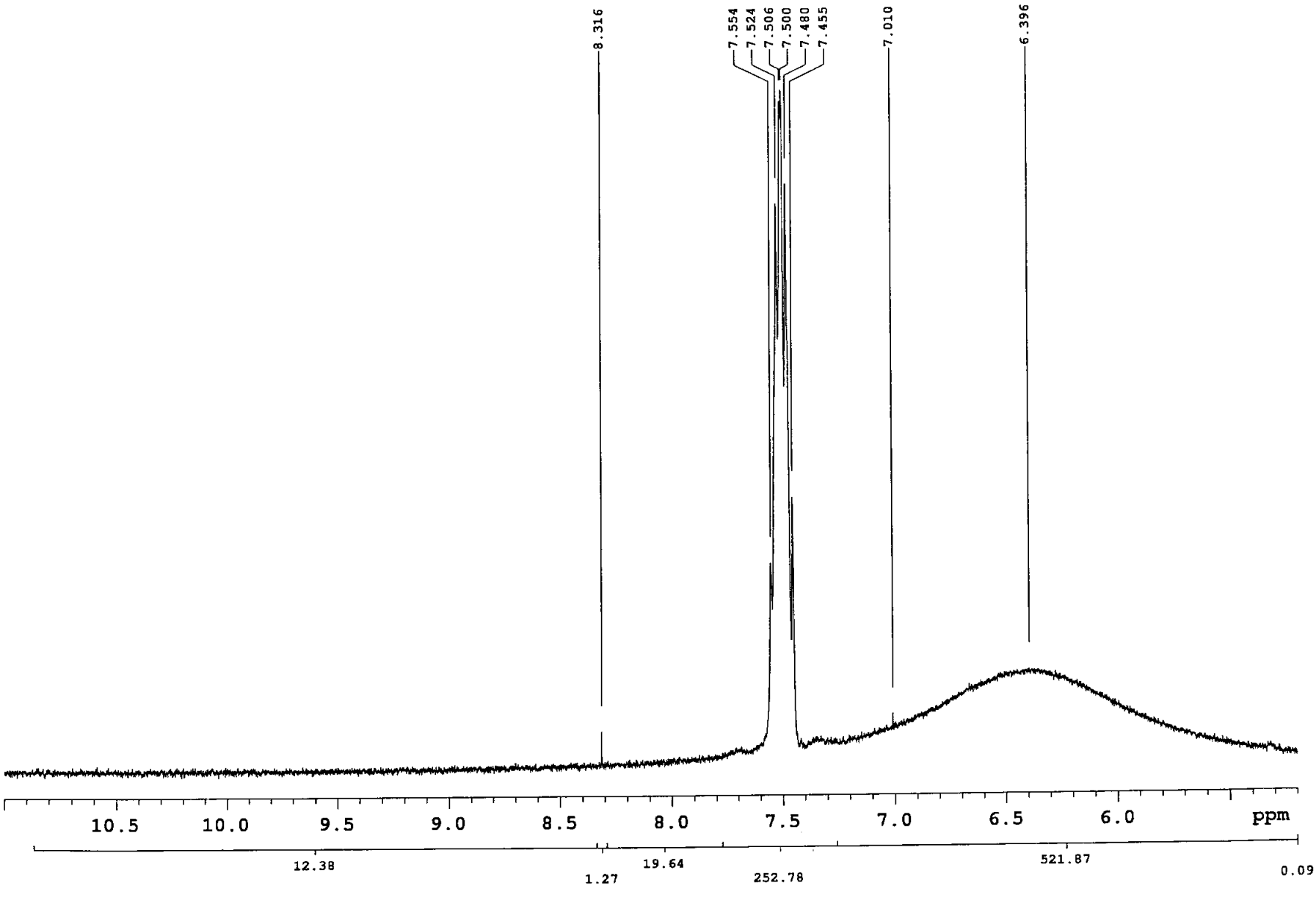
Acq. Date: May 20 2010
Probe: 5mm_VDBP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 9.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 400891-1

233142, 4063-04-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

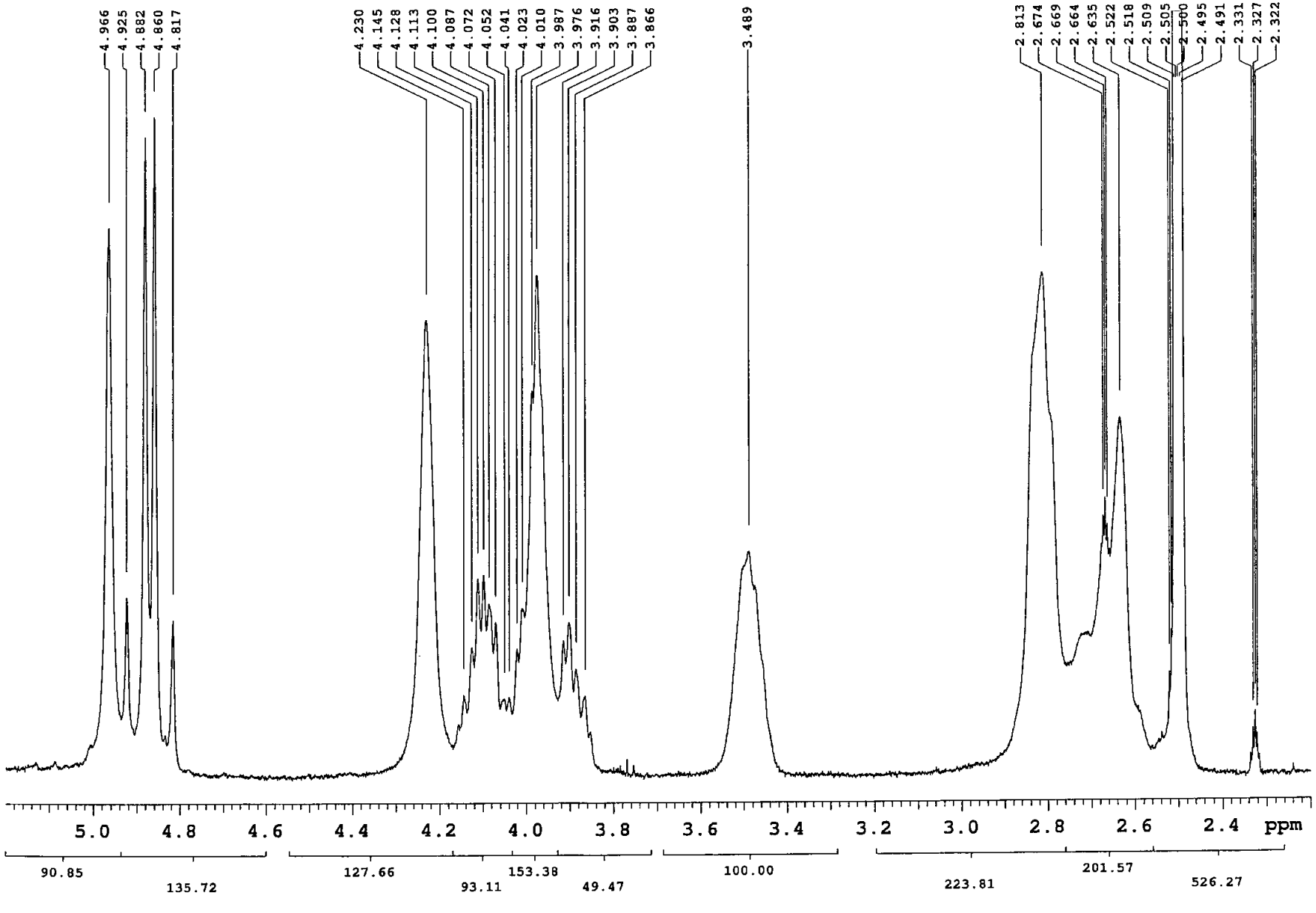
File: 400891



Plot file: 400891-2

233142, 4063-04-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

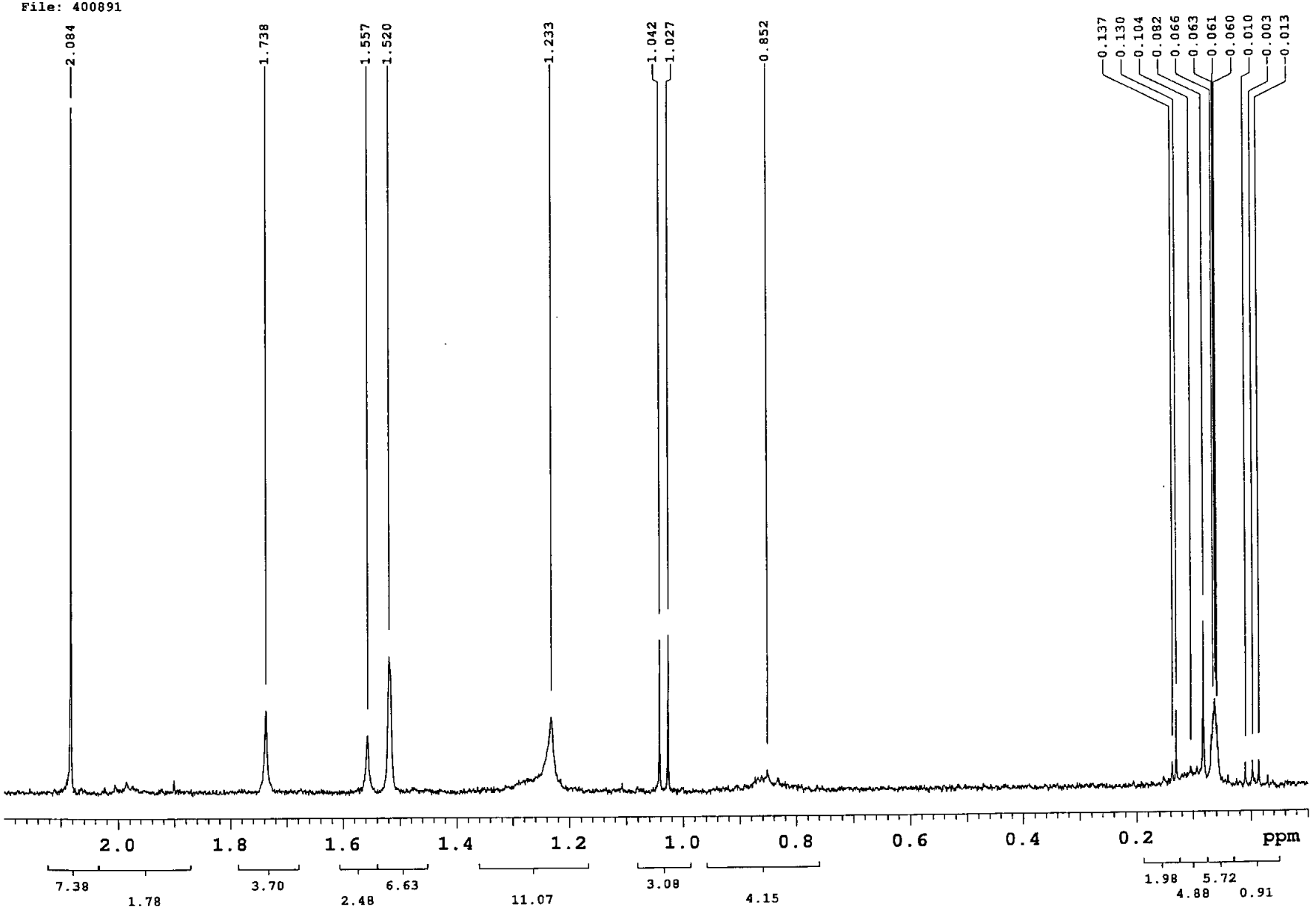
File: 400891



Plot file: 400891-3

233142, 4063-04-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 400891



Plot file: 400891-4

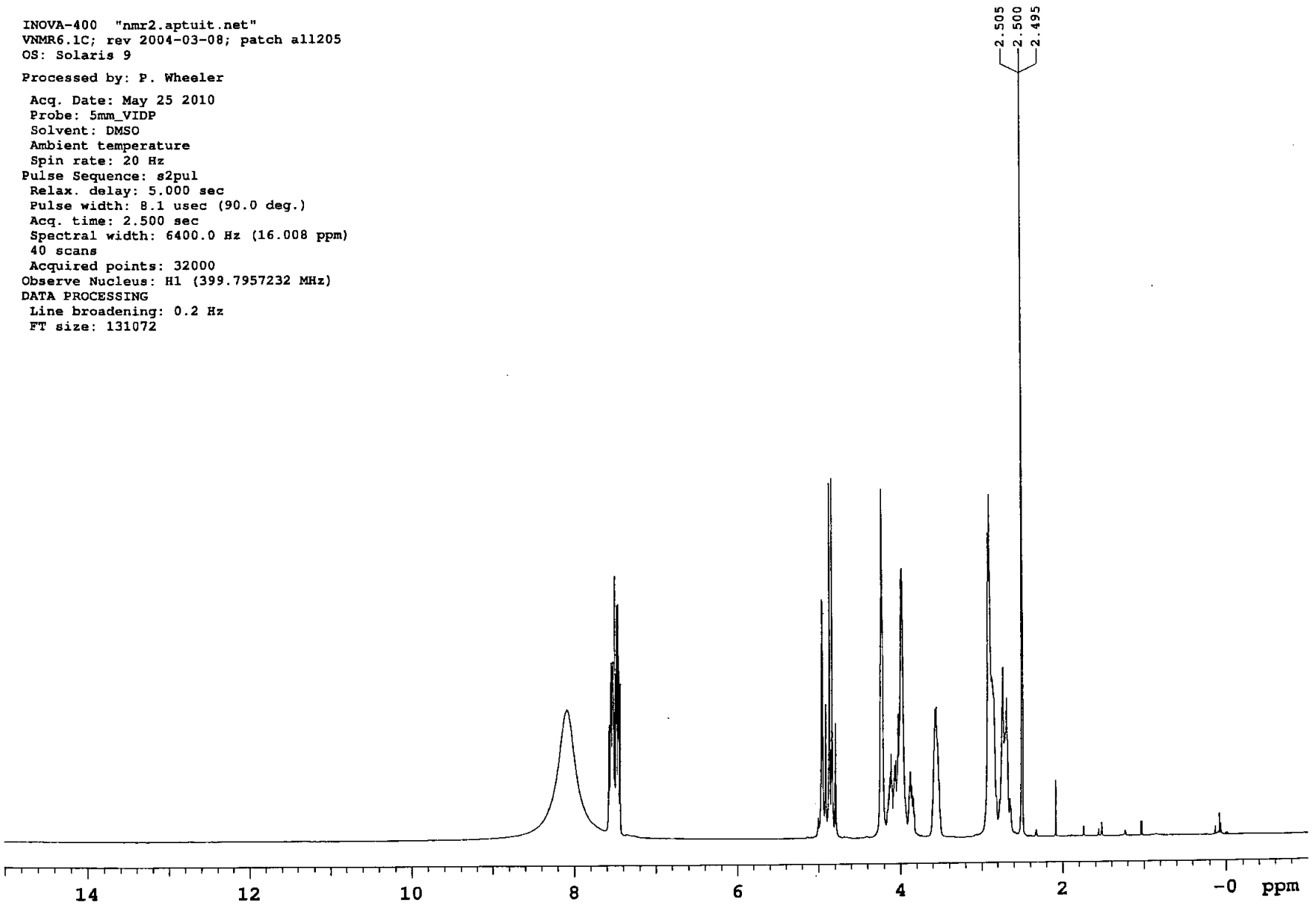
234584, 4063-19-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401064

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch a11205
OS: Solaris 9

Processed by: P. Wheeler

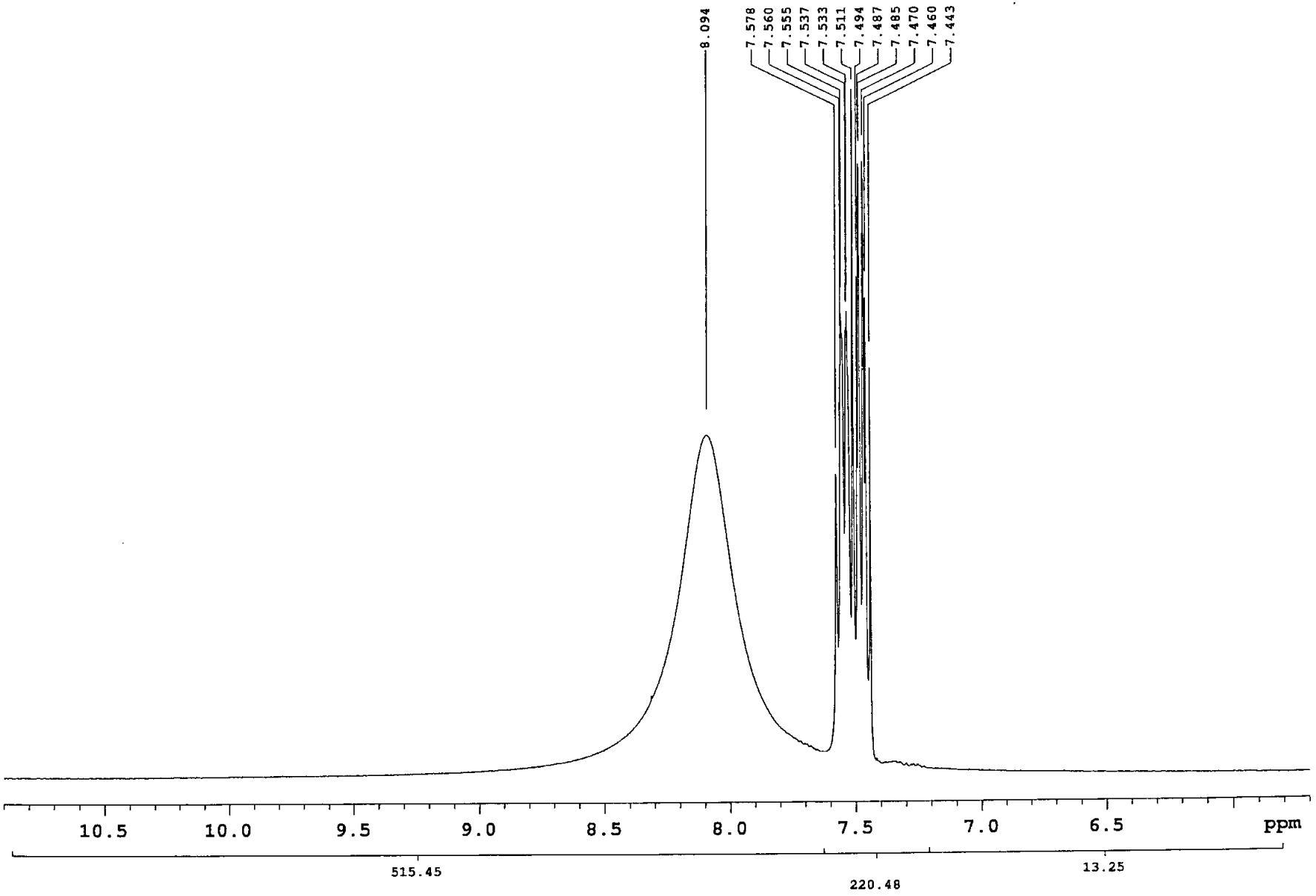
Acq. Date: May 25 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 401064-1

234584, 4063-19-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

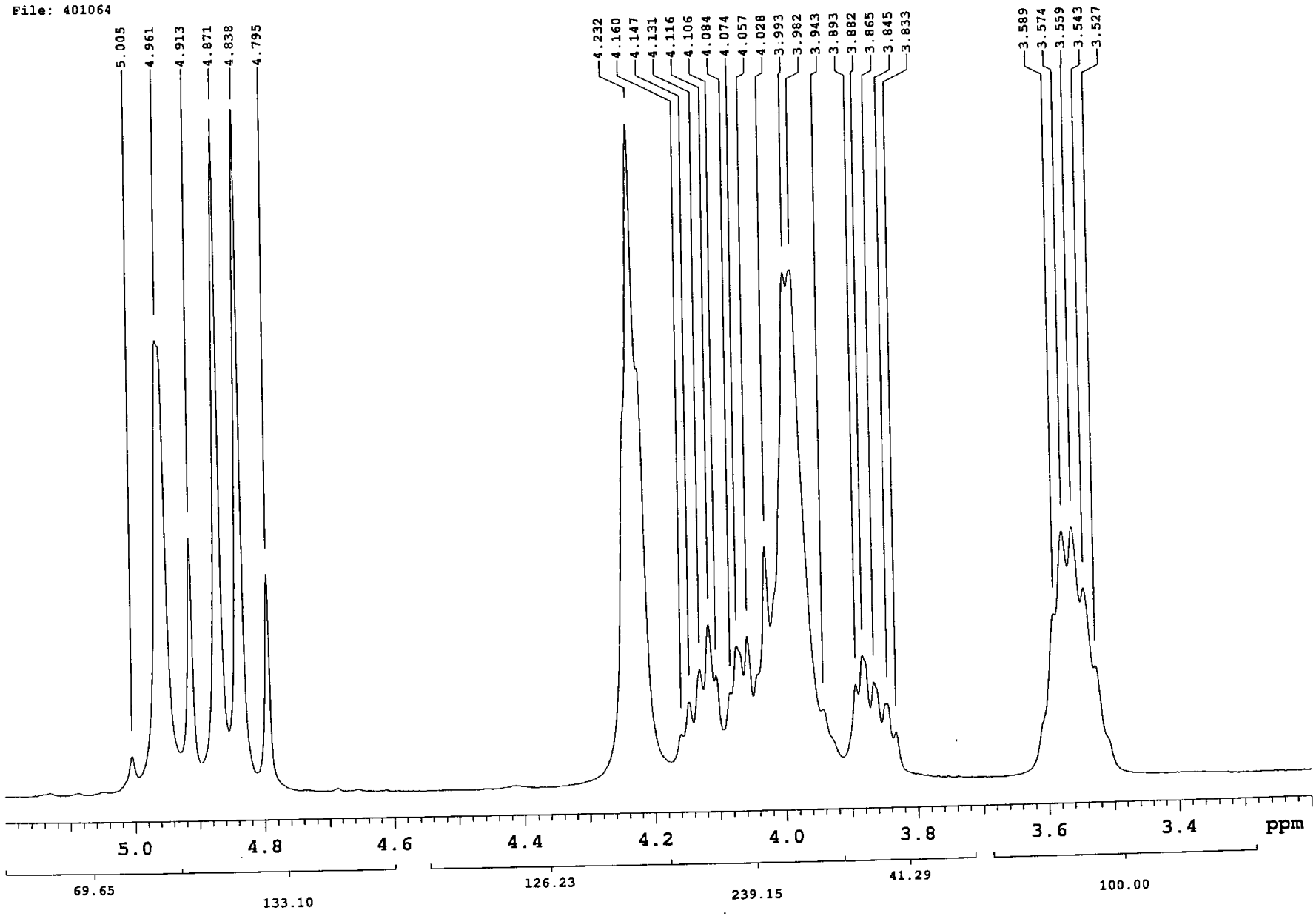
File: 401064



Plot file: 401064-2

234584, 4063-19-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

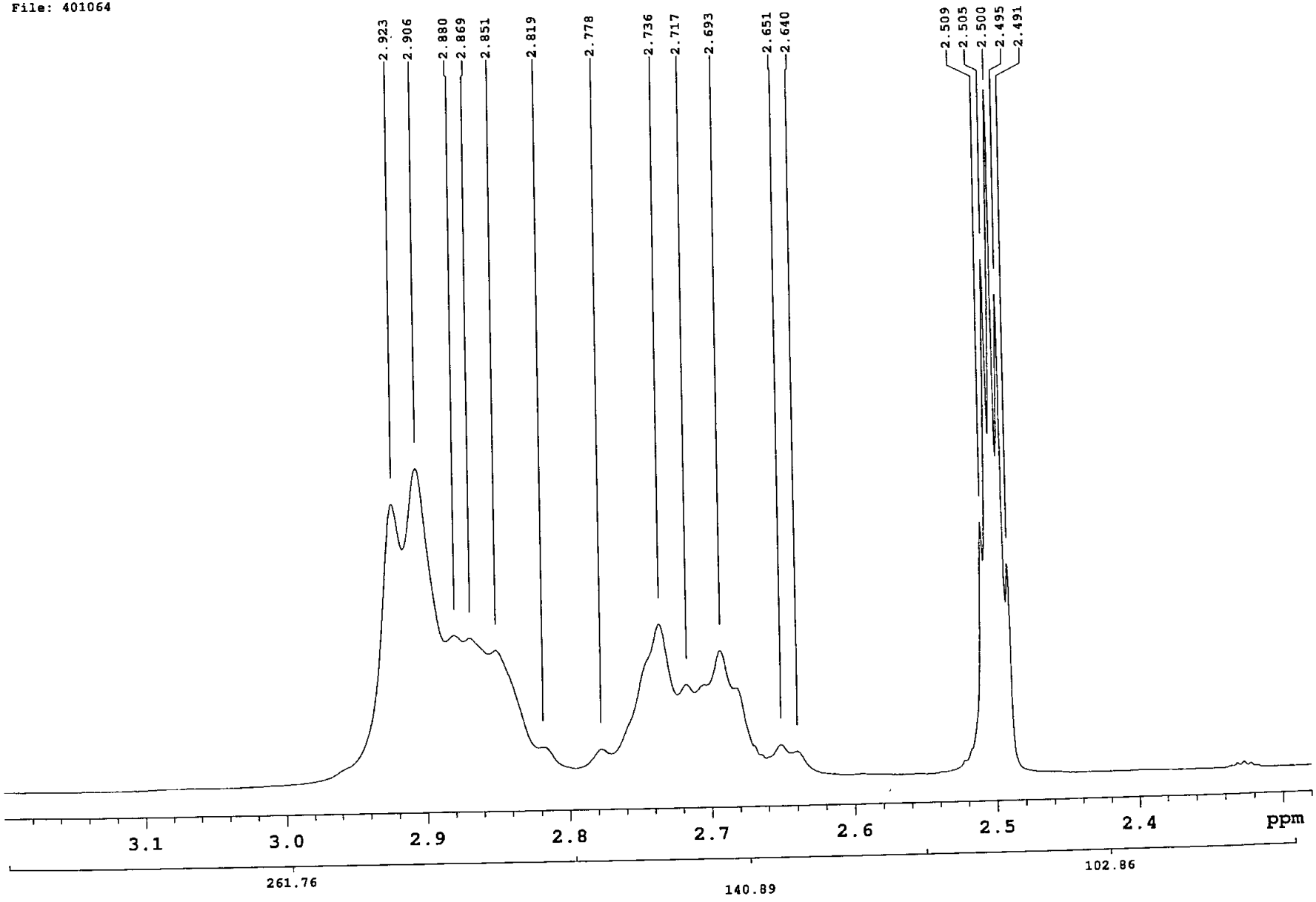
File: 401064



Plot file: 401064-3

234584, 4063-19-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

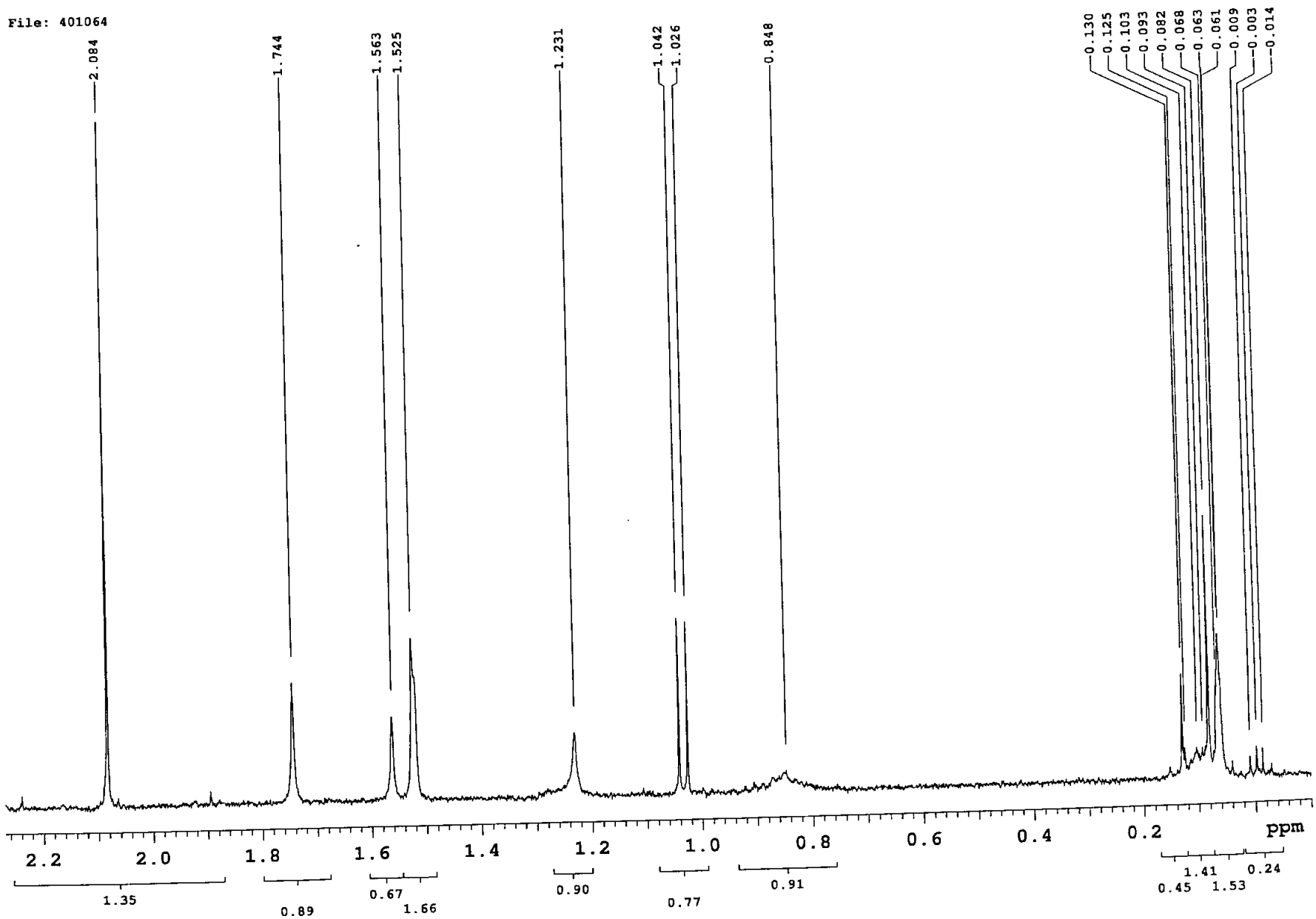
File: 401064



Plot file: 401064-4

234584, 4063-19-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401064



Plot file: 401064-5

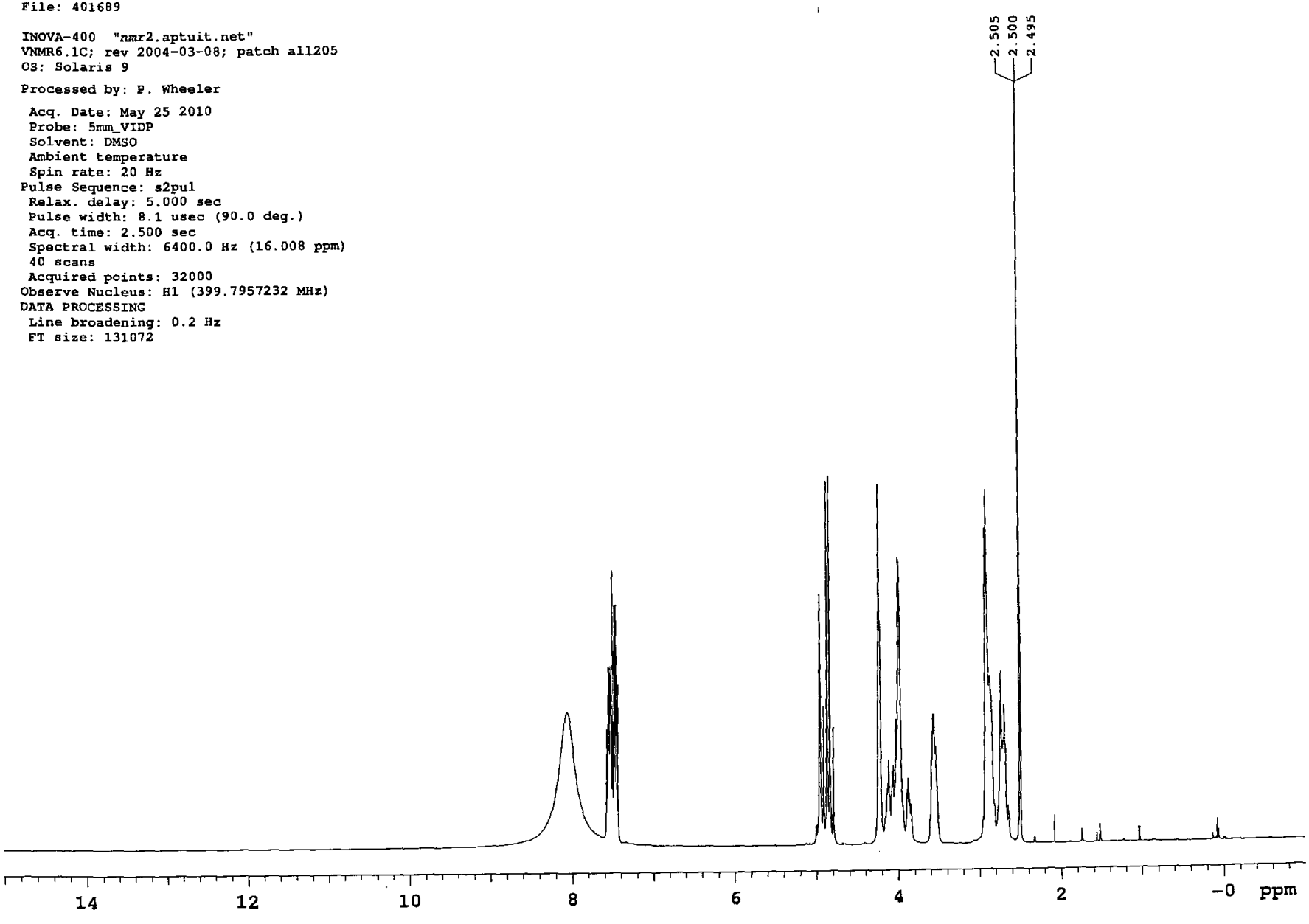
234874, 4063-35-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401689

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

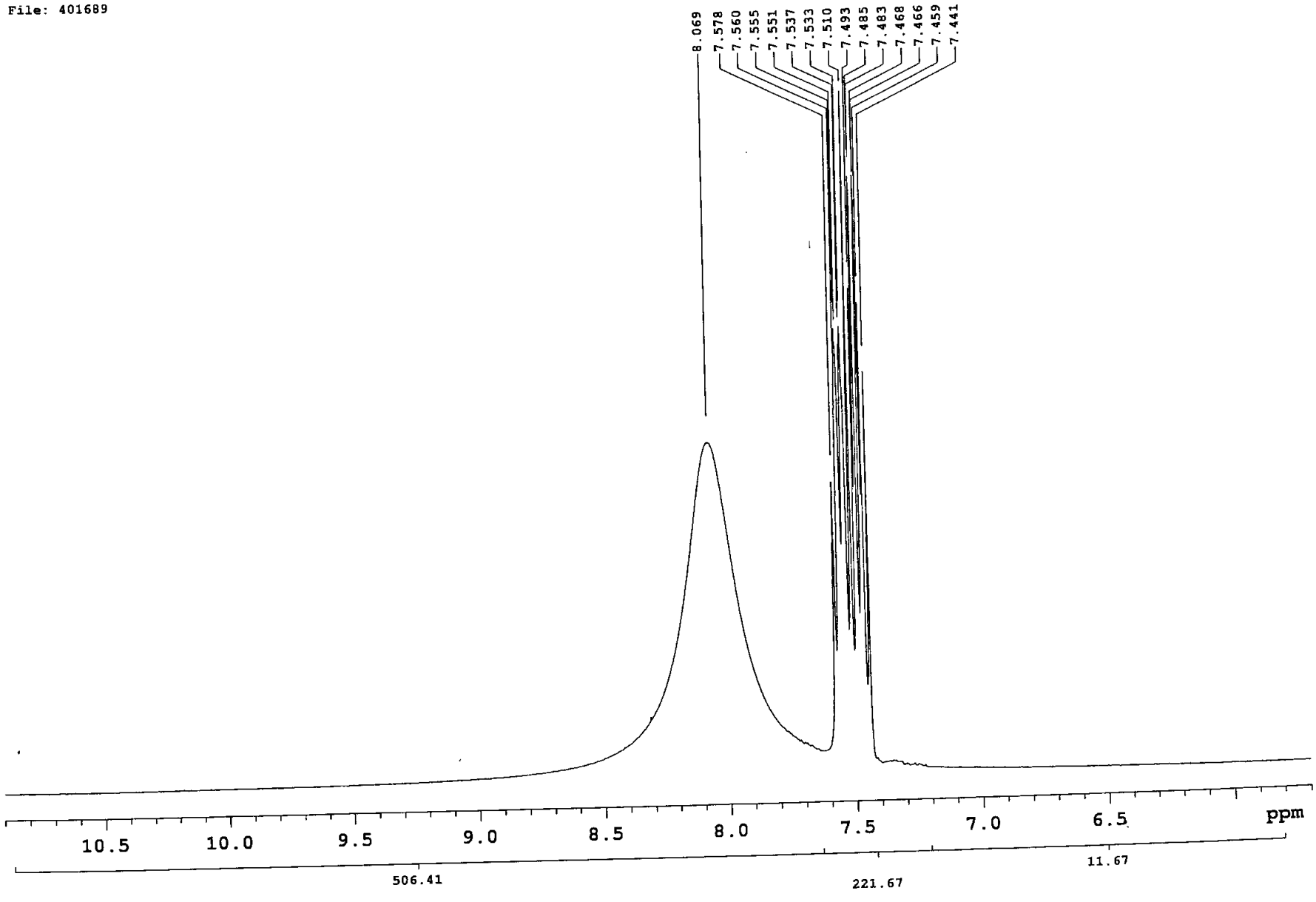
Acq. Date: May 25 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 401689-1

234874, 4063-35-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

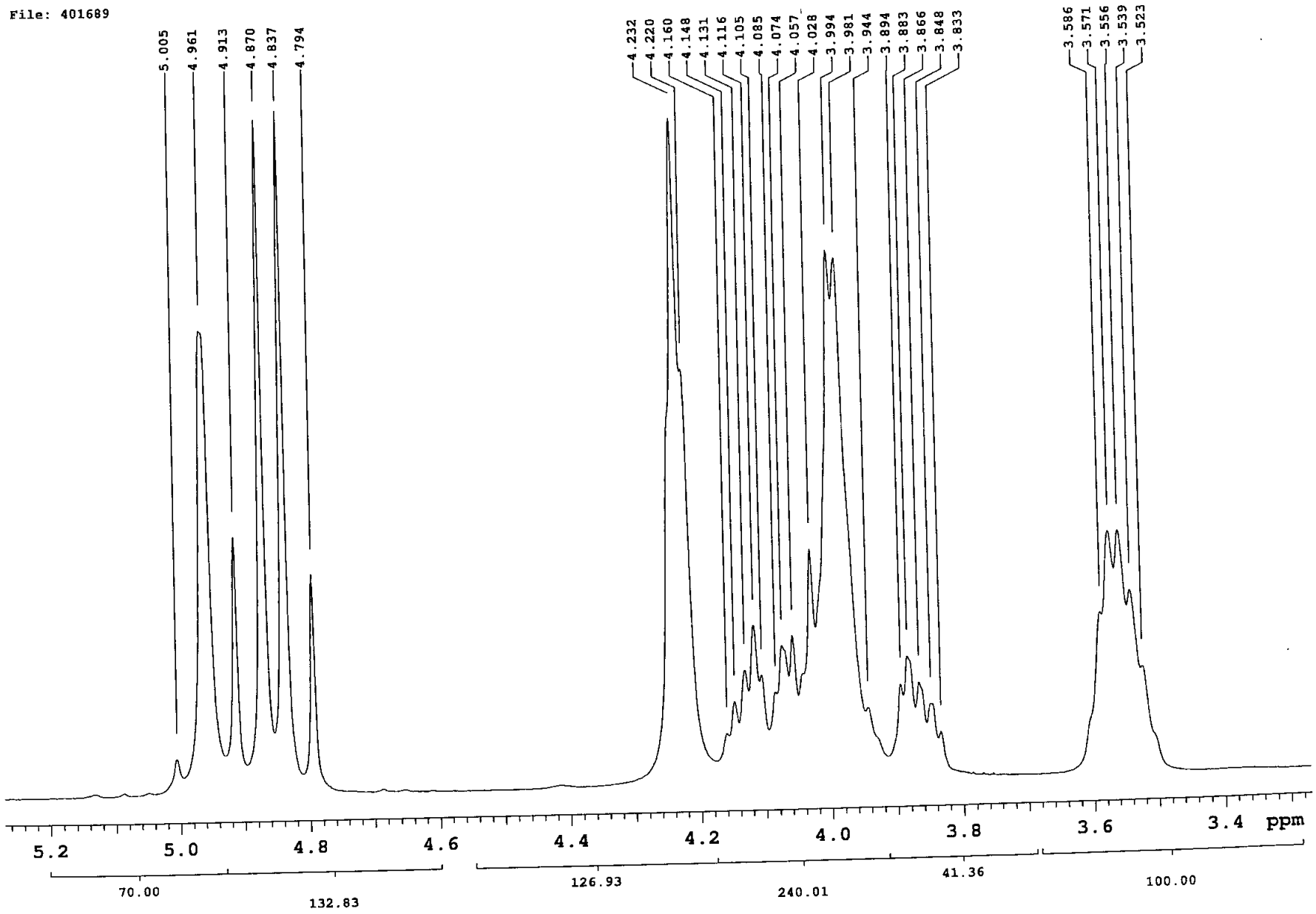
File: 401689



Plot file: 401689-2

234874, 4063-35-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

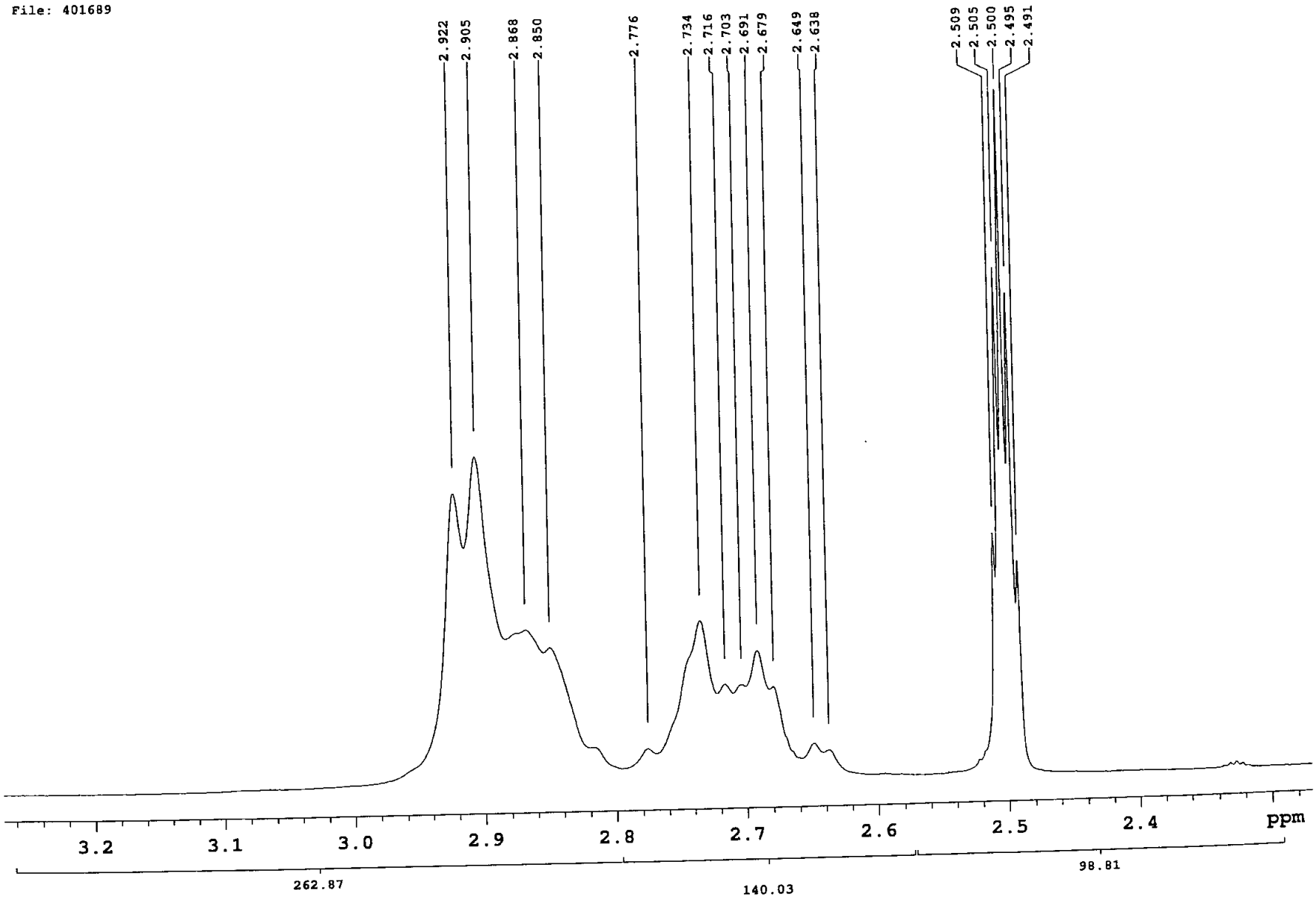
File: 401689



Plot file: 401689-3

234874, 4063-35-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

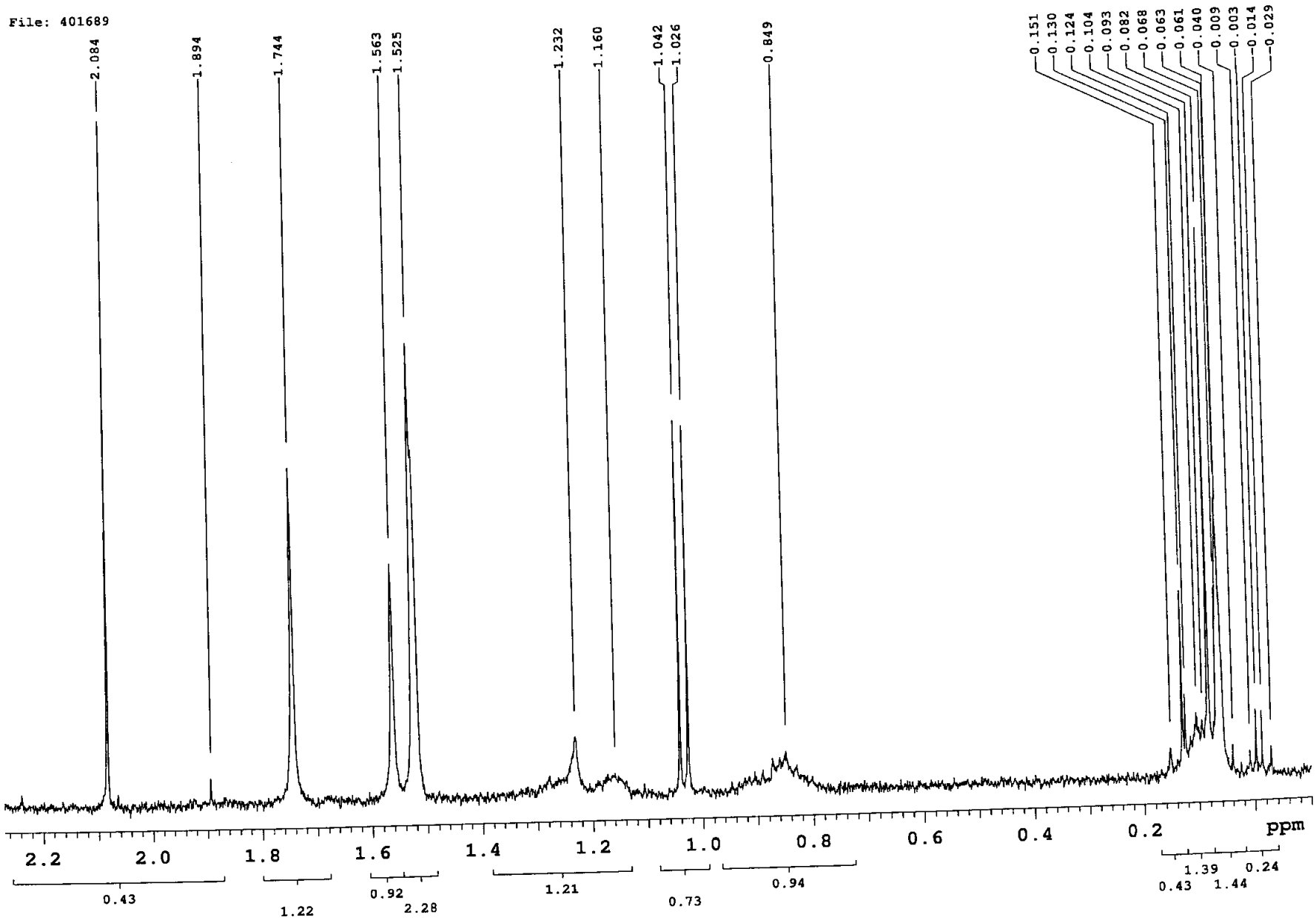
File: 401689



Plot file: 401689-4

234874, 4063-35-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401689



Plot file: 401689-5

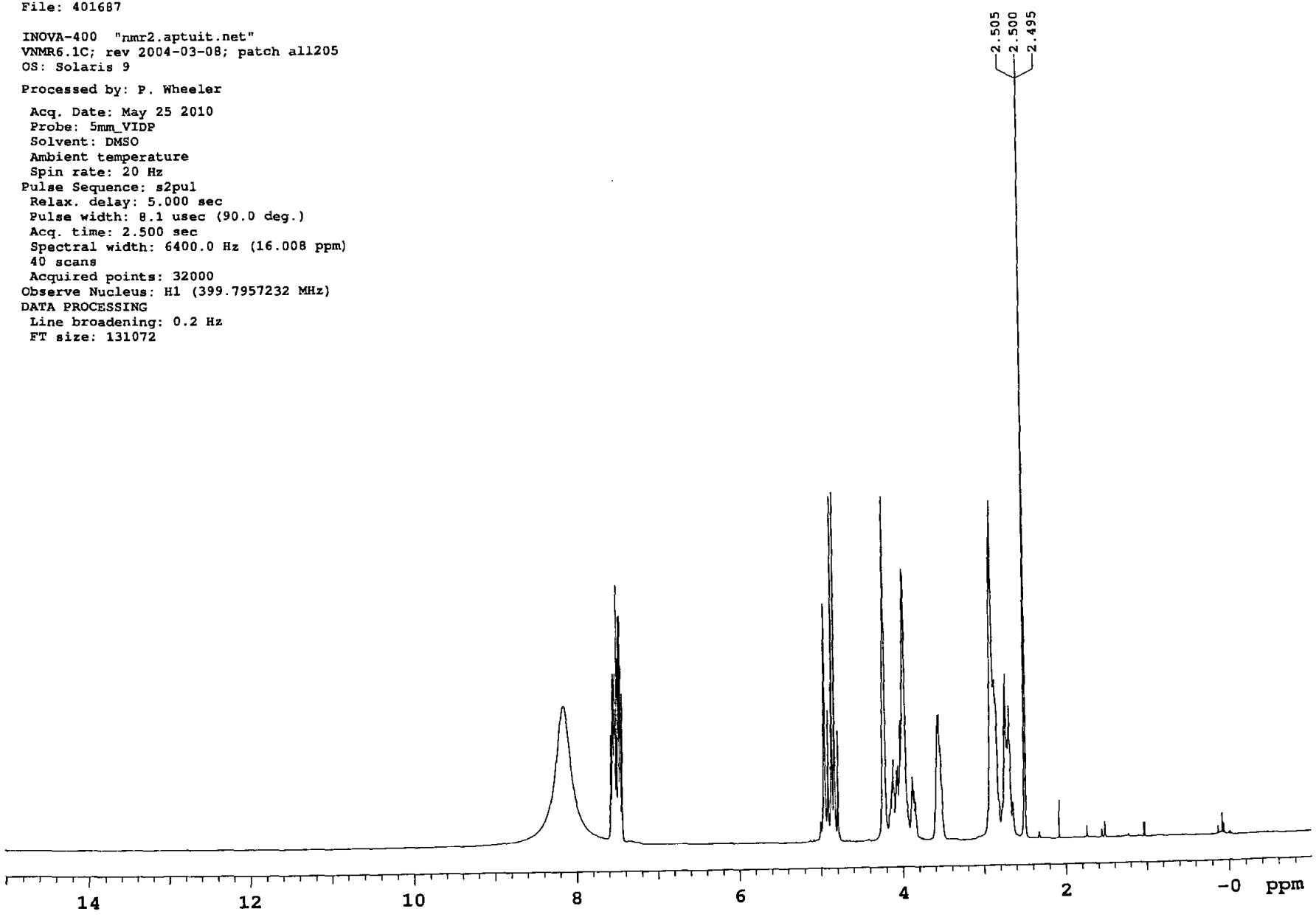
234872, 4063-34-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401687

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

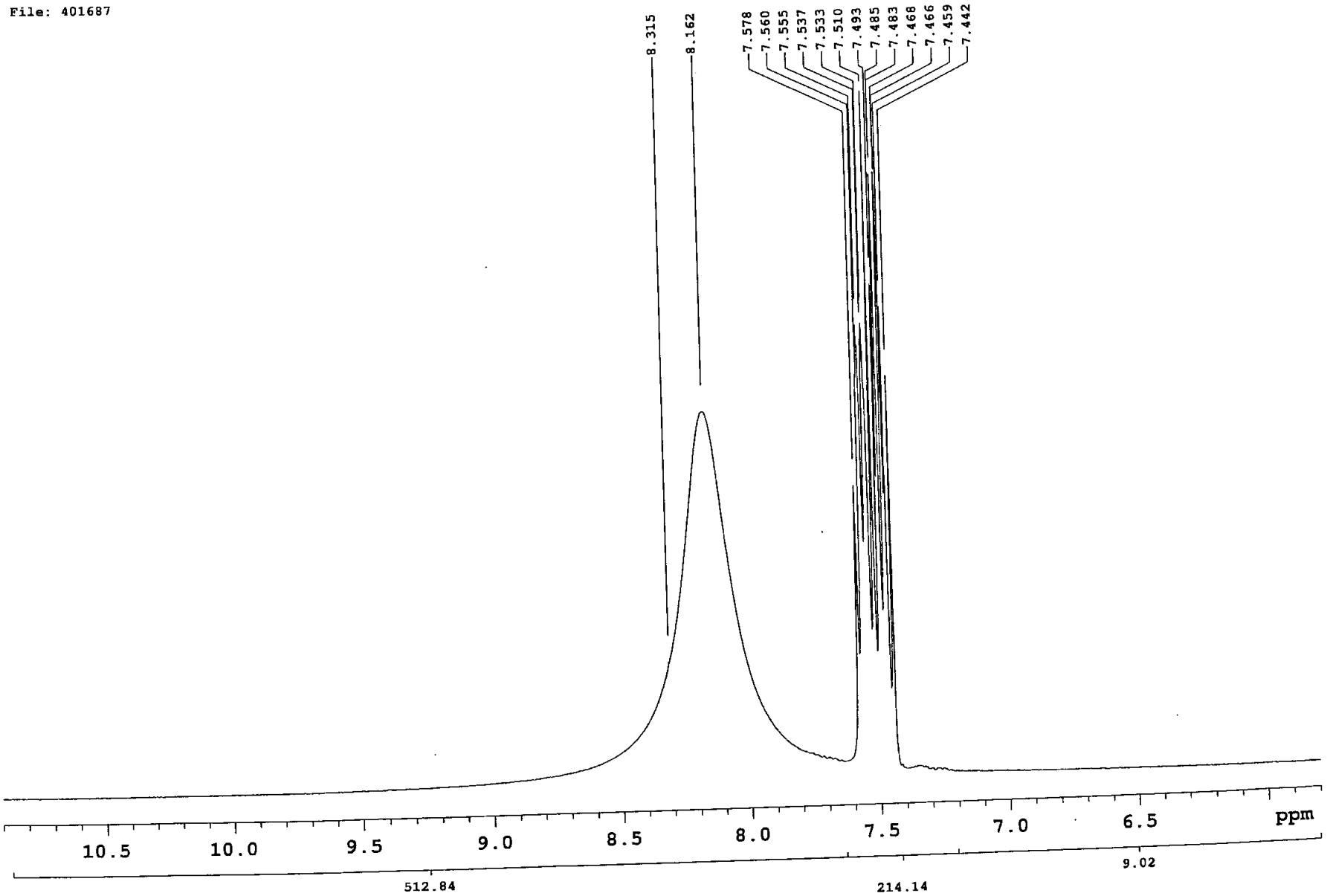
Acq. Date: May 25 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 401687-1

234872, 4063-34-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

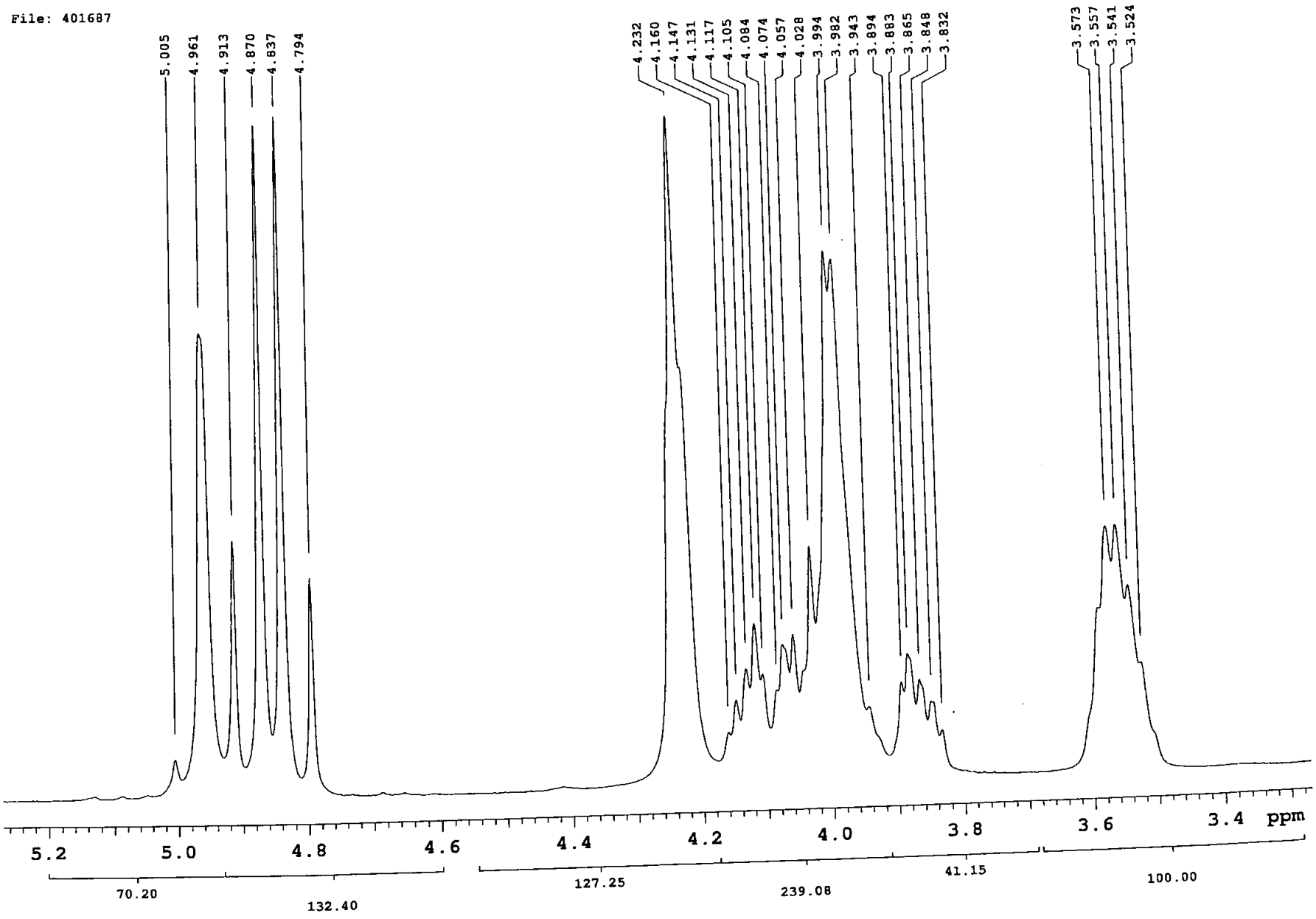
File: 401687



Plot file: 401687-2

234872, 4063-34-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

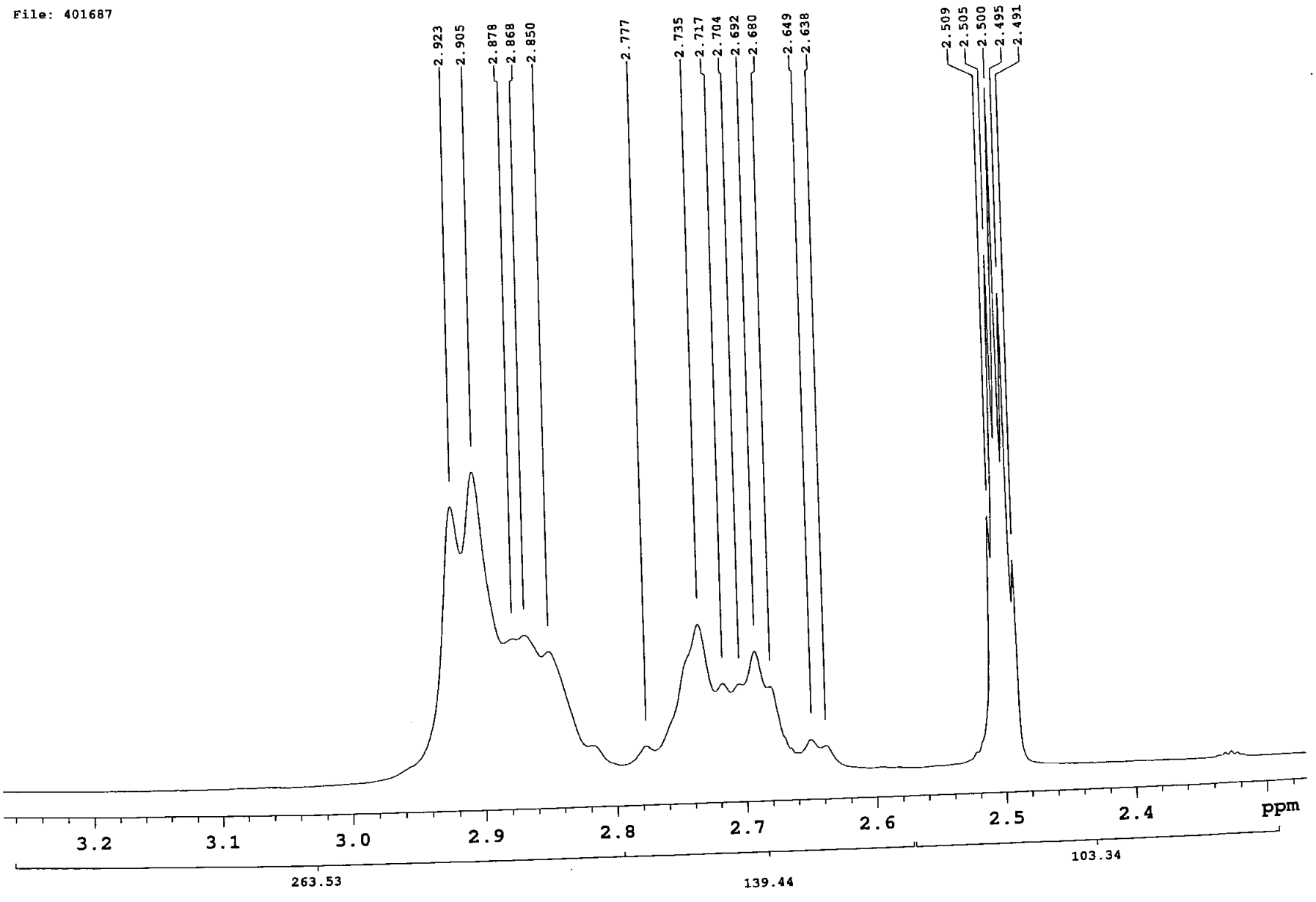
File: 401687



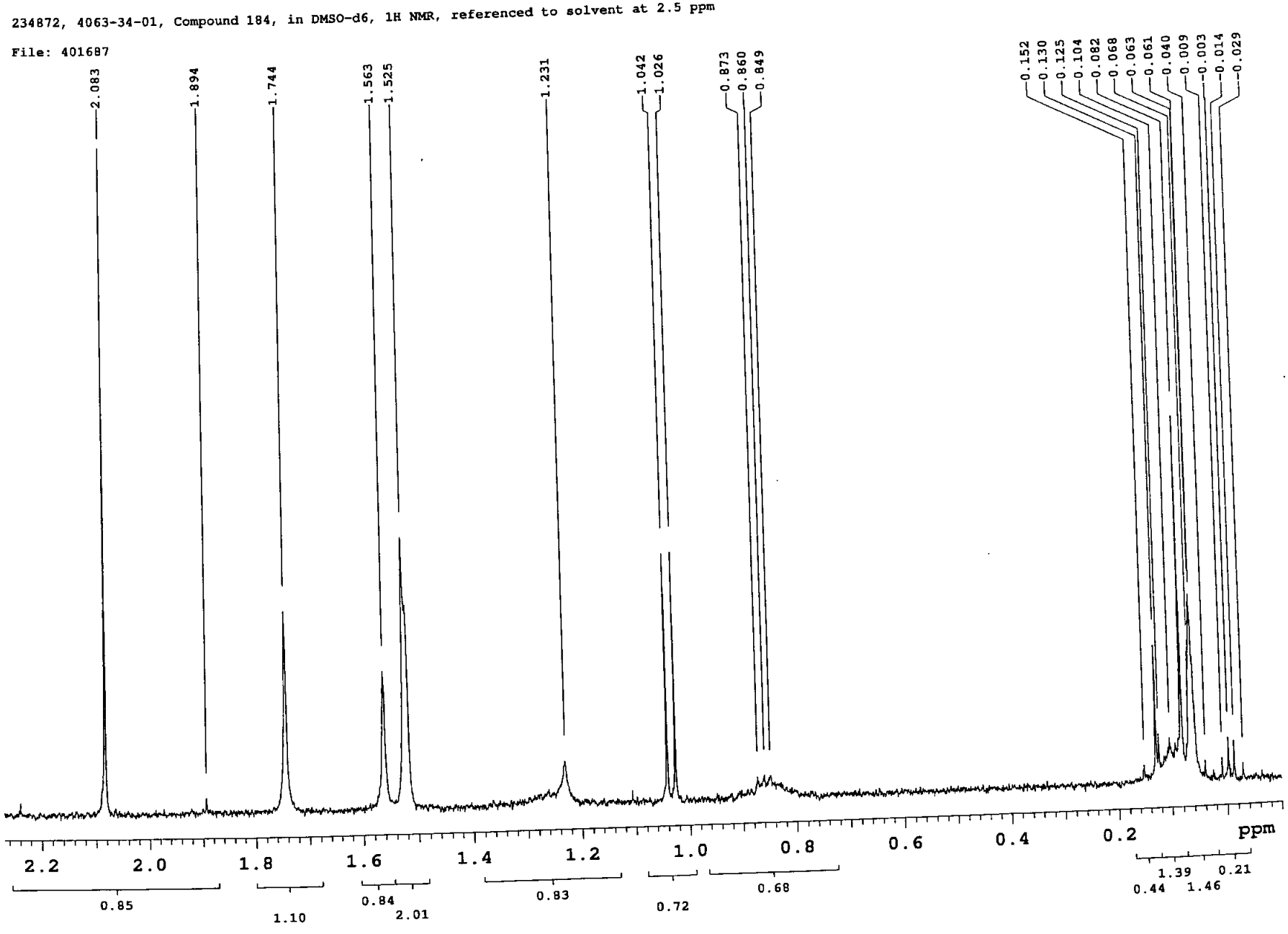
Plot file: 401687-3

234872, 4063-34-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401687



Plot file: 401687-4



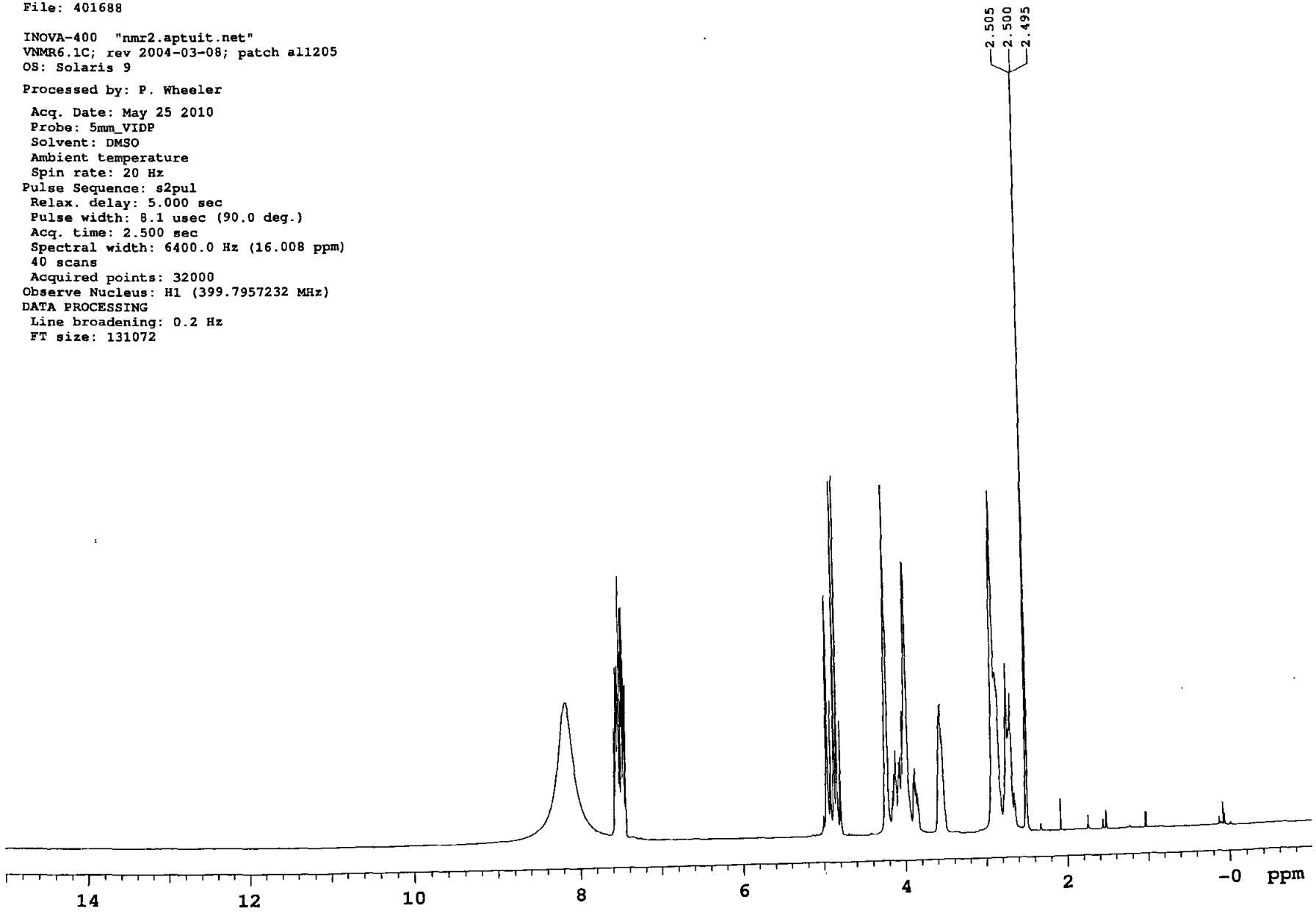
234873, 4063-32-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401688

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch a11205
OS: Solaris 9

Processed by: P. Wheeler

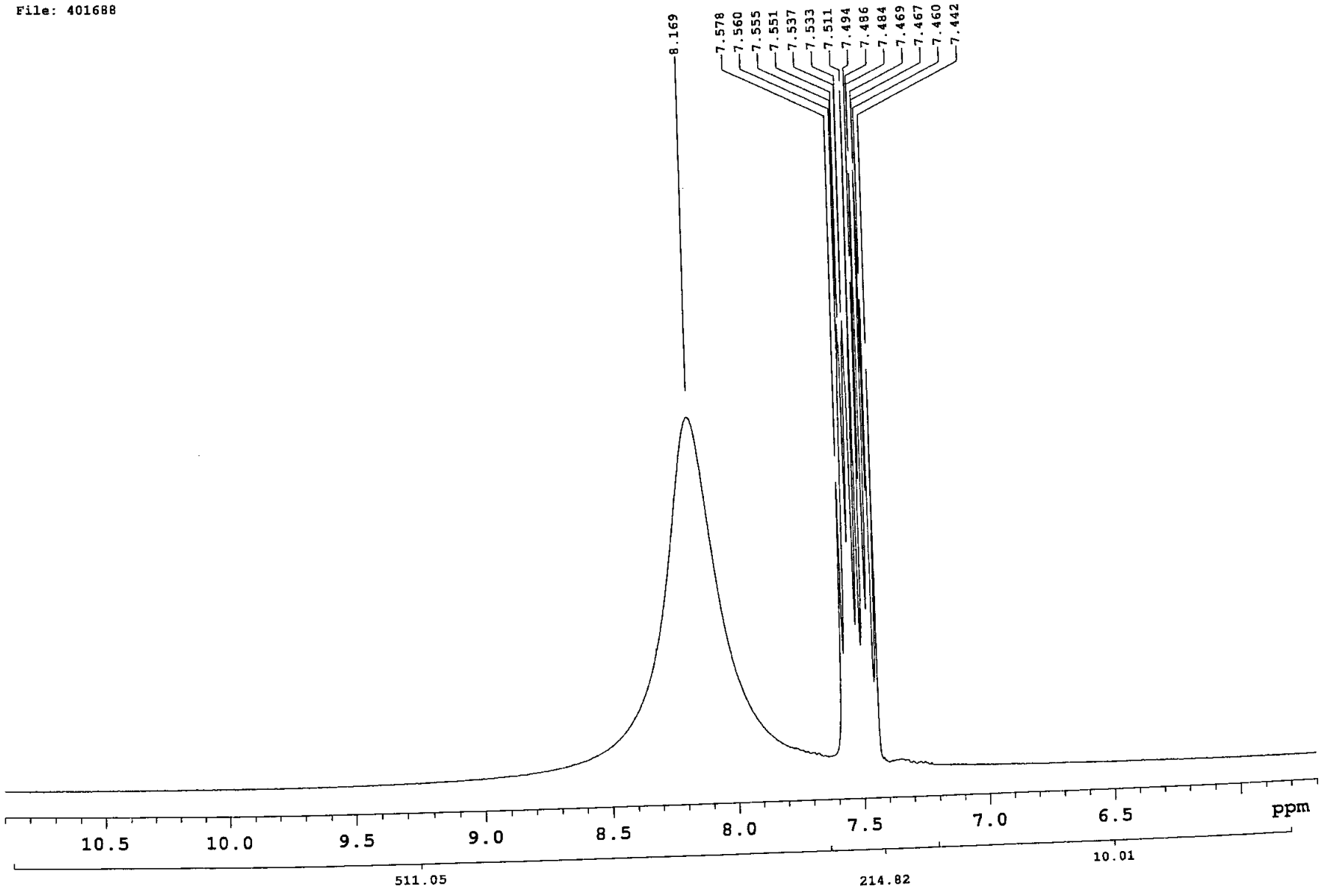
Acq. Date: May 25 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 401688-1

234873, 4063-32-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

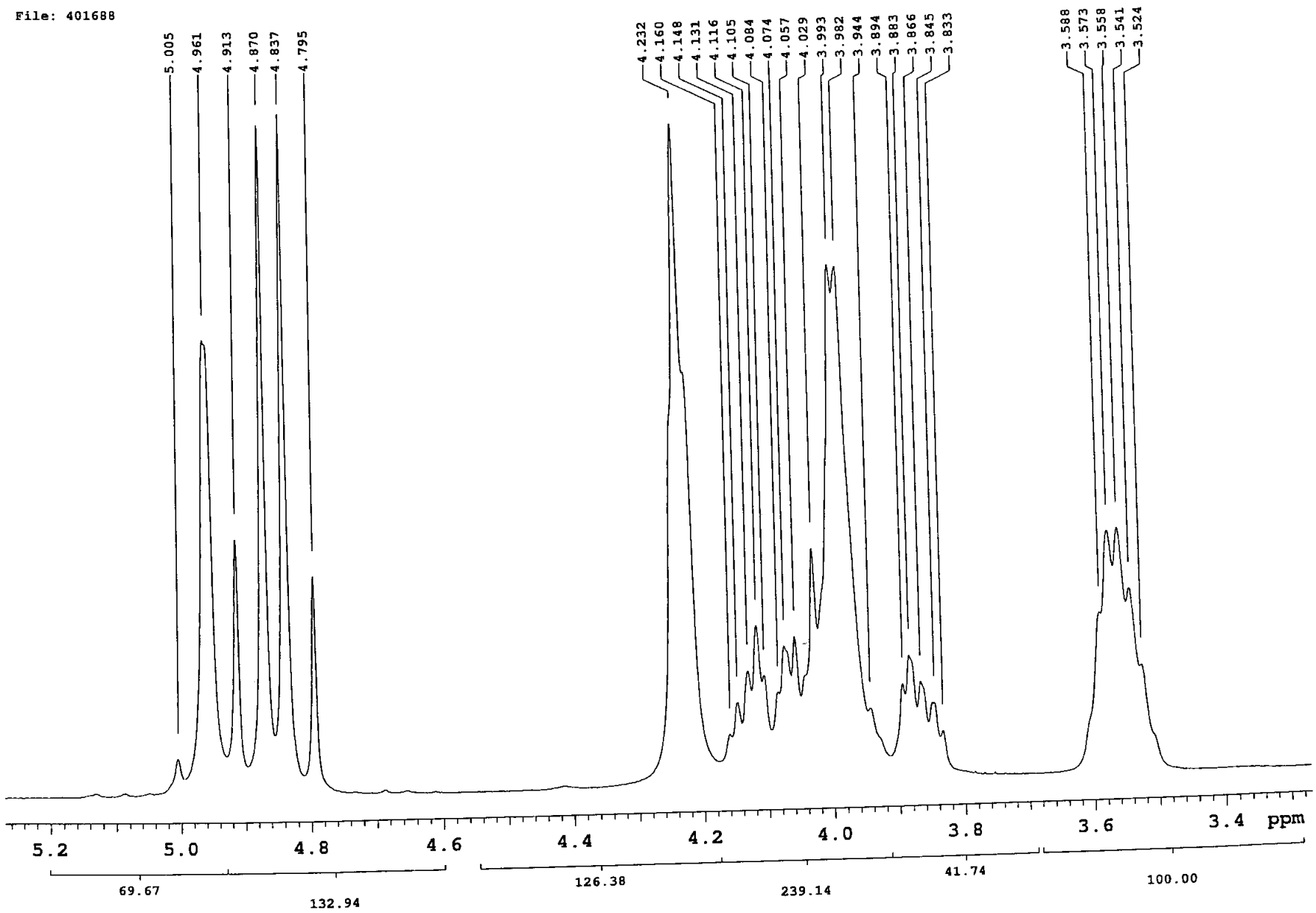
File: 401688



Plot file: 401688-2

234873, 4063-32-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

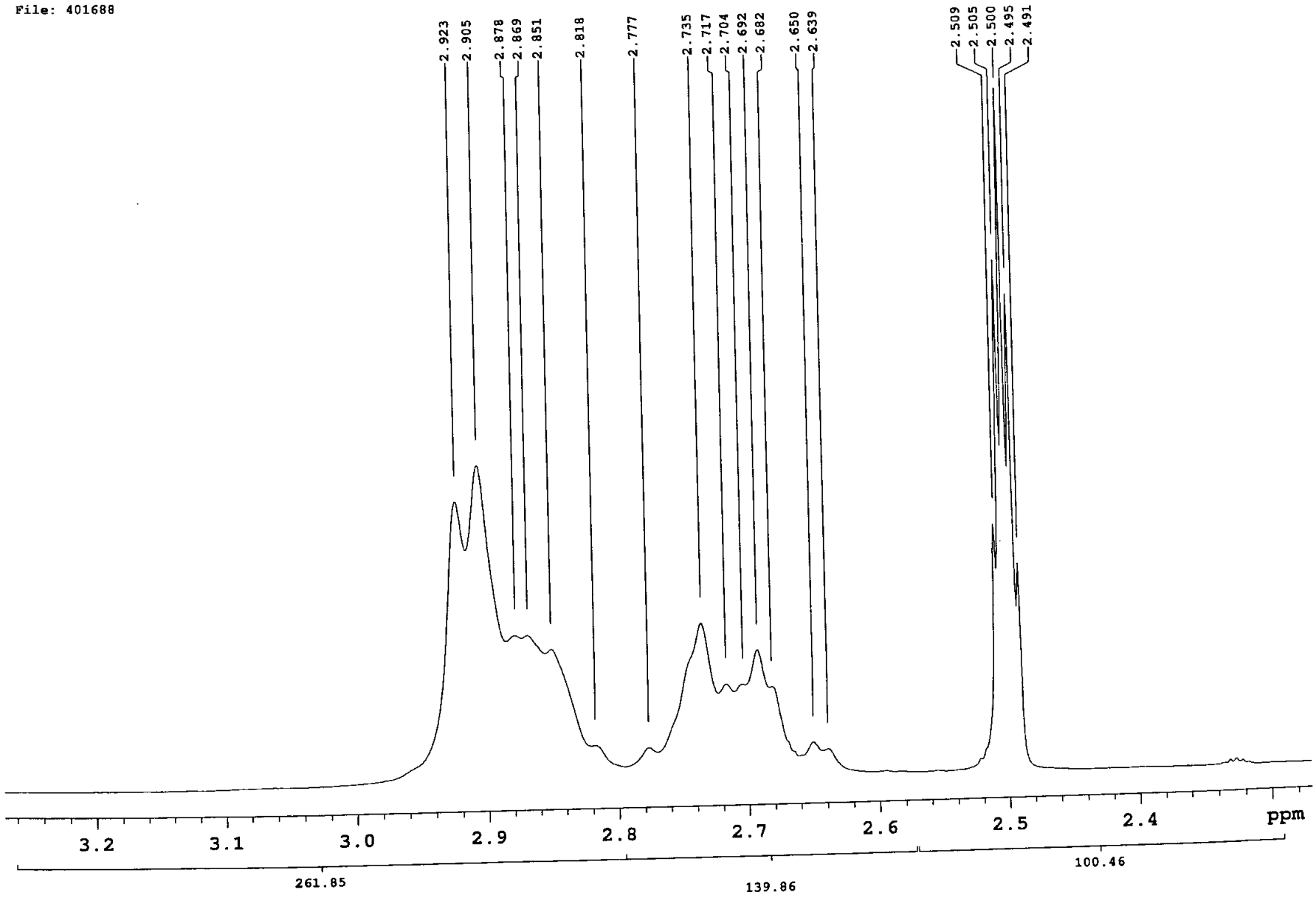
File: 401688



Plot file: 401688-3

234873, 4063-32-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

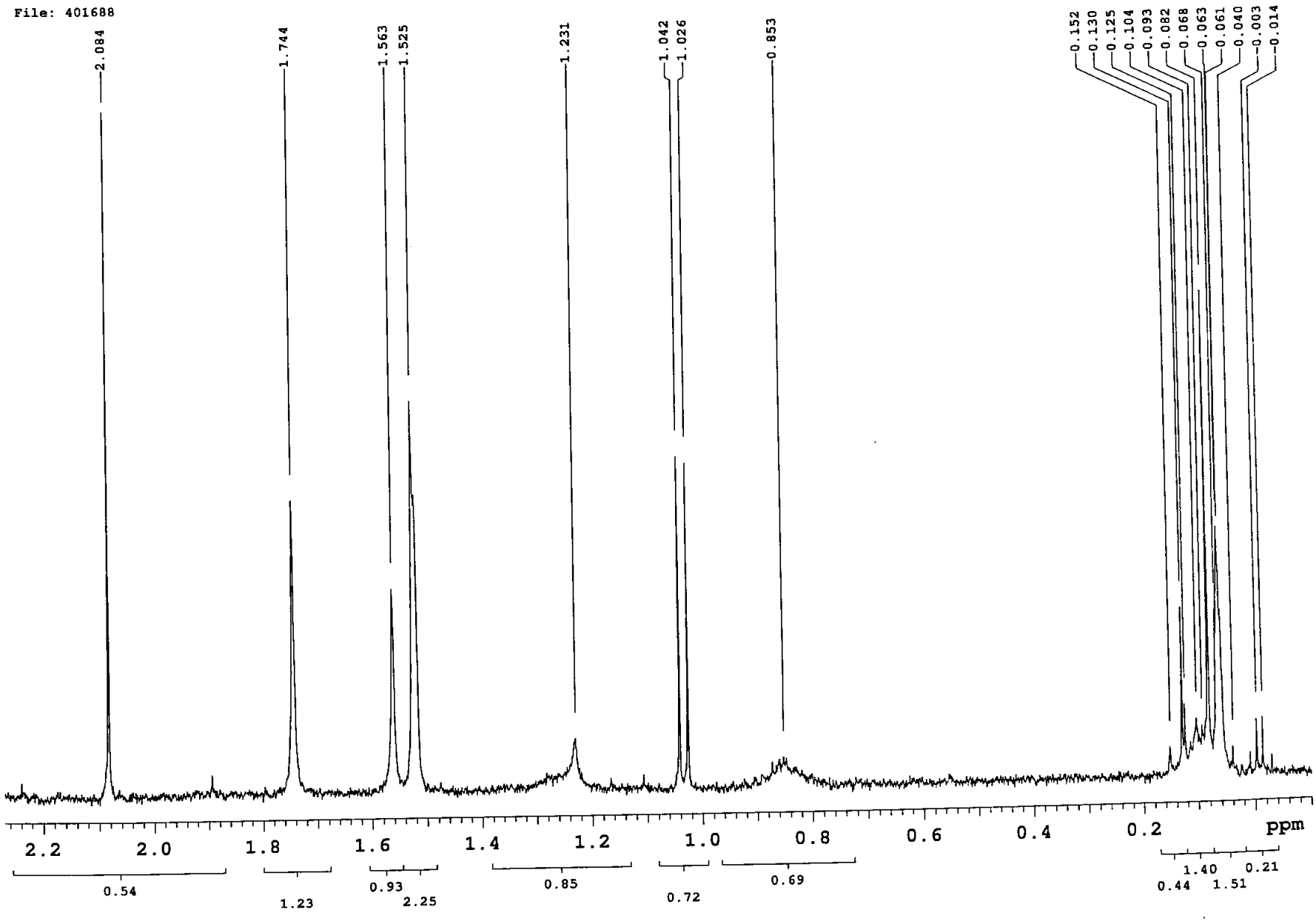
File: 401688



Plot file: 401688-4

234873, 4063-32-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 401688



Plot file: 401688-5

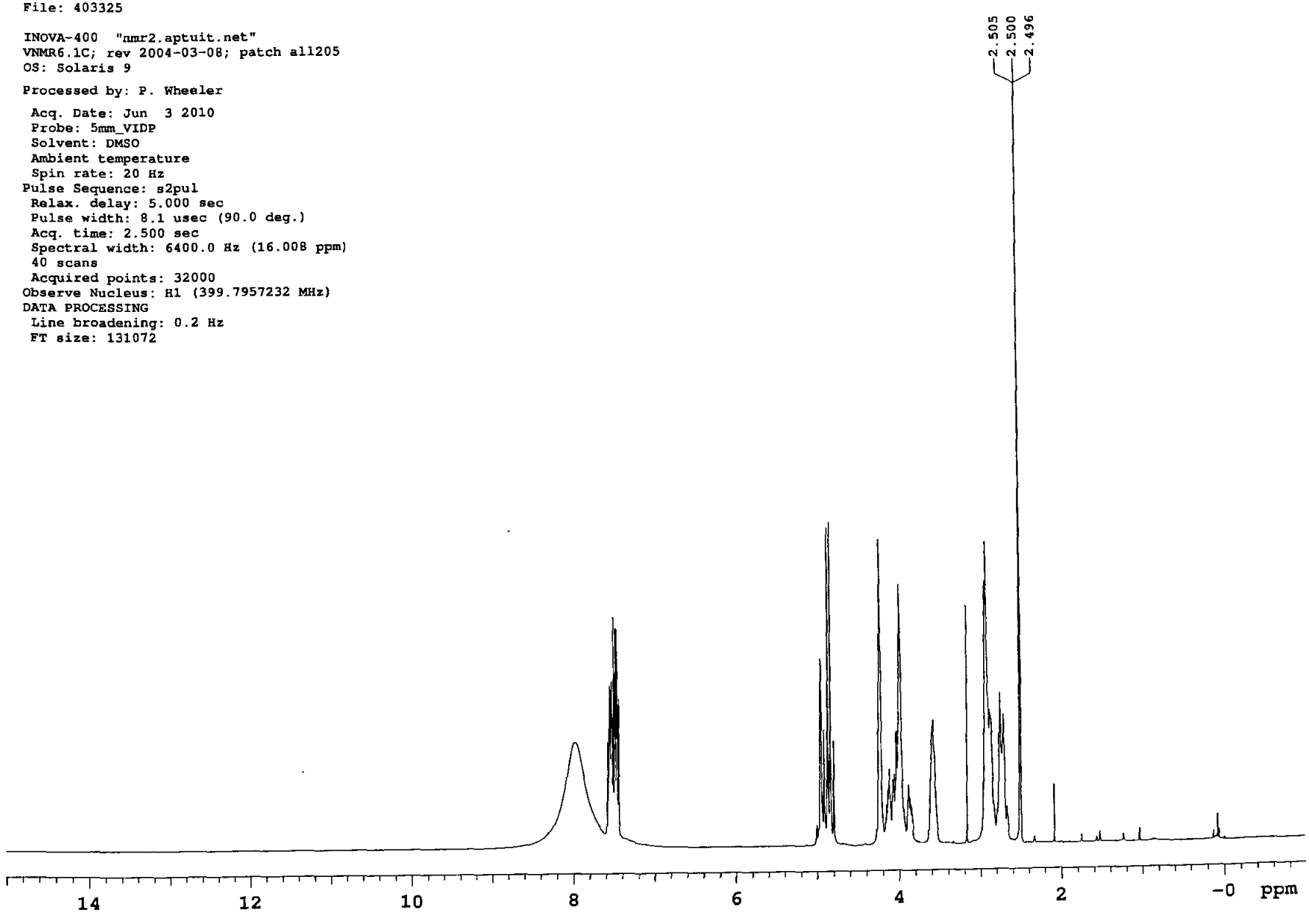
235805, 4063-50-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403325

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

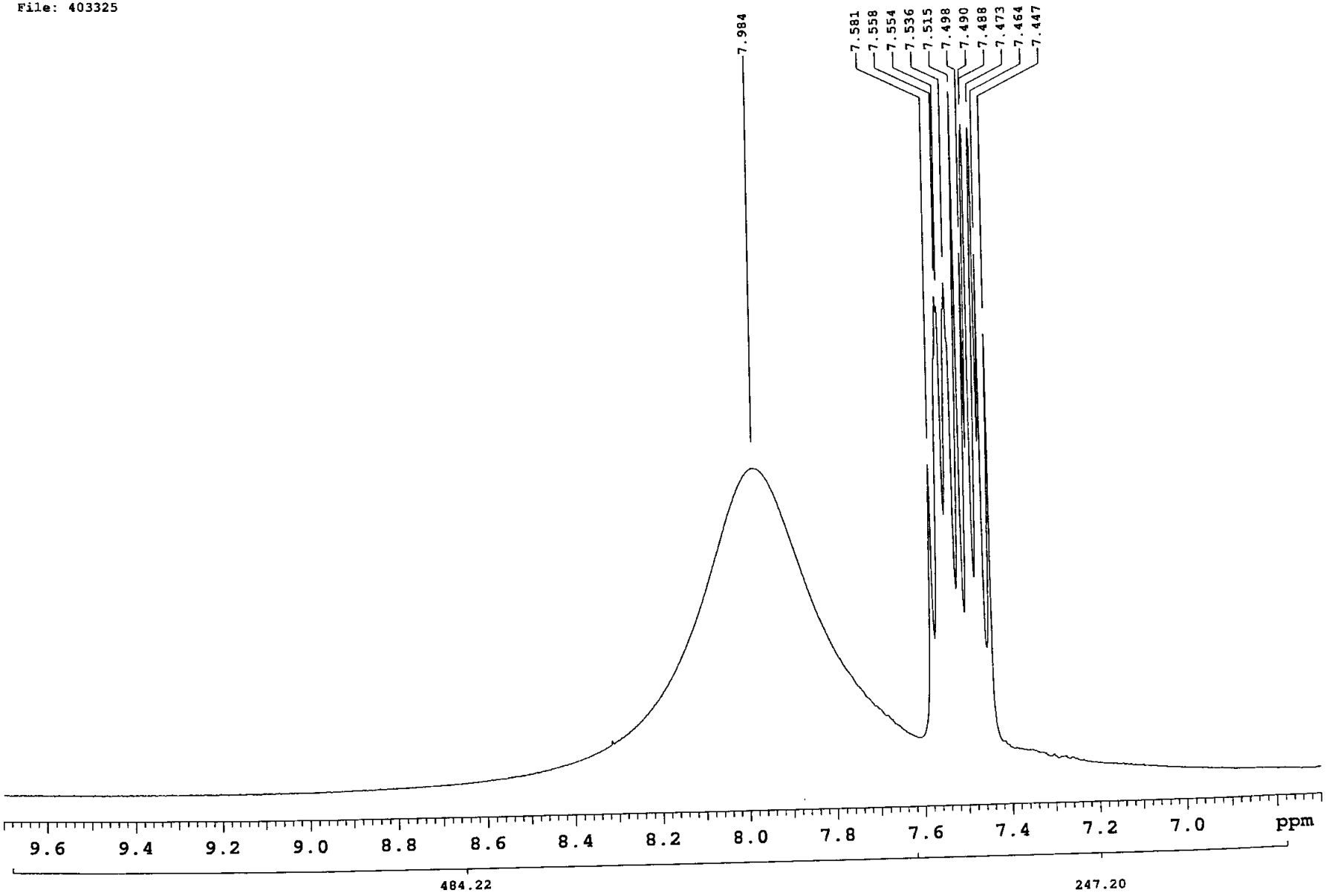
Acq. Date: Jun 3 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 403325-1

235805, 4063-50-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

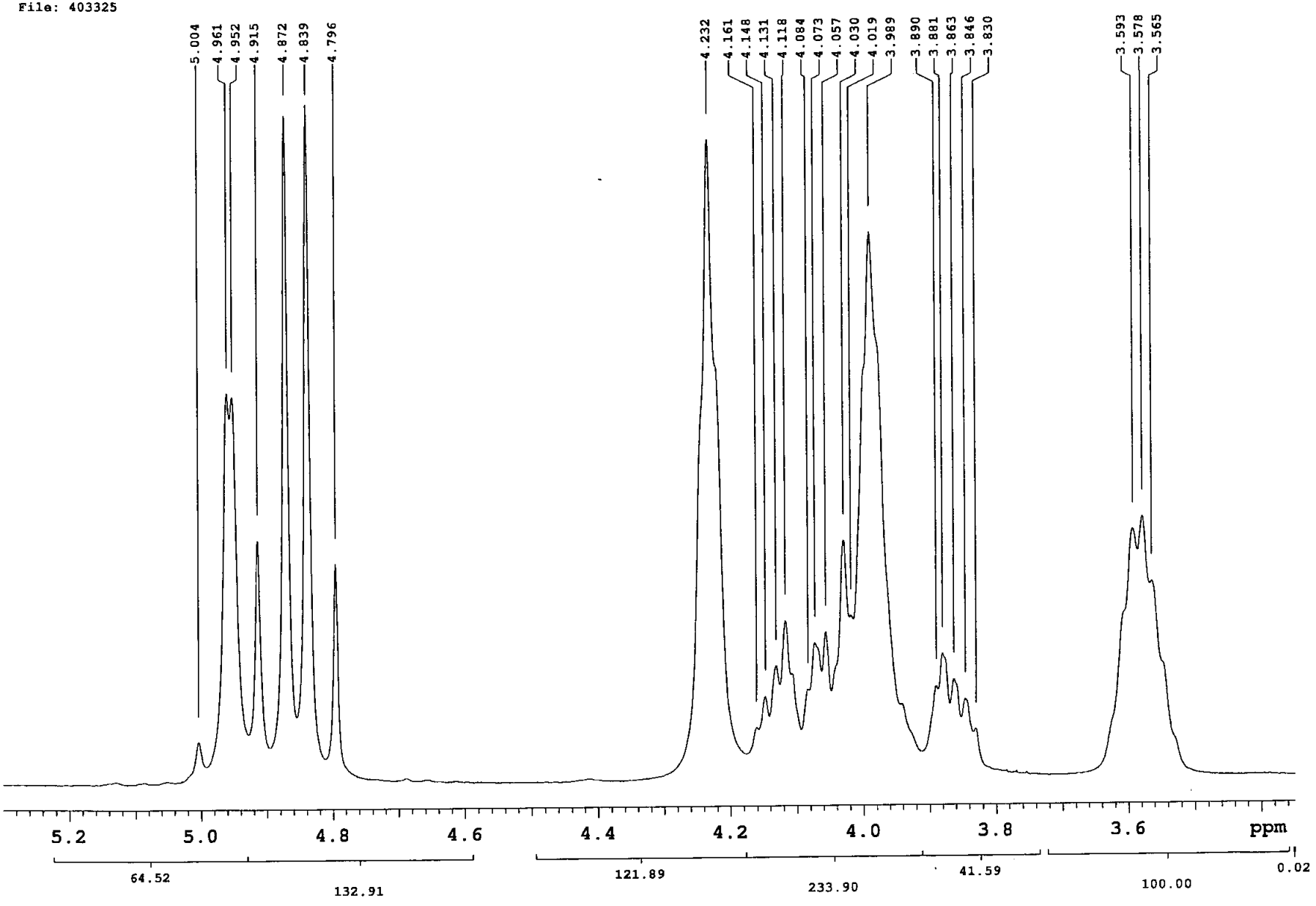
File: 403325



Plot file: 403325-2

235805, 4063-50-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

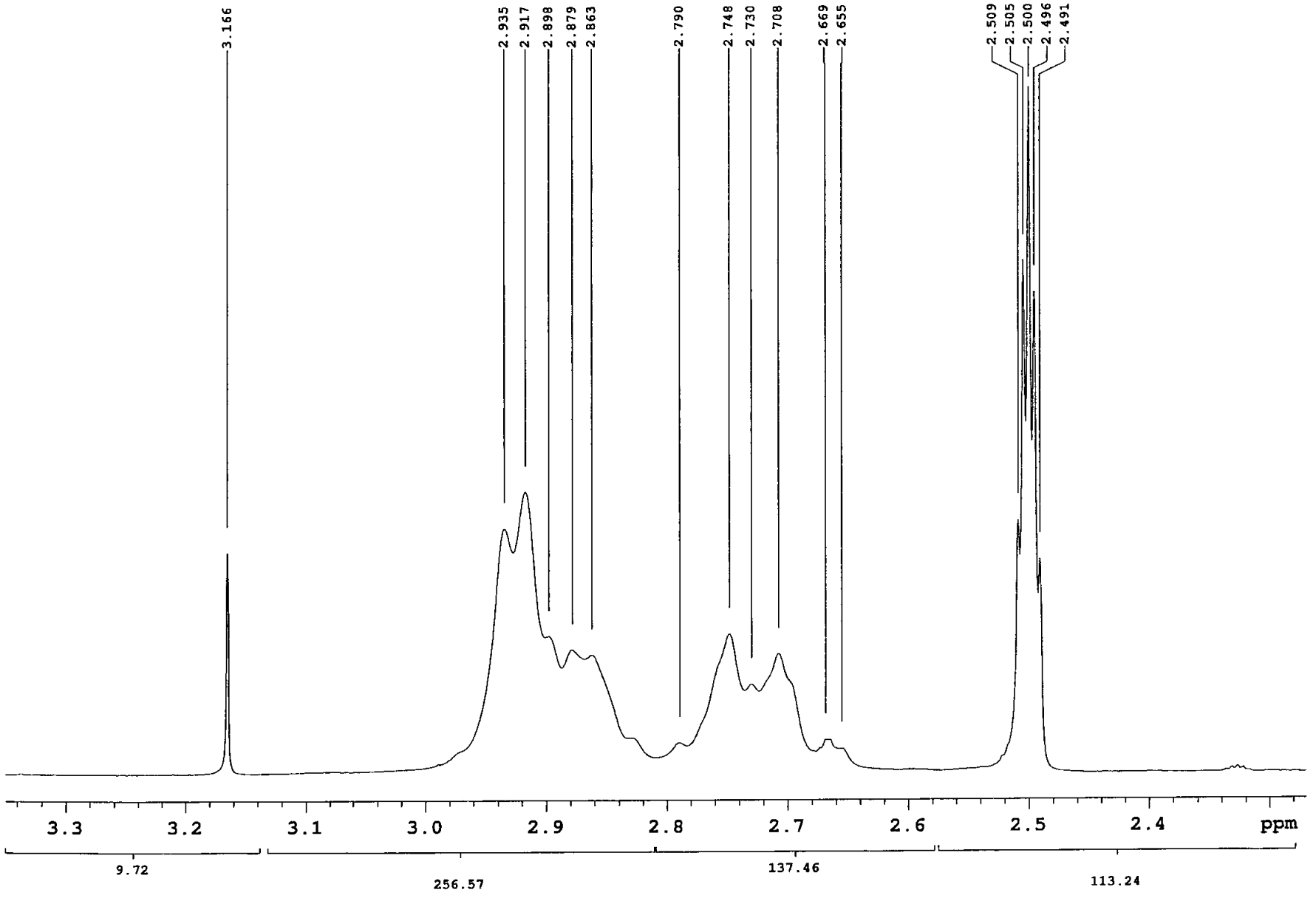
File: 403325



Plot file: 403325-3

235805, 4063-50-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

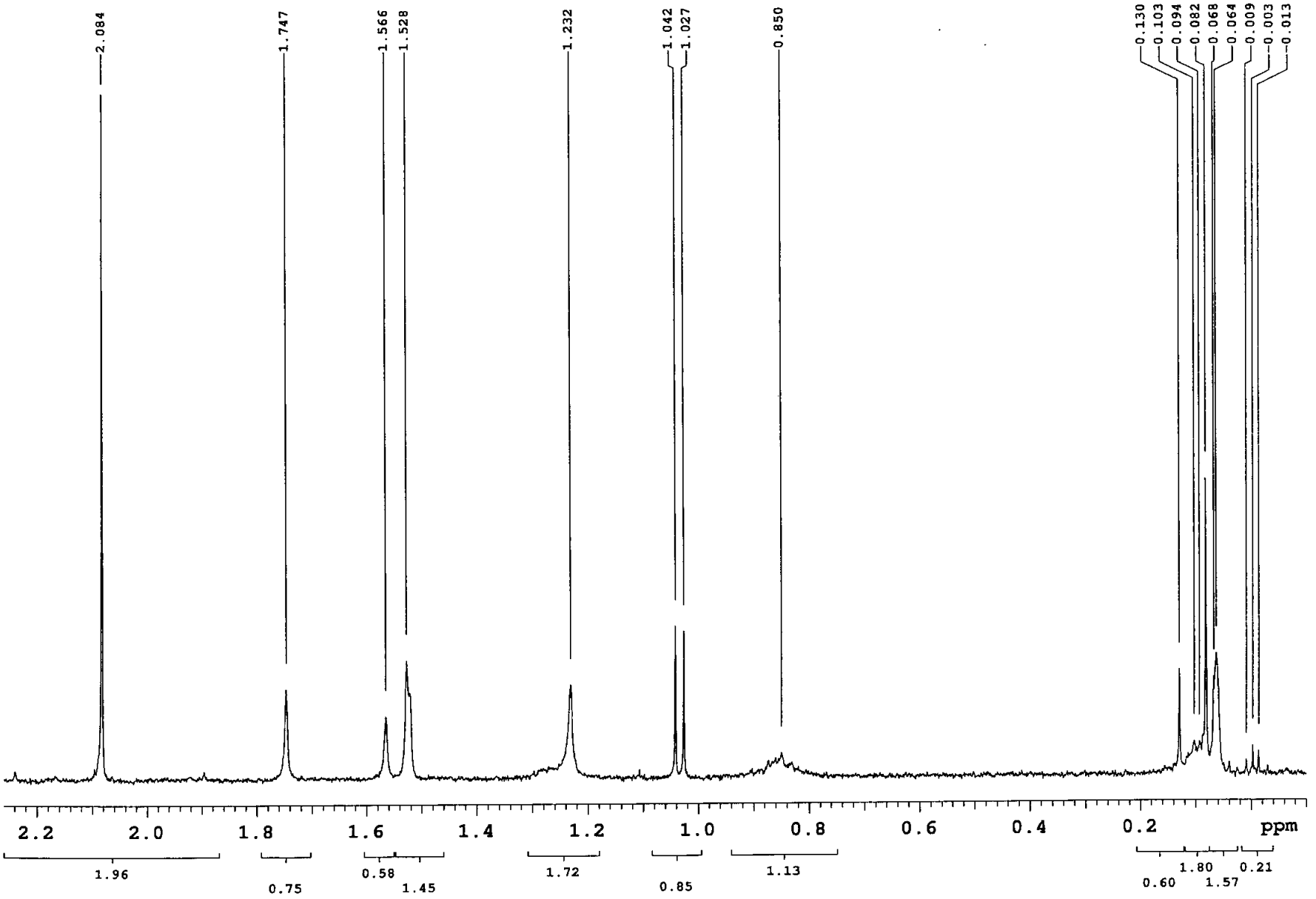
File: 403325



Plot file: 403325-4

235805, 4063-50-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403325



Plot file: 403325-5

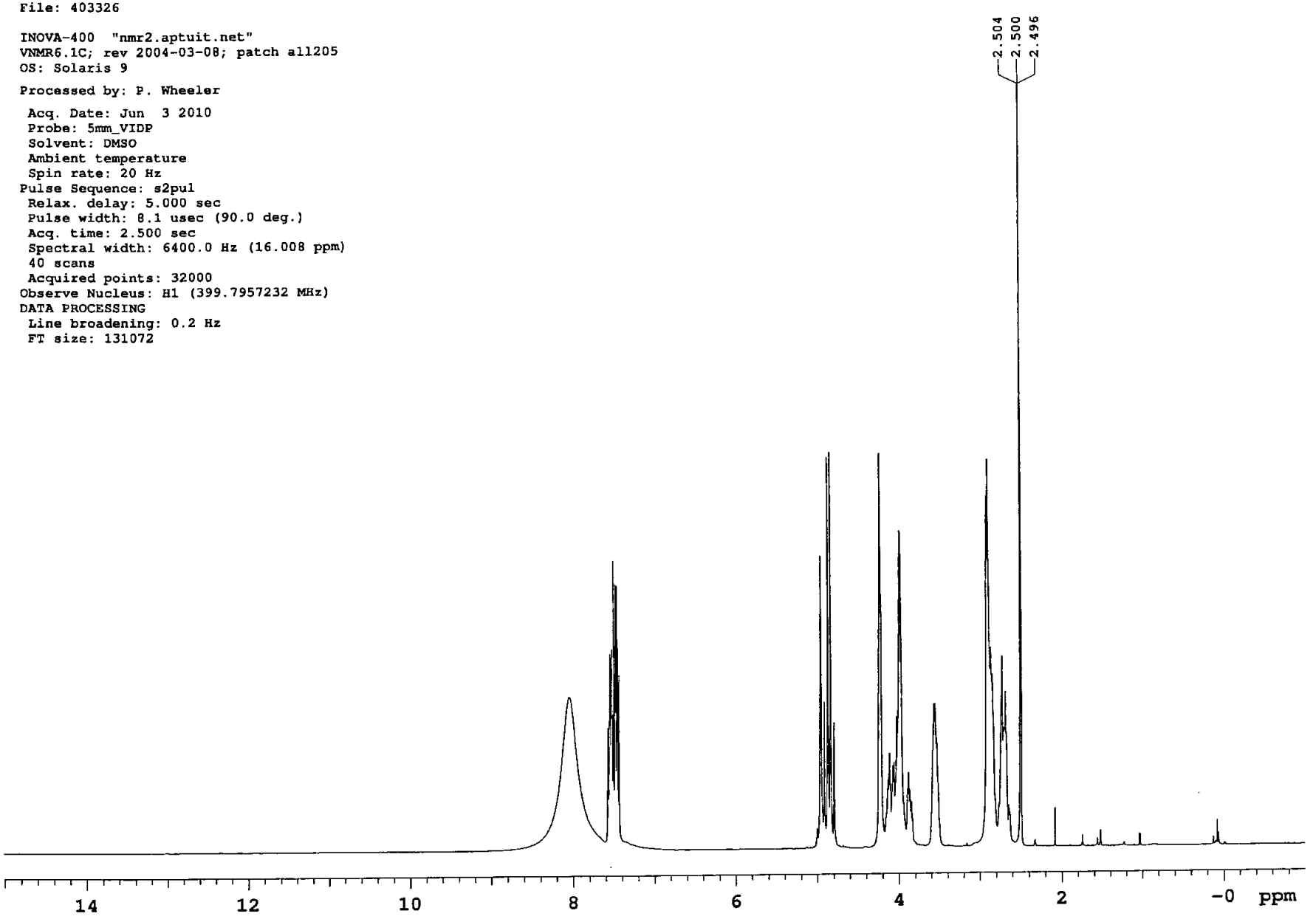
235806, 4063-51-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403326

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

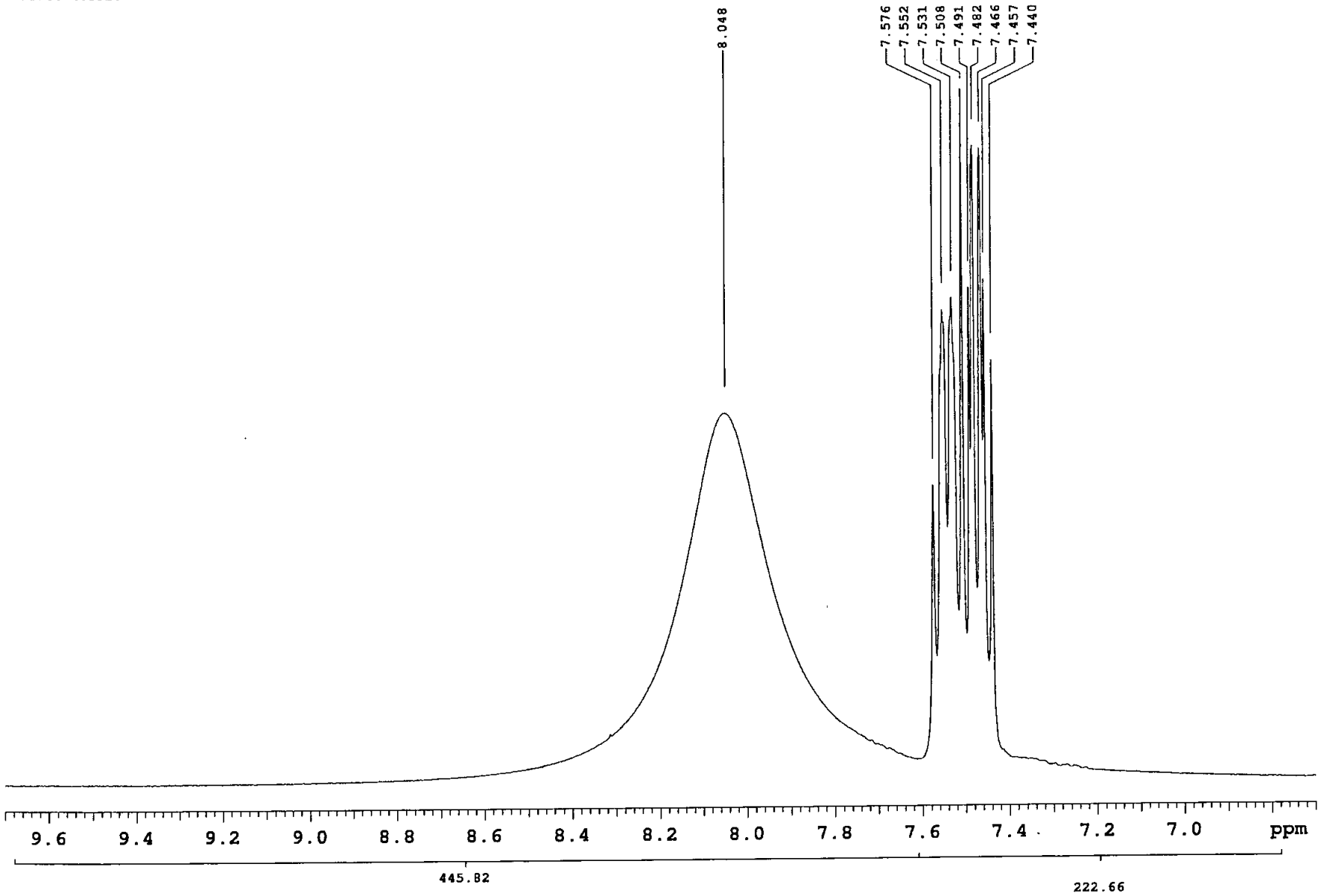
Acq. Date: Jun 3 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 403326-1

235806, 4063-51-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

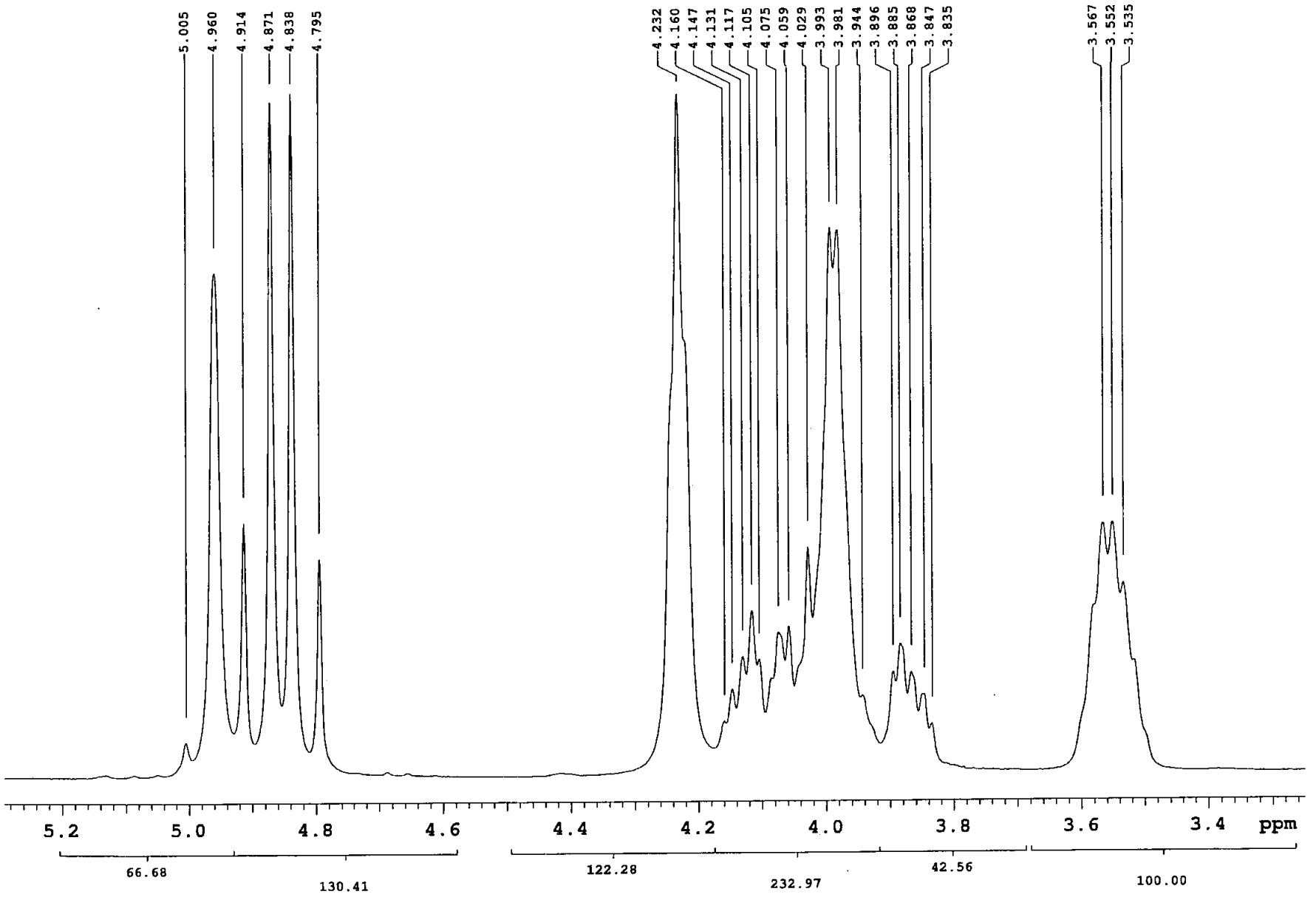
File: 403326



Plot file: 403326-2

235806, 4063-51-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

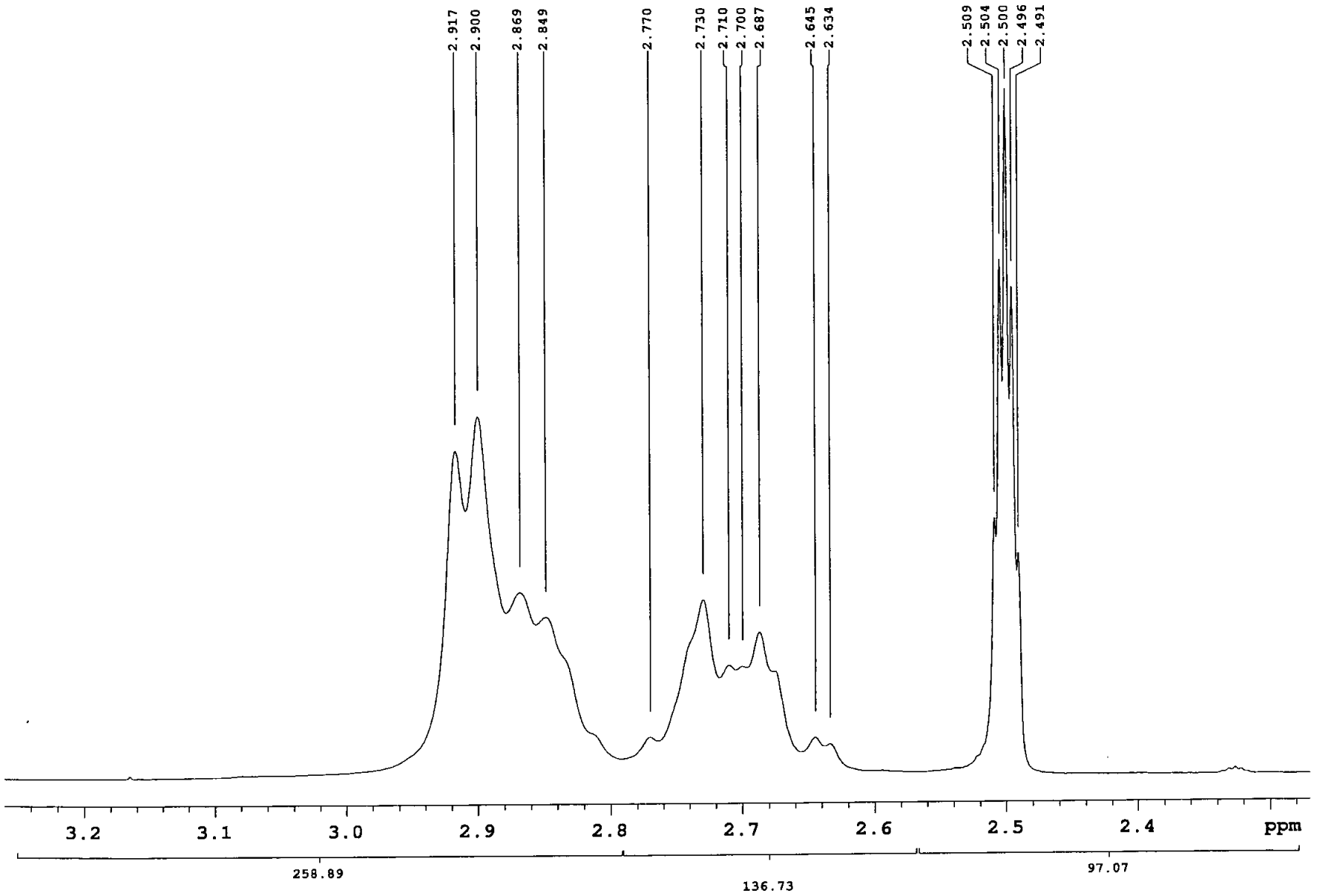
File: 403326



Plot file: 403326-3

235806, 4063-51-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

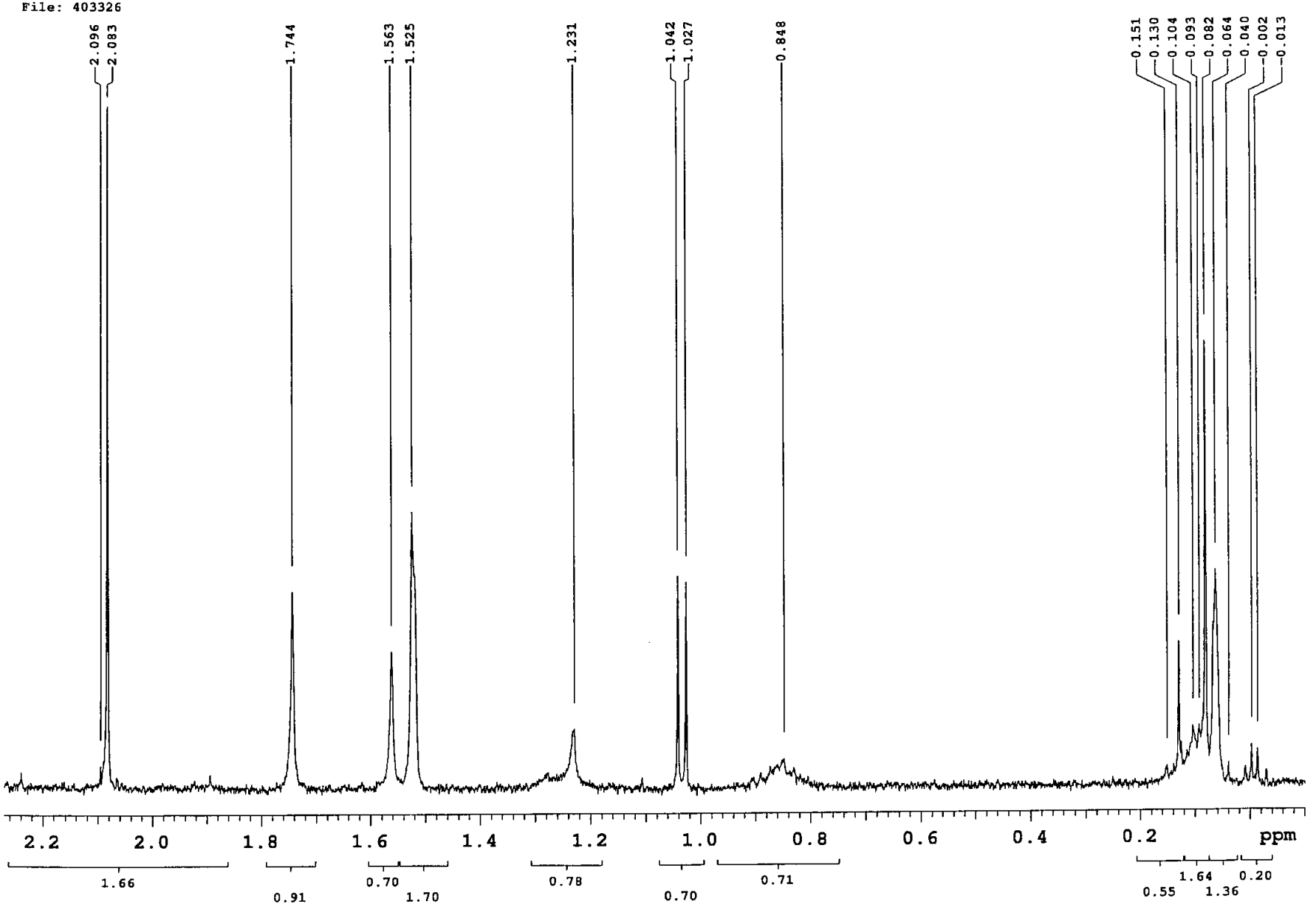
File: 403326



Plot file: 403326-4

235806, 4063-51-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403326



Plot file: 403326-5

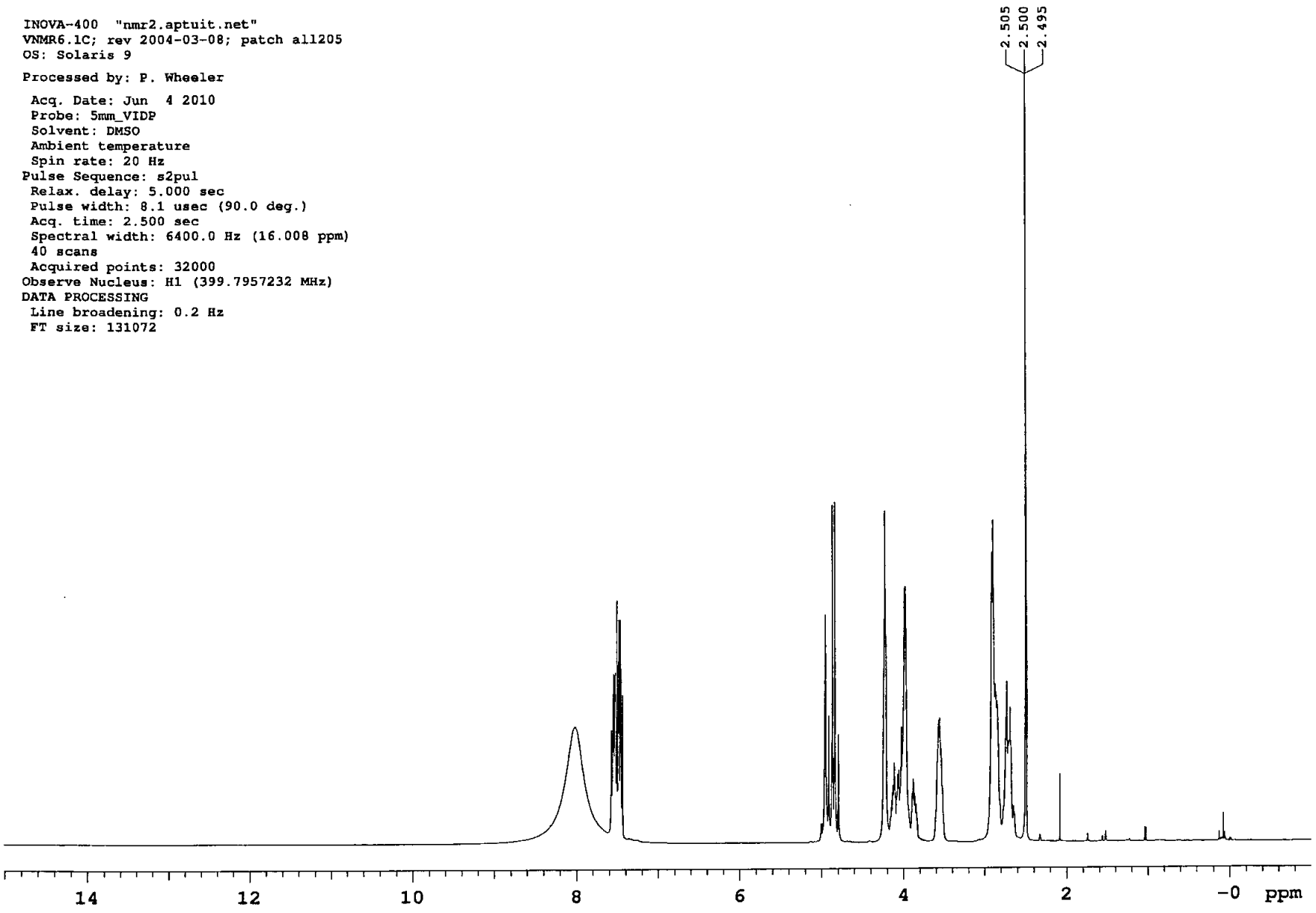
235848, 4063-56-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403553

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

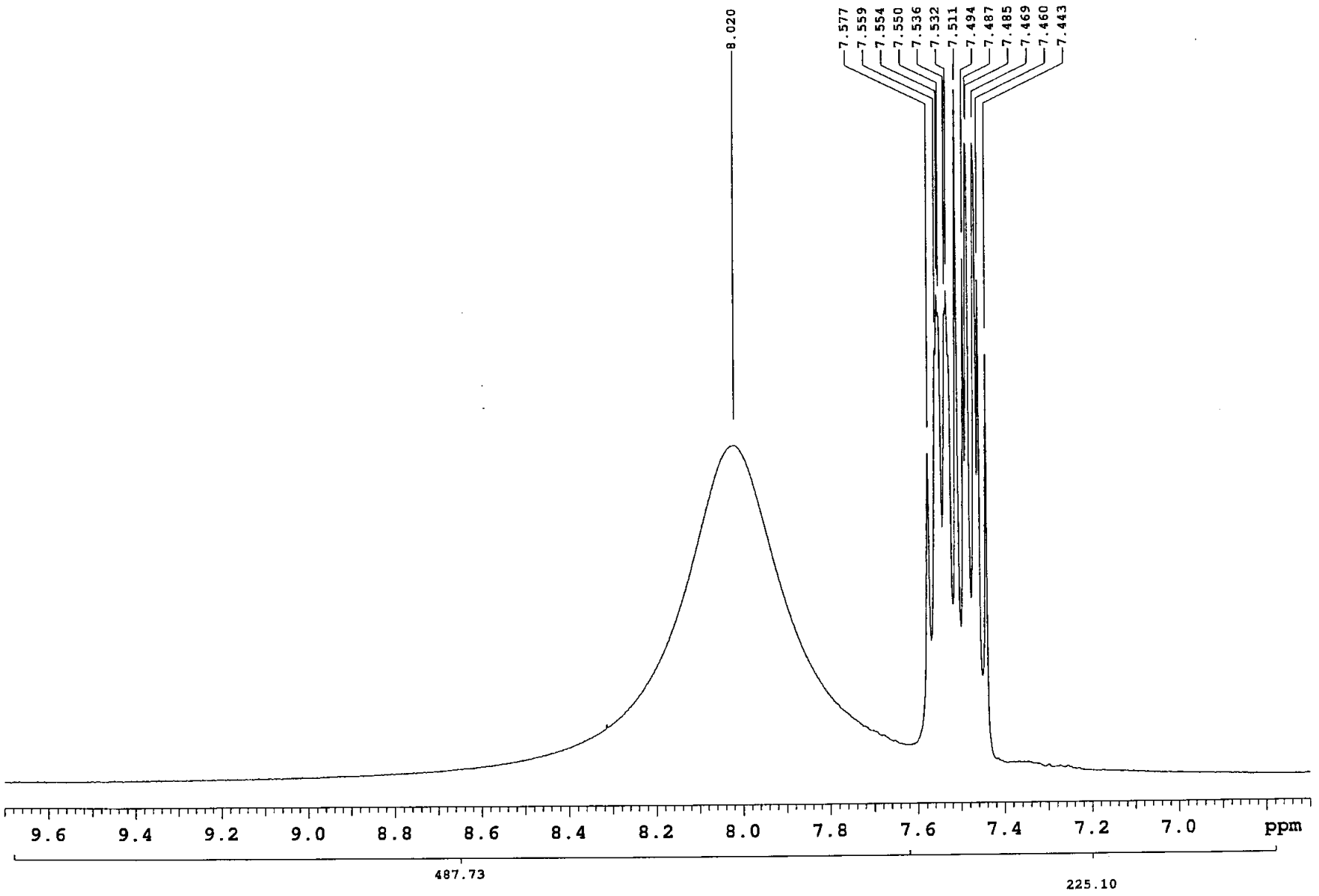
Acq. Date: Jun 4 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



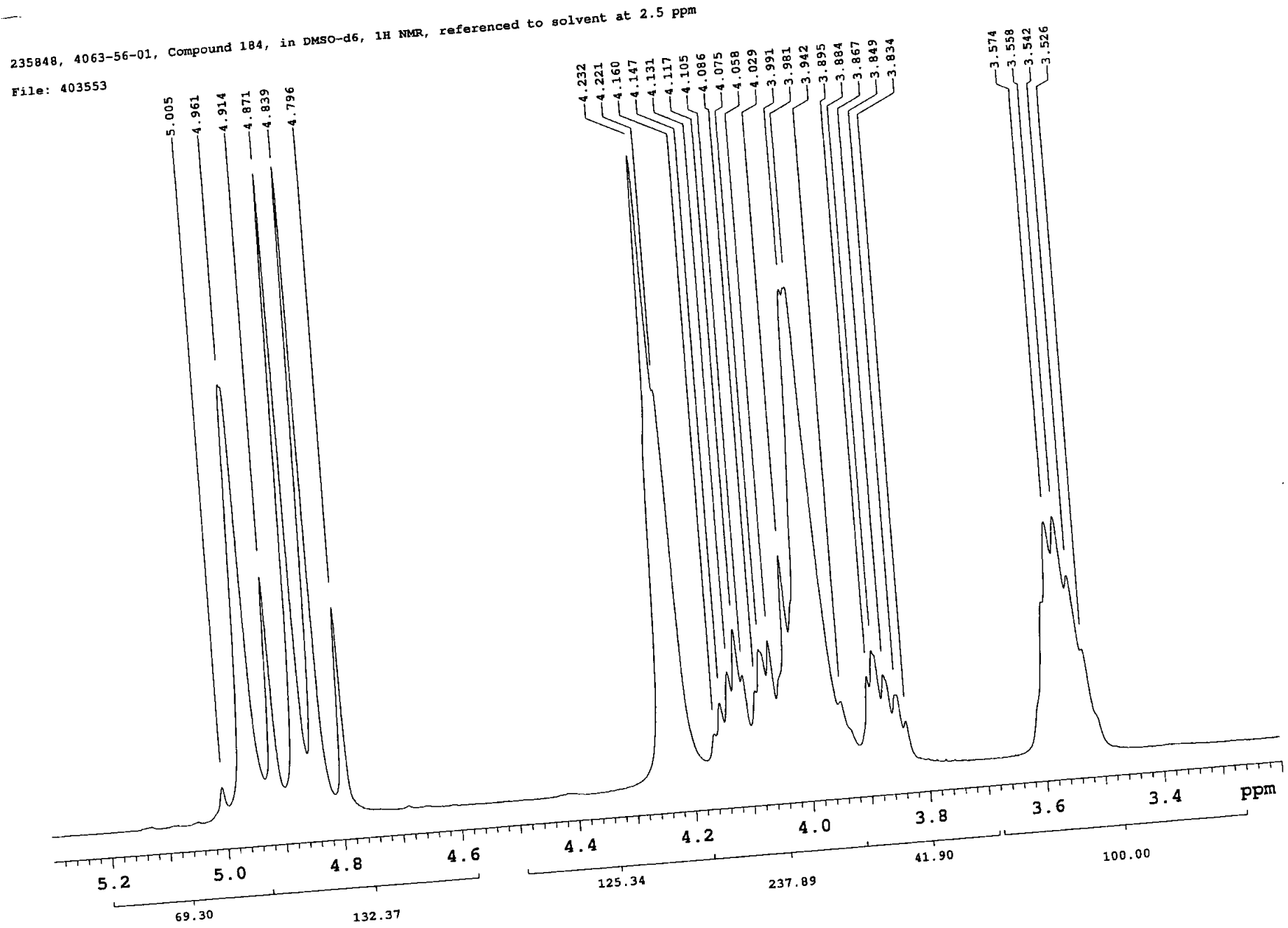
Plot file: 403553-1

235848, 4063-56-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403553

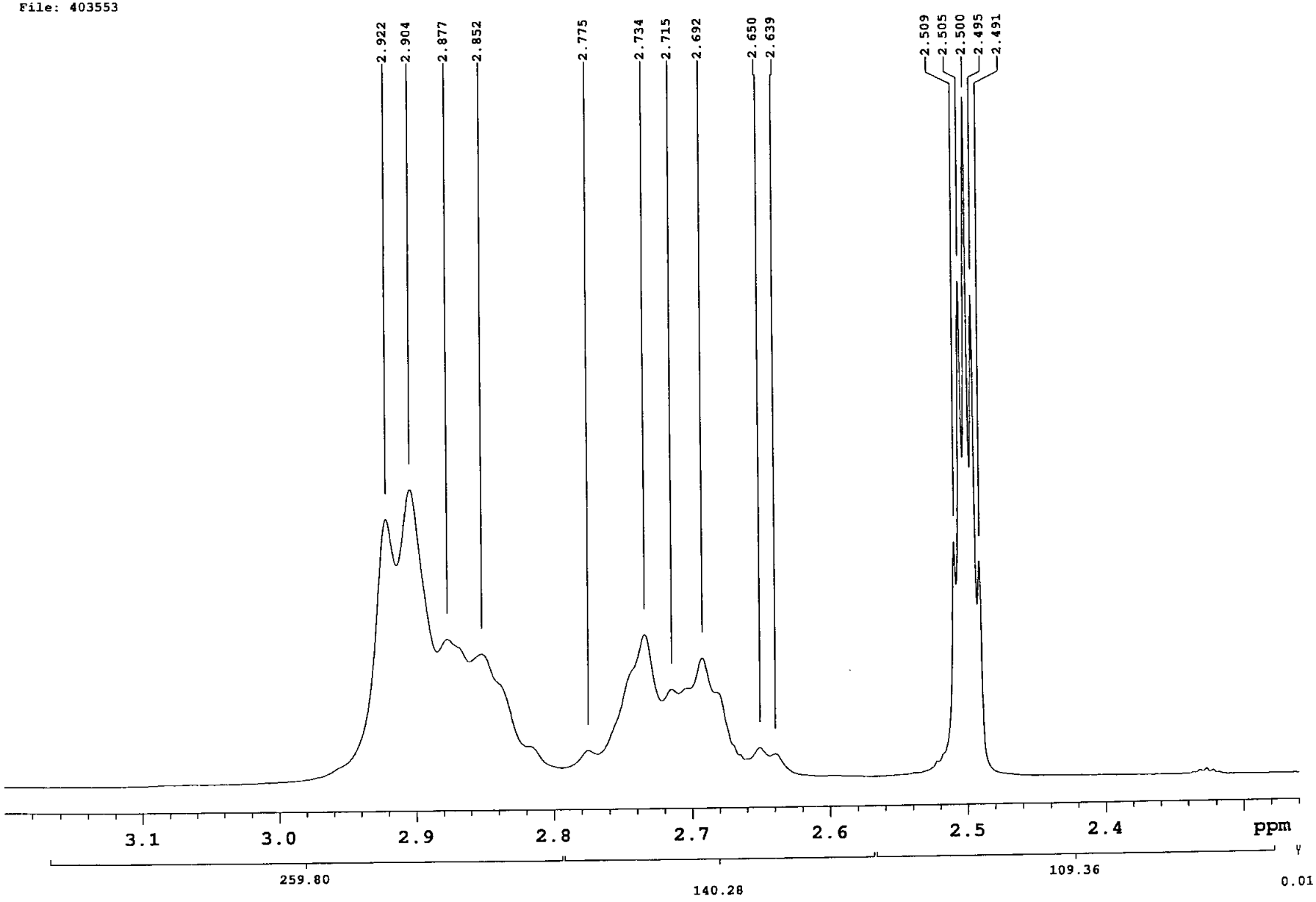


Plot file: 403553-2



235848, 4063-56-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

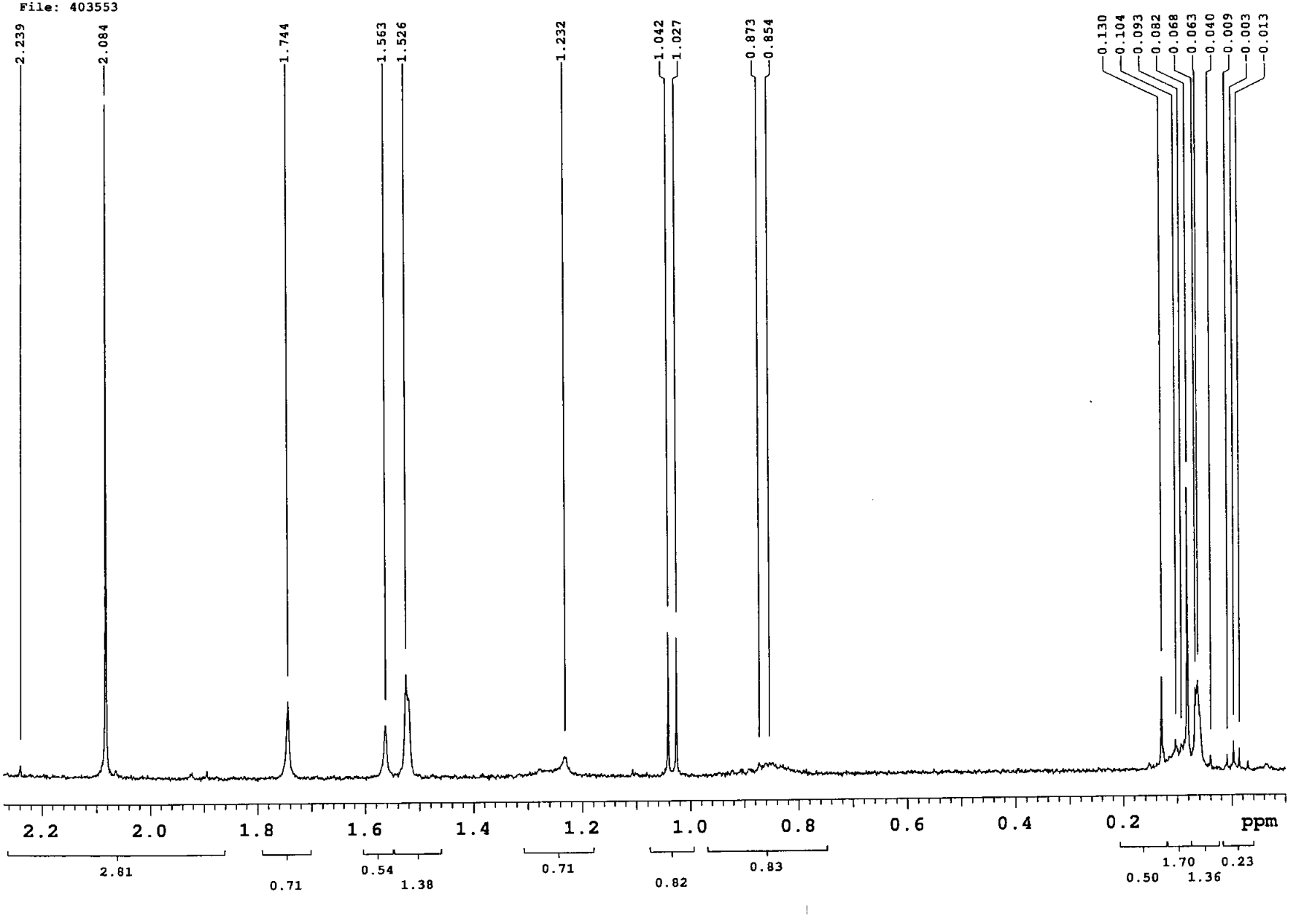
File: 403553



Plot file: 403553-4

235848, 4063-56-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403553



Plot file: 403553-5

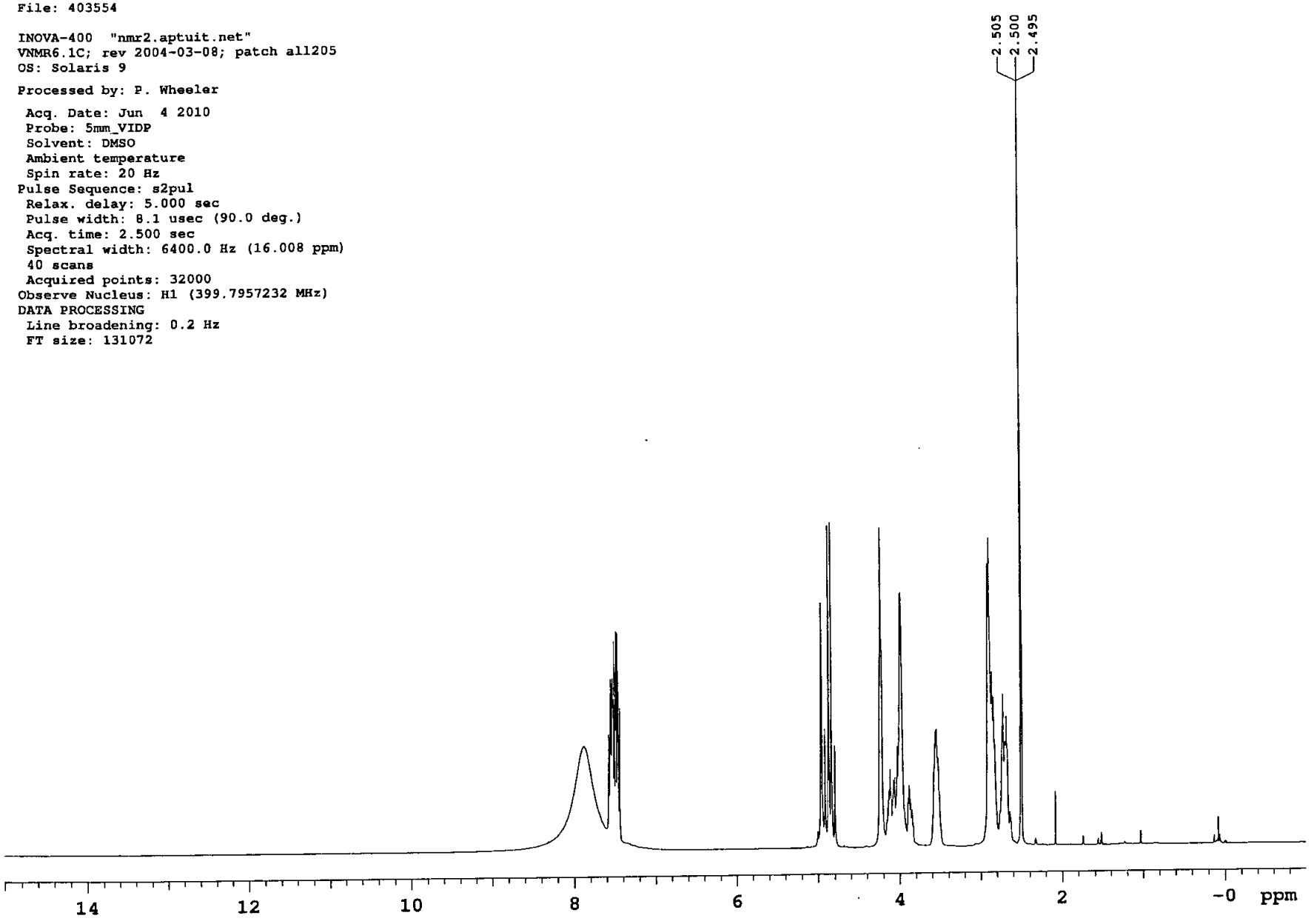
235849, 4063-57-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403554

INOVA-400 "nmr2.aptuit.net"
VNMR6.1C; rev 2004-03-08; patch all205
OS: Solaris 9

Processed by: P. Wheeler

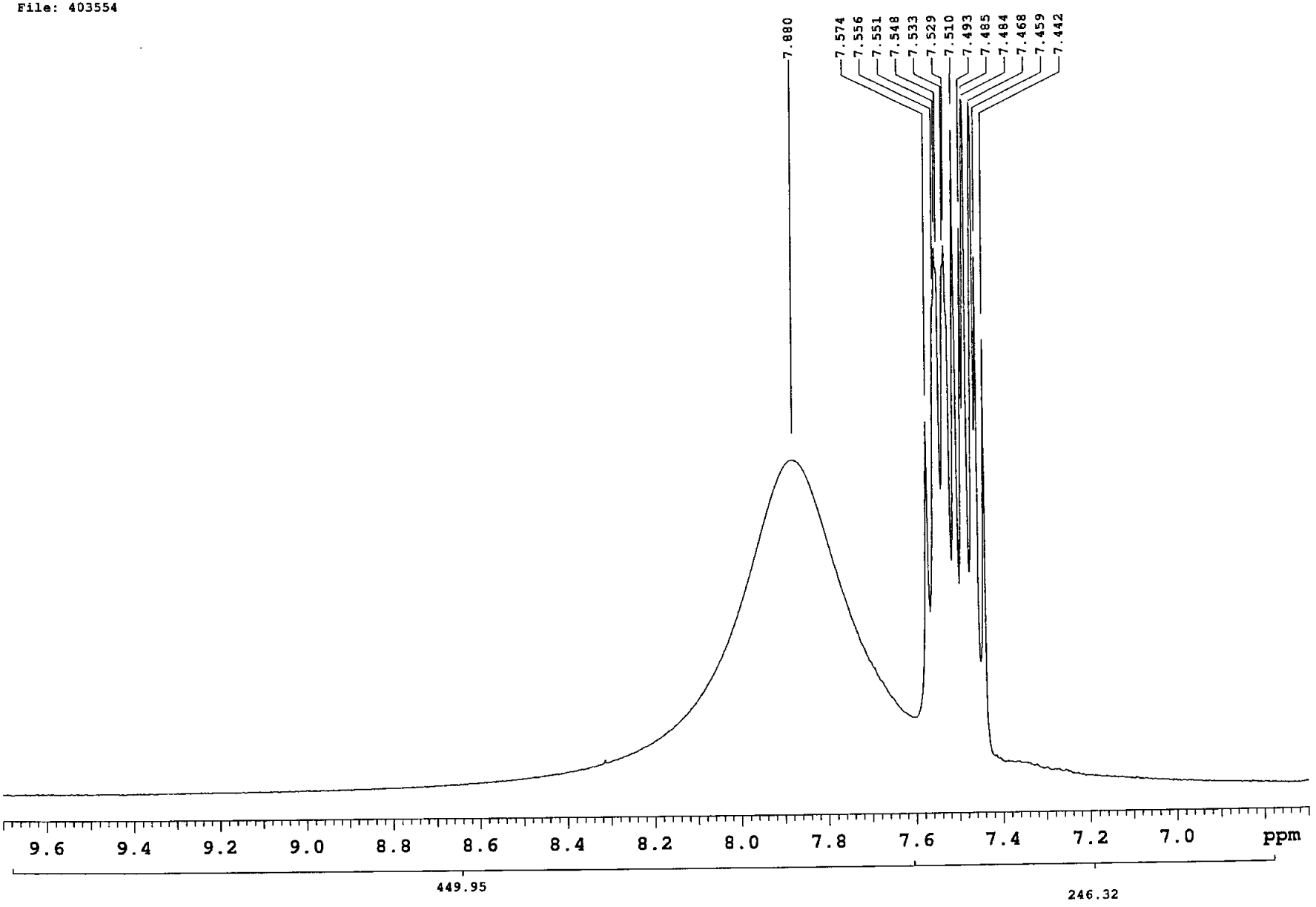
Acq. Date: Jun 4 2010
Probe: 5mm_VIDP
Solvent: DMSO
Ambient temperature
Spin rate: 20 Hz
Pulse Sequence: s2pul
Relax. delay: 5.000 sec
Pulse width: 8.1 usec (90.0 deg.)
Acq. time: 2.500 sec
Spectral width: 6400.0 Hz (16.008 ppm)
40 scans
Acquired points: 32000
Observe Nucleus: H1 (399.7957232 MHz)
DATA PROCESSING
Line broadening: 0.2 Hz
FT size: 131072



Plot file: 403554-1

235849, 4063-57-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

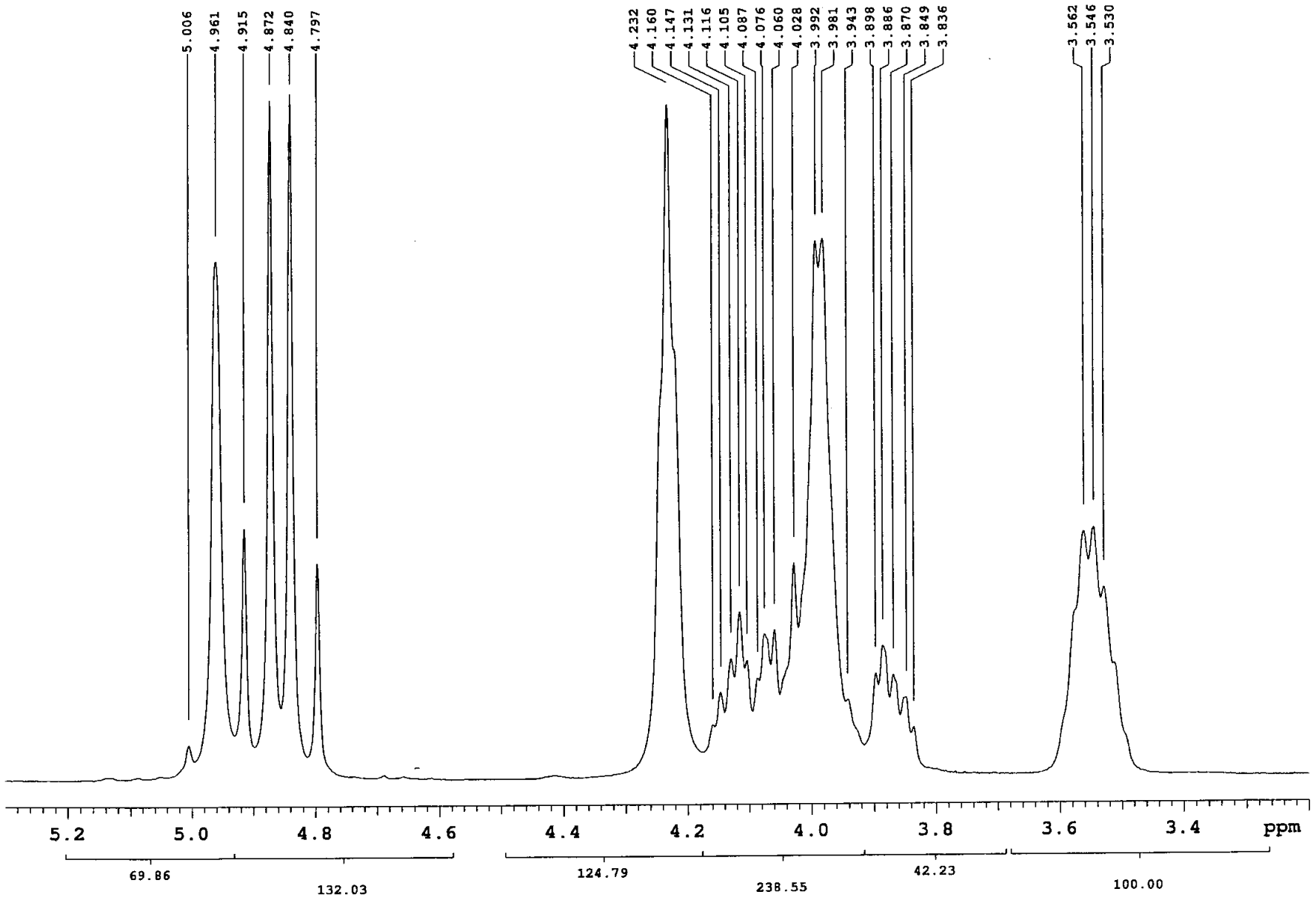
File: 403554



Plot file: 403554-2

235849, 4063-57-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

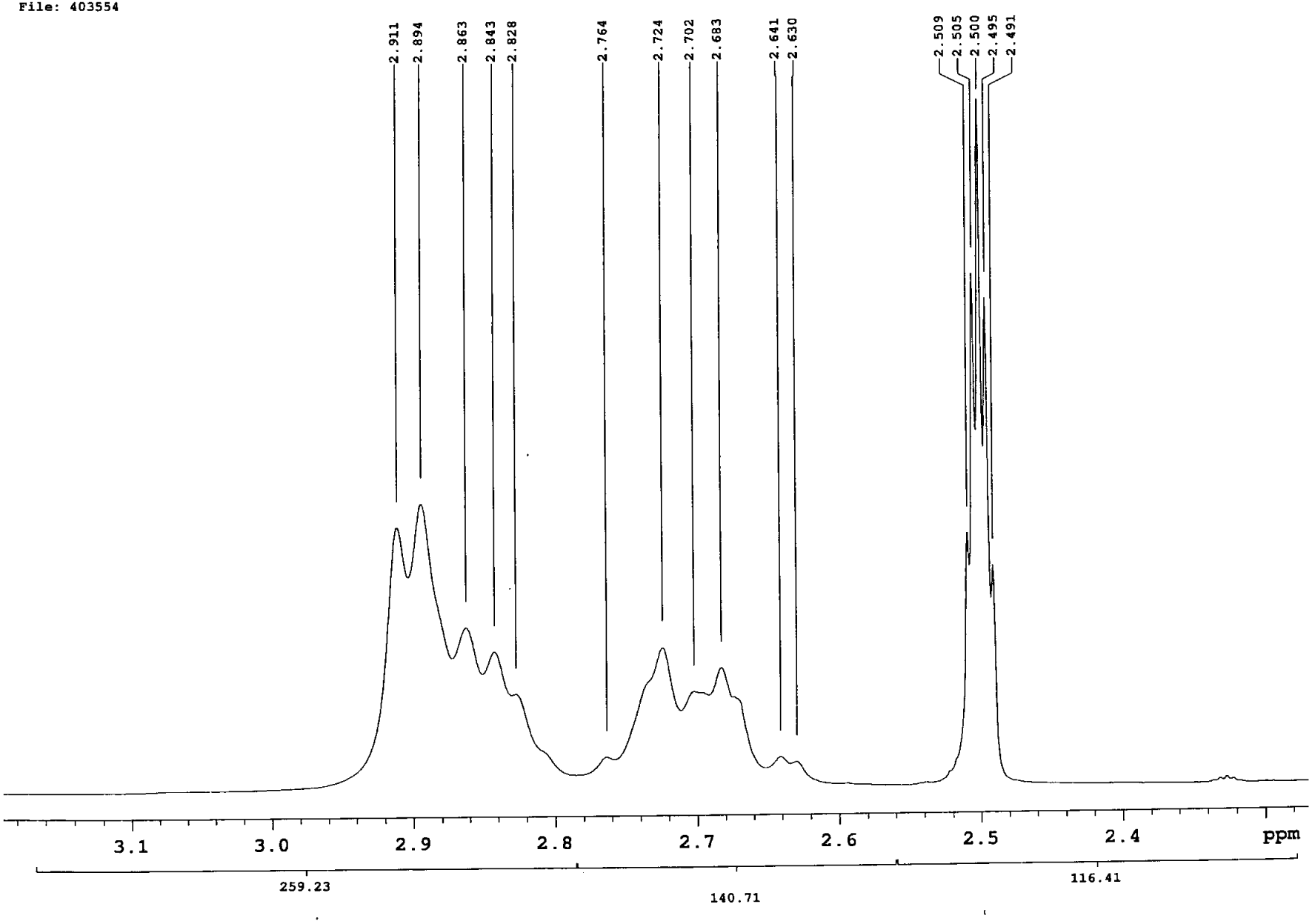
File: 403554



Plot file: 403554-3

235849, 4063-57-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

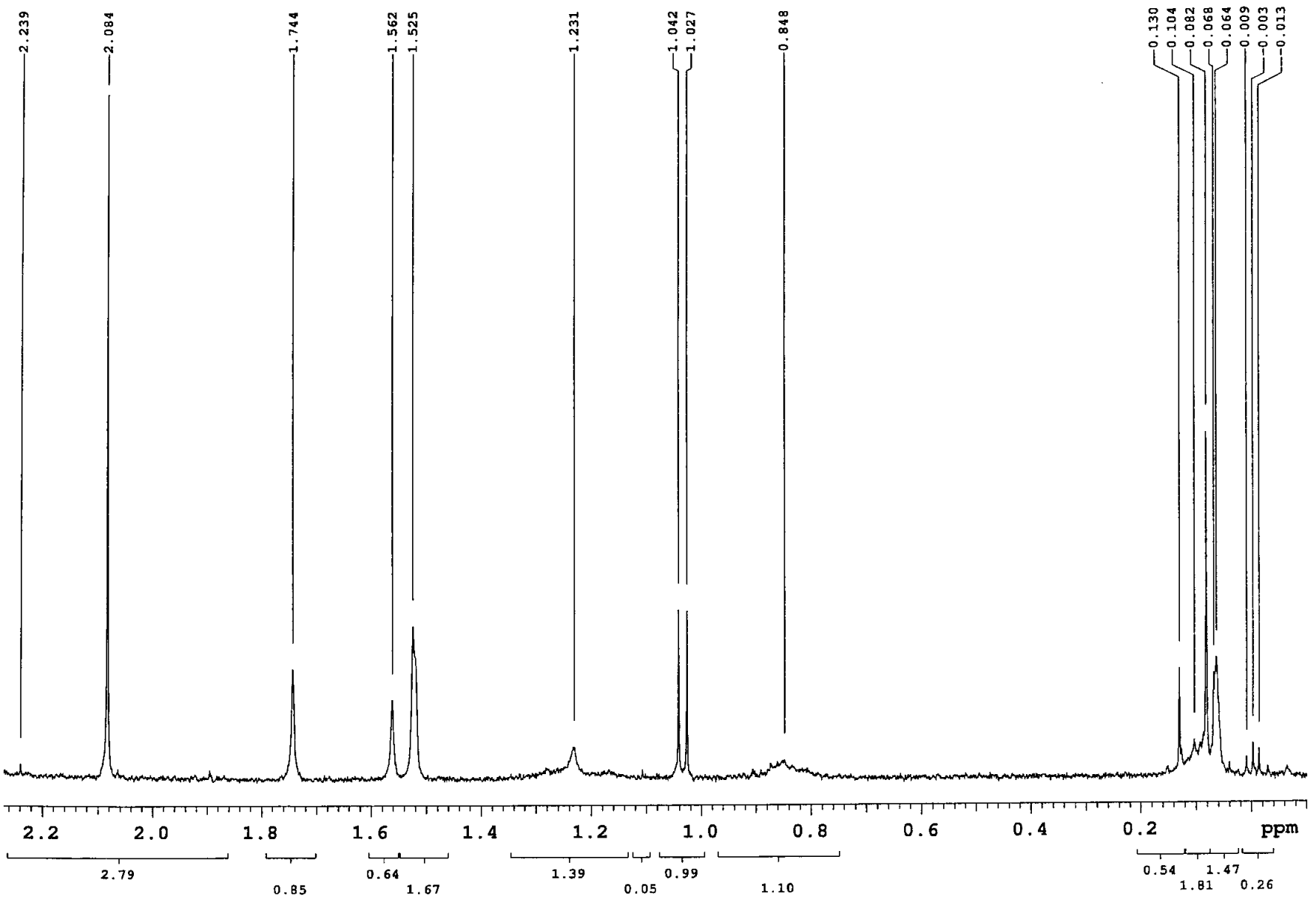
File: 403554



Plot file: 403554-4

235849, 4063-57-01, Compound 184, in DMSO-d6, 1H NMR, referenced to solvent at 2.5 ppm

File: 403554



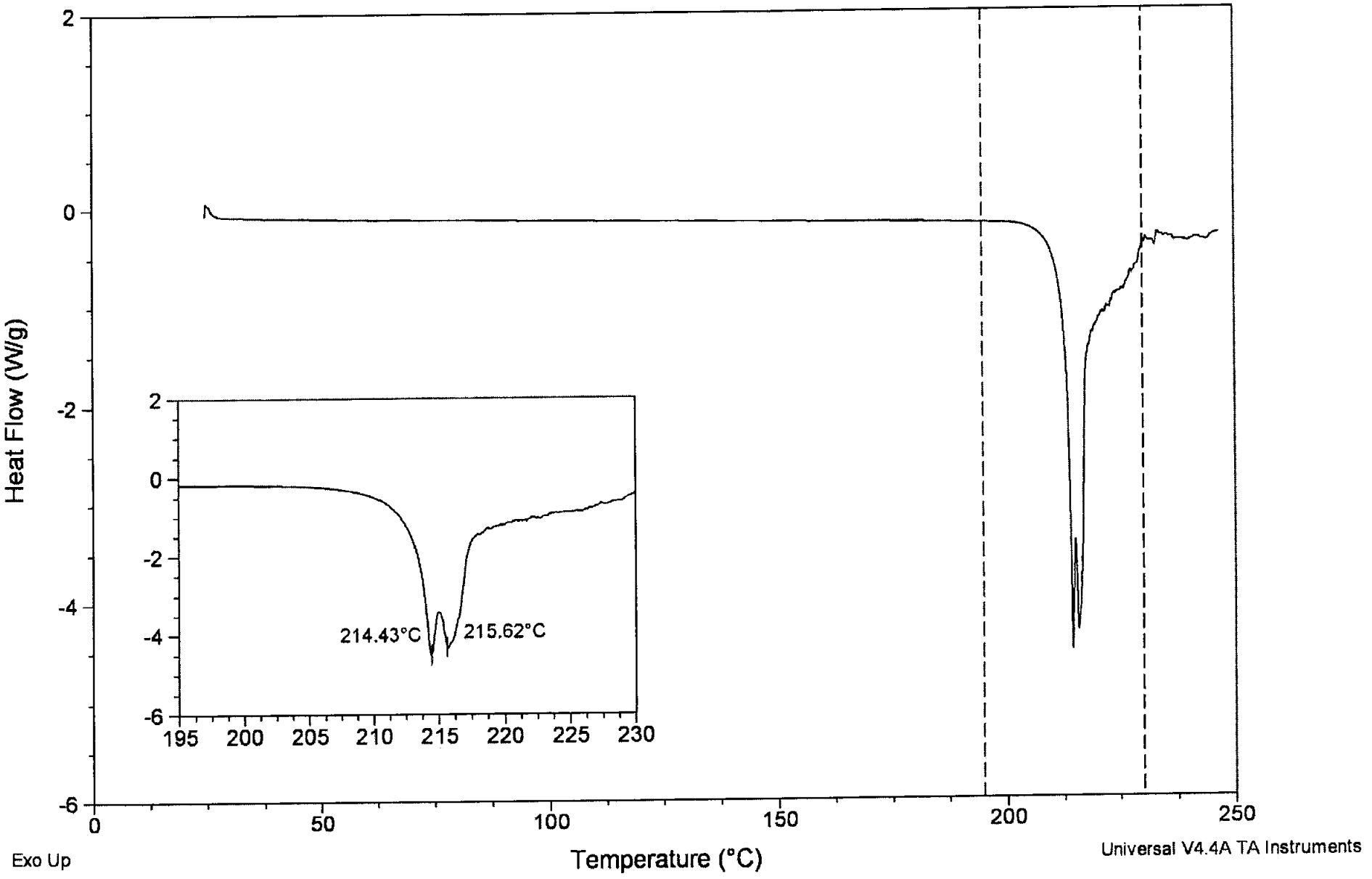
Plot file: 403554-5

EXHIBIT K

Sample: Compound 184
Size: 4.4400 mg
Method: 25-250-10
Comment: 233140, 4063-02-01, 10°C/min, T0HSLP, R2 P1

DSC

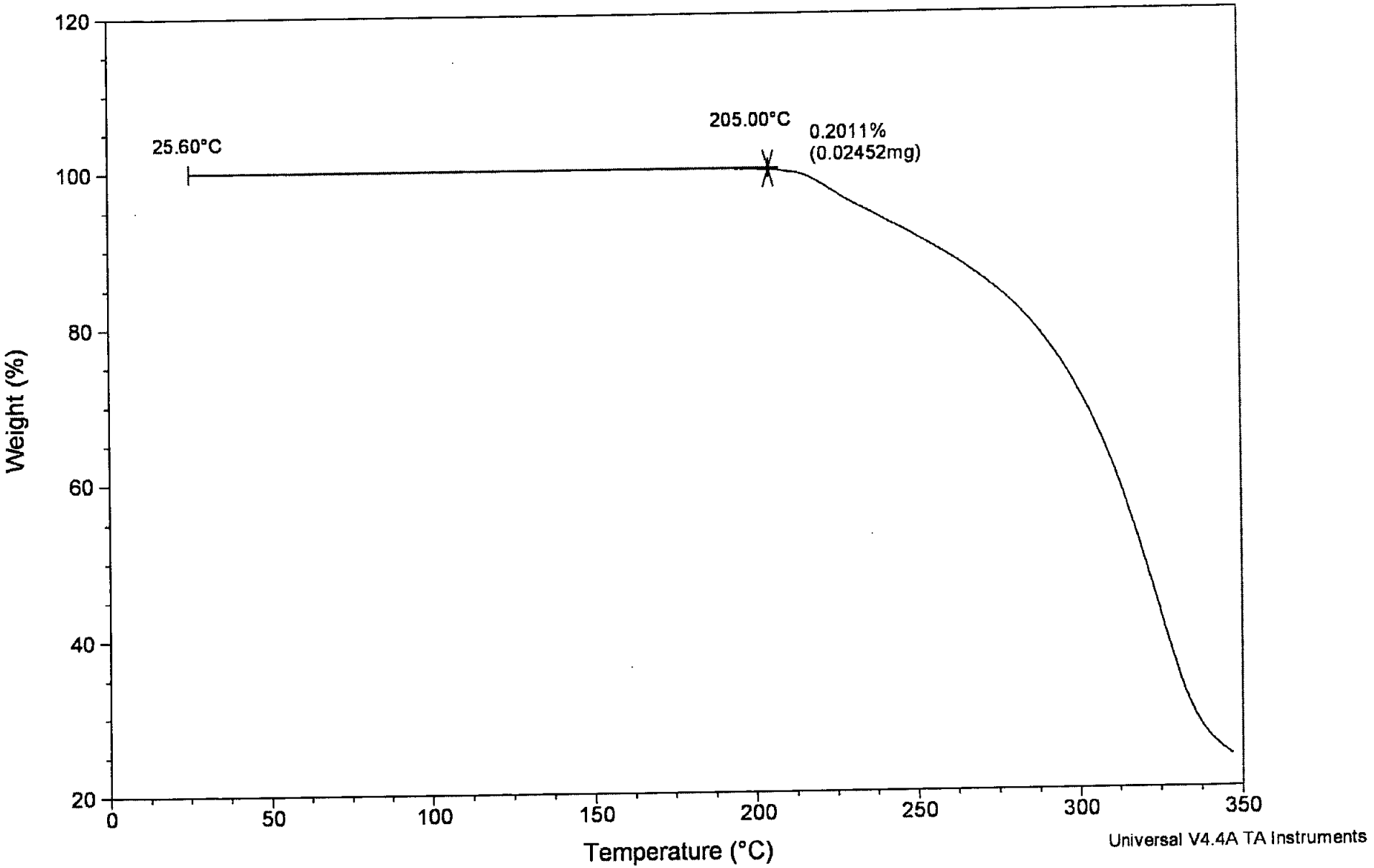
File: I:...\EL20100011\DSC\399011.dsc
Operator: DMP
Run Date: 10-May-2010 15:04
Instrument: DSC Q2000 V23.10 Build 79



Sample: Compound 184
Size: 12.1950 mg
Method: 00-350-10
Comment: 233140, 4063-02-01, 10°C/min, P1

TGA

File: I:...\EL20100011\TGA\398706.tga
Operator: DMP
Run Date: 07-May-2010 14:33
Instrument: AutoTGA 2950 V5.4A

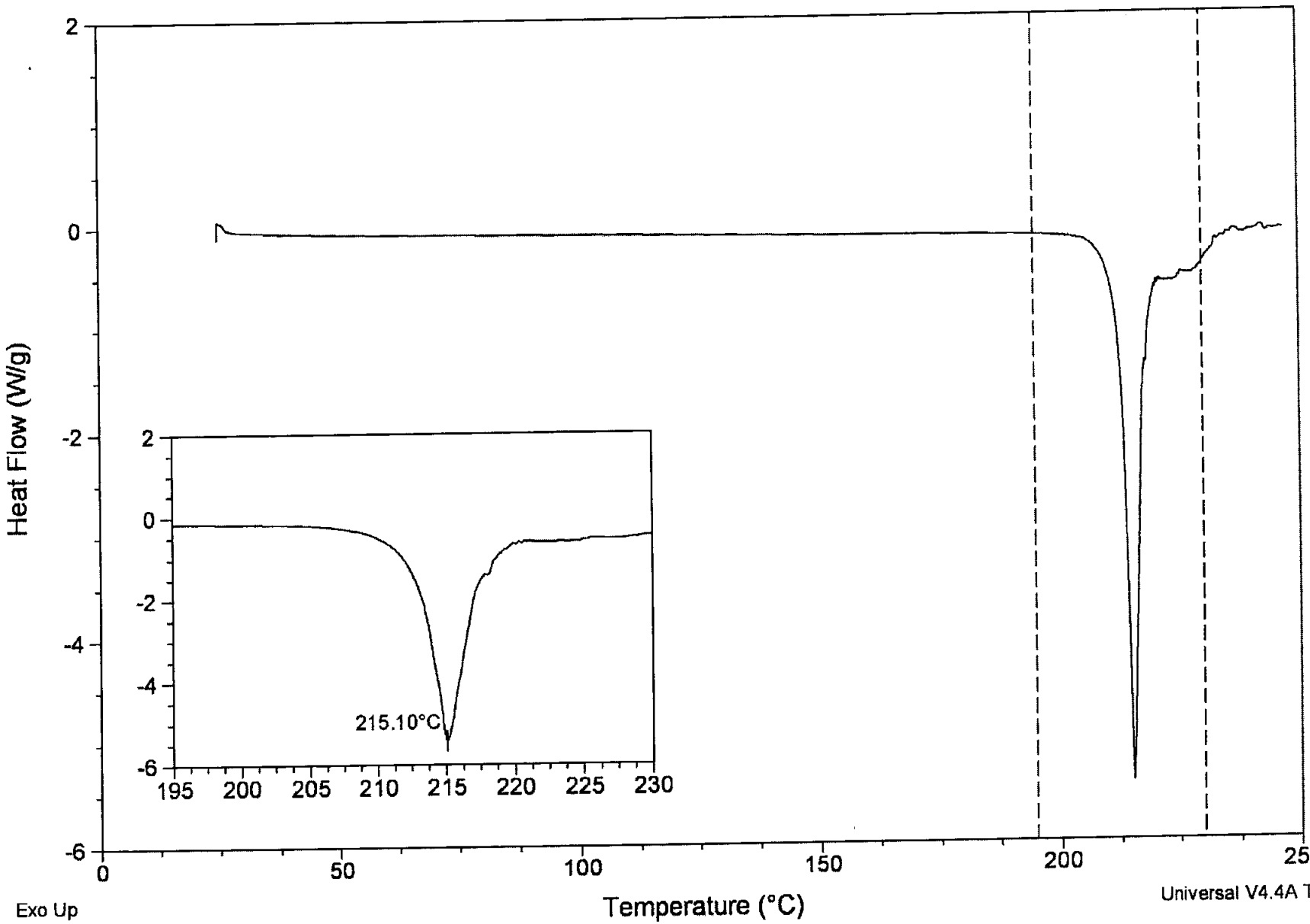


Universal V4.4A TA Instruments

Sample: Compound 184
Size: 2.8200 mg
Method: 25-250-10
Comment: 233141, 4063-03-01, 10°C/min, T0HSLP, R2 P2

DSC

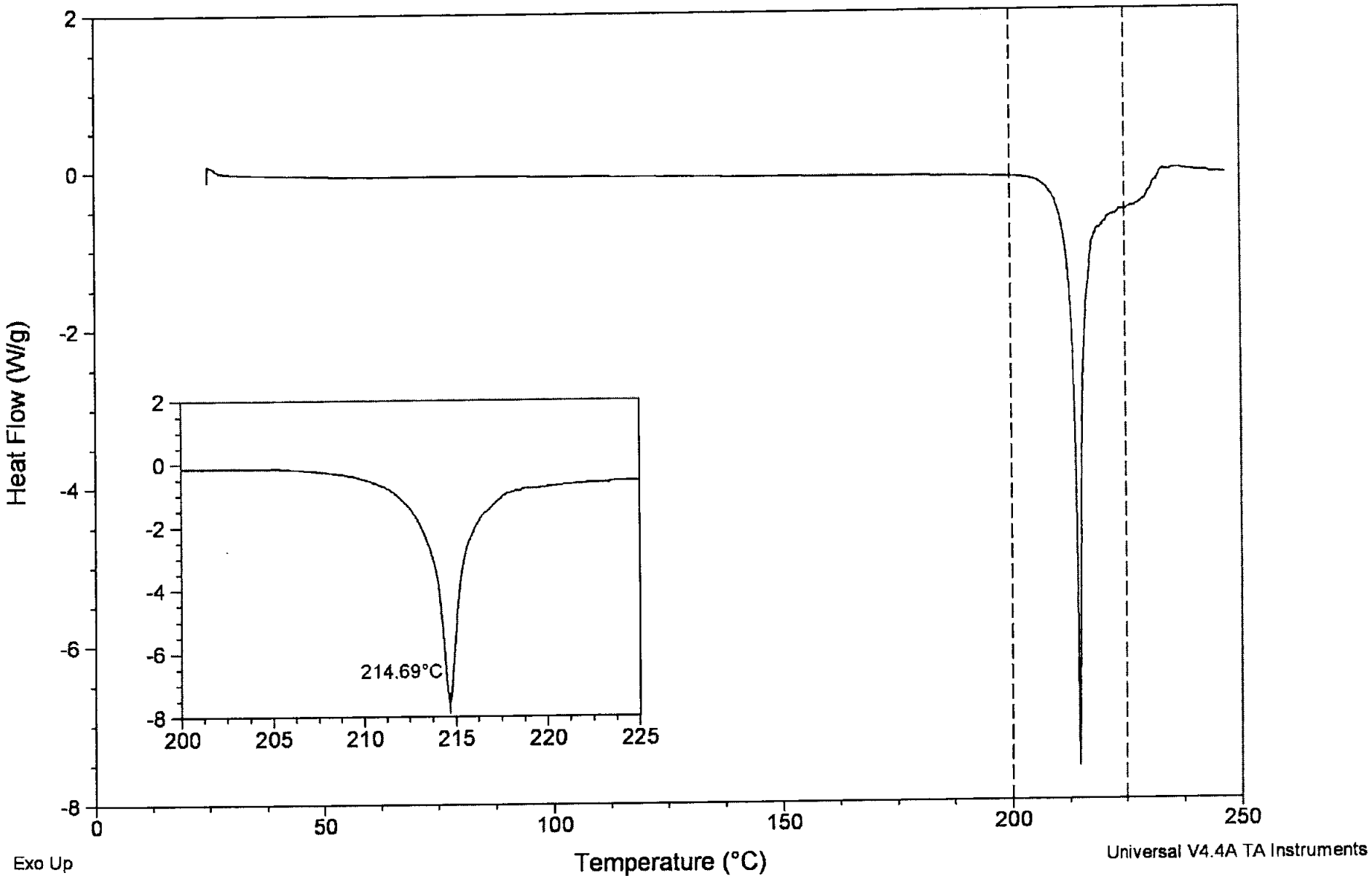
File: I:...\EL20100011\DSC\399012.dsc
Operator: DMP
Run Date: 10-May-2010 15:34
Instrument: DSC Q2000 V23.10 Build 79



Sample: Compound 184
Size: 2.5800 mg
Method: 25-250-10
Comment: 233142, 4063-04-01, 10°C/min, T0HSLP, R2 P3

DSC

File: I:...\EL20100011\DSC\399013.dsc
Operator: DMP
Run Date: 10-May-2010 16:04
Instrument: DSC Q2000 V23.10 Build 79

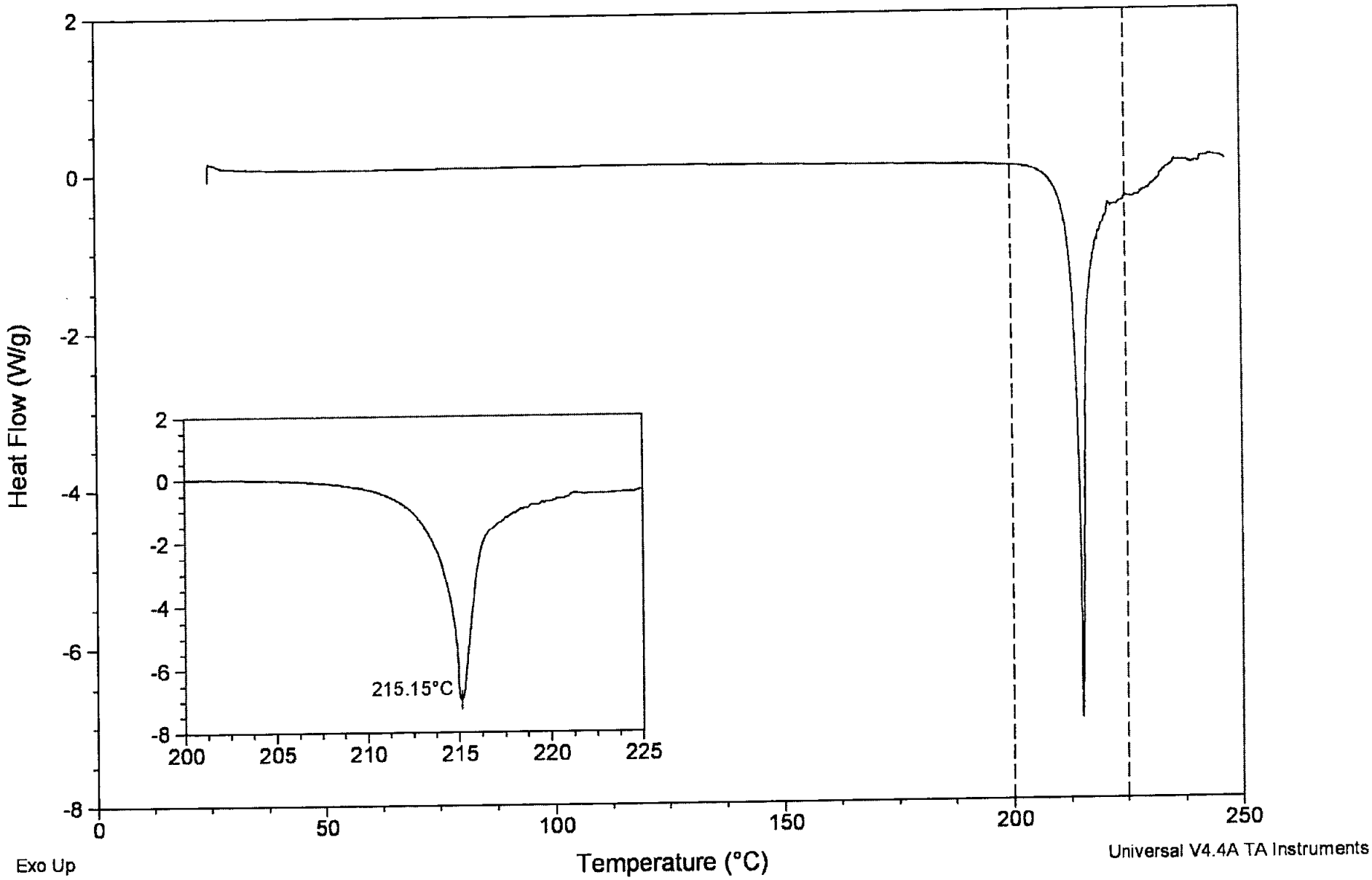


Universal V4.4A TA Instruments

Sample: Compound 184
Size: 2.7200 mg
Method: 25-250-10
Comment: 234584, 4063-19-01, 10°C/min, T0HSLP, R1 P1

DSC

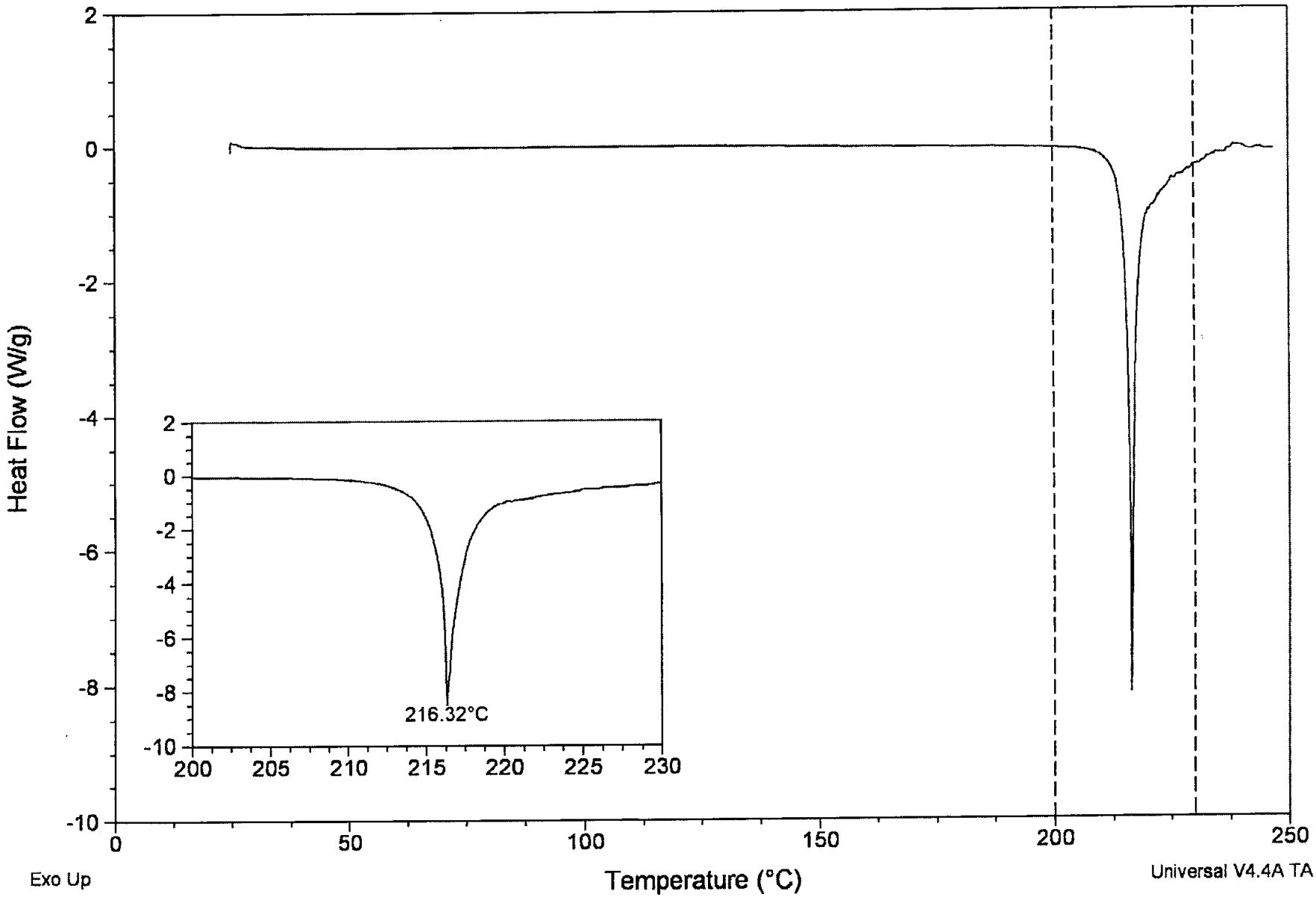
File: J:...\Thermal\401063.dsc
Operator: KEL
Run Date: 20-May-2010 12:15
Instrument: DSC Q2000 V23.10 Build 79



Sample: Compound 184
Size: 3.0500 mg
Method: 25-250-10
Comment: 234636, 4063-18-01, 10°C/min, T0HSLP, R1 P2

DSC

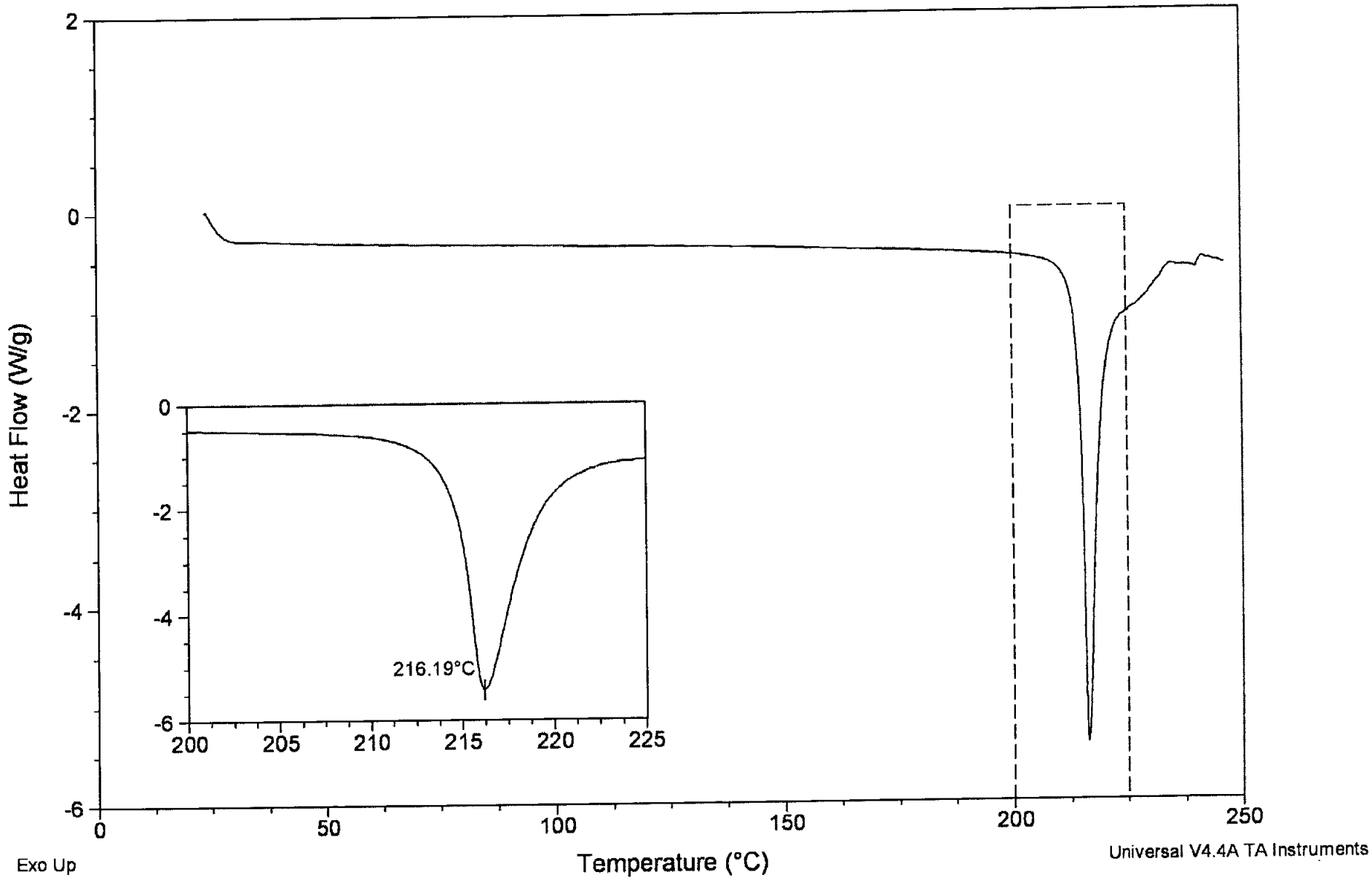
File: J:...\Thermal\401166.dsc
Operator: KEL
Run Date: 20-May-2010 12:45
Instrument: DSC Q2000 V23.10 Build 79



Sample: Compound 184
Size: 4.2500 mg
Method: 25-250-10
Comment: 234872, 4063-34-01, 10°C/min, T0HSLP

DSC

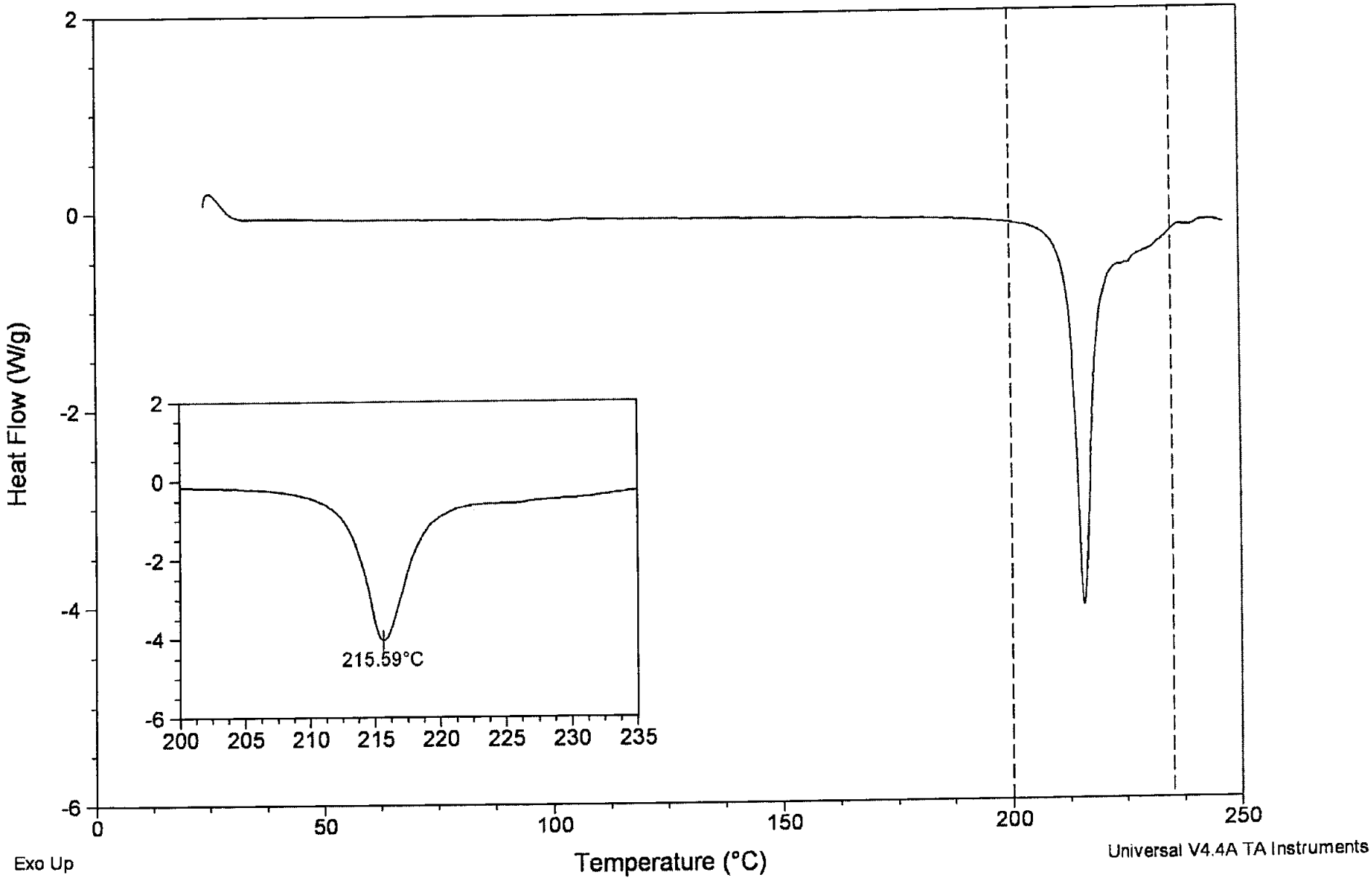
File: I:...\EL20100011\DSC\401684.DSC
Operator: DMP
Run Date: 25-May-2010 10:57
Instrument: 2920 MDSC V2.6A



Sample: Compound 184
Size: 2.4600 mg
Method: 25-250-10
Comment: 234873, 4063-32-01, 10°C/min, T0HSLP

DSC

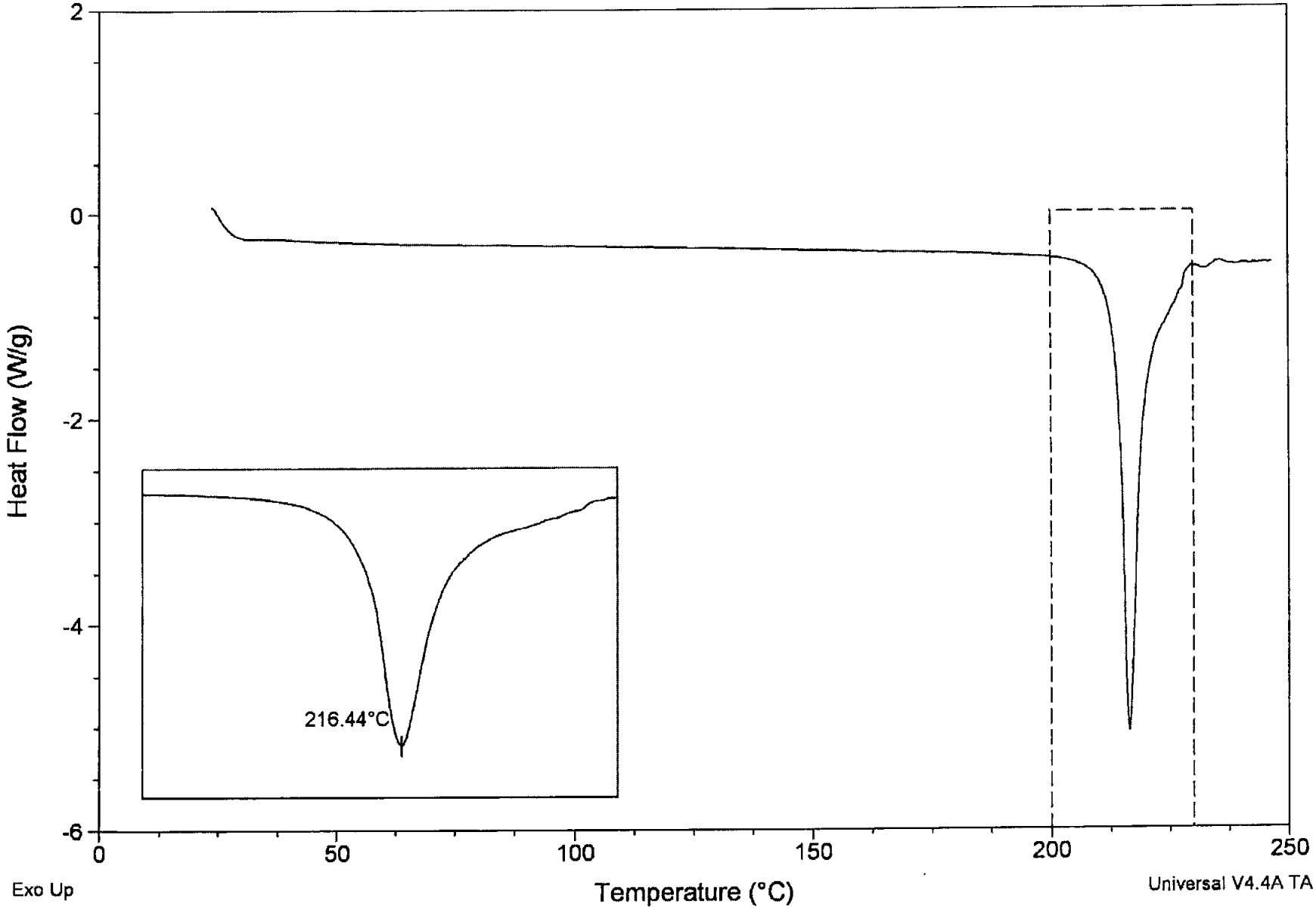
File: I:...\EL20100011\DSC\401685.DSC
Operator: DMP
Run Date: 25-May-2010 11:37
Instrument: 2920 MDSC V2.6A



Sample: Compound 184
Size: 2.6100 mg
Method: 25-250-10
Comment: 234874, 4063-35-01, 10°C/min, T0HSLP

DSC

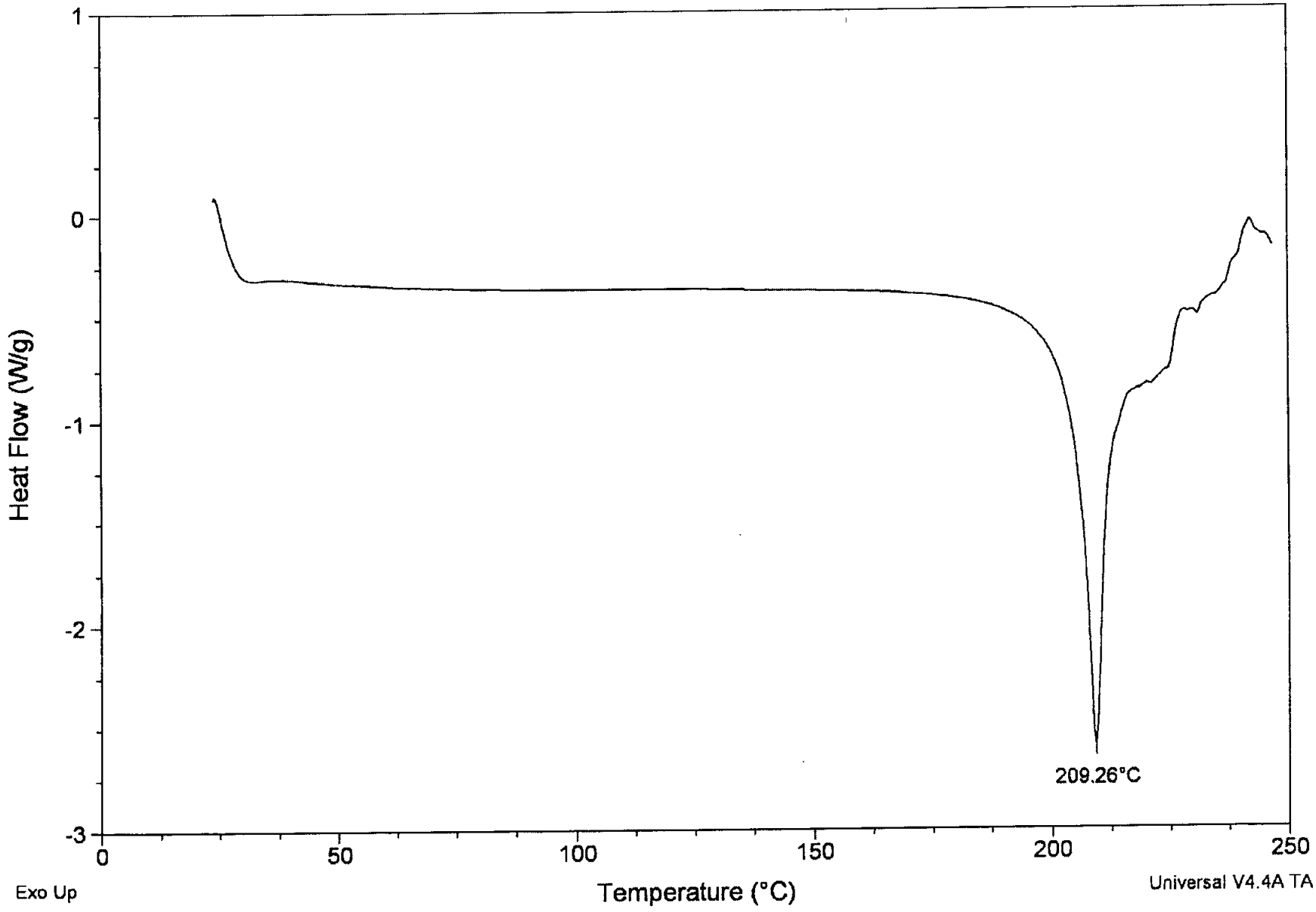
File: I:...\EL20100011\DSC\401686.DSC
Operator: DMP
Run Date: 25-May-2010 12:14
Instrument: 2920 MDSC V2.6A



Sample: Compound 184
Size: 2.0900 mg
Method: 25-250-10
Comment: 235805, 4063-50-01, 10°C/min, T0HSLP

DSC

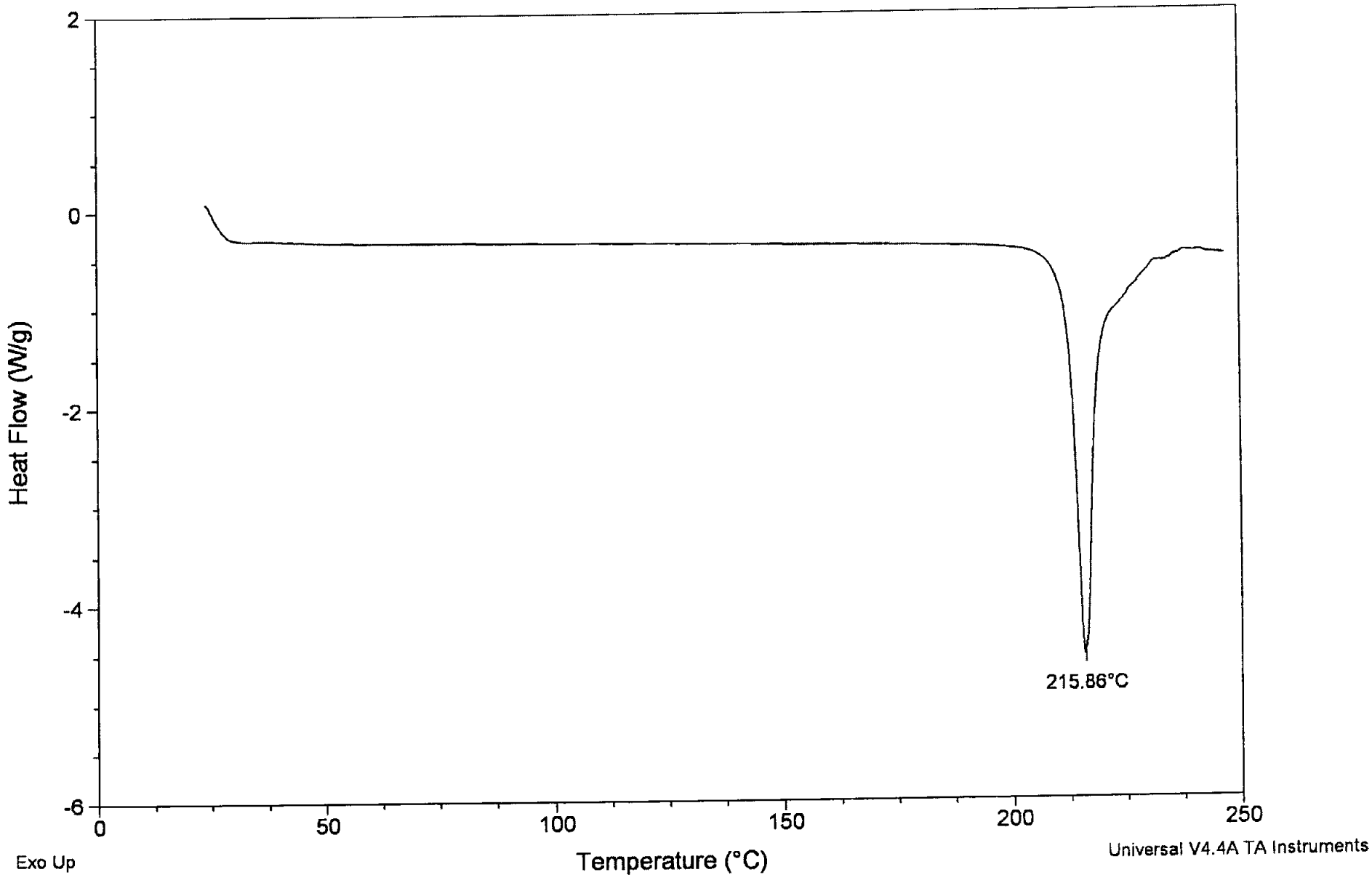
File: I:...\EL20100011\DSC\403329.DSC
Operator: DMP
Run Date: 04-Jun-2010 09:32
Instrument: 2920 MDSC V2.6A



Sample: Compound 184
Size: 2.1800 mg
Method: 25-250-10
Comment: 235806, 4063-51-01, 10°C/min, T0HSLP

DSC

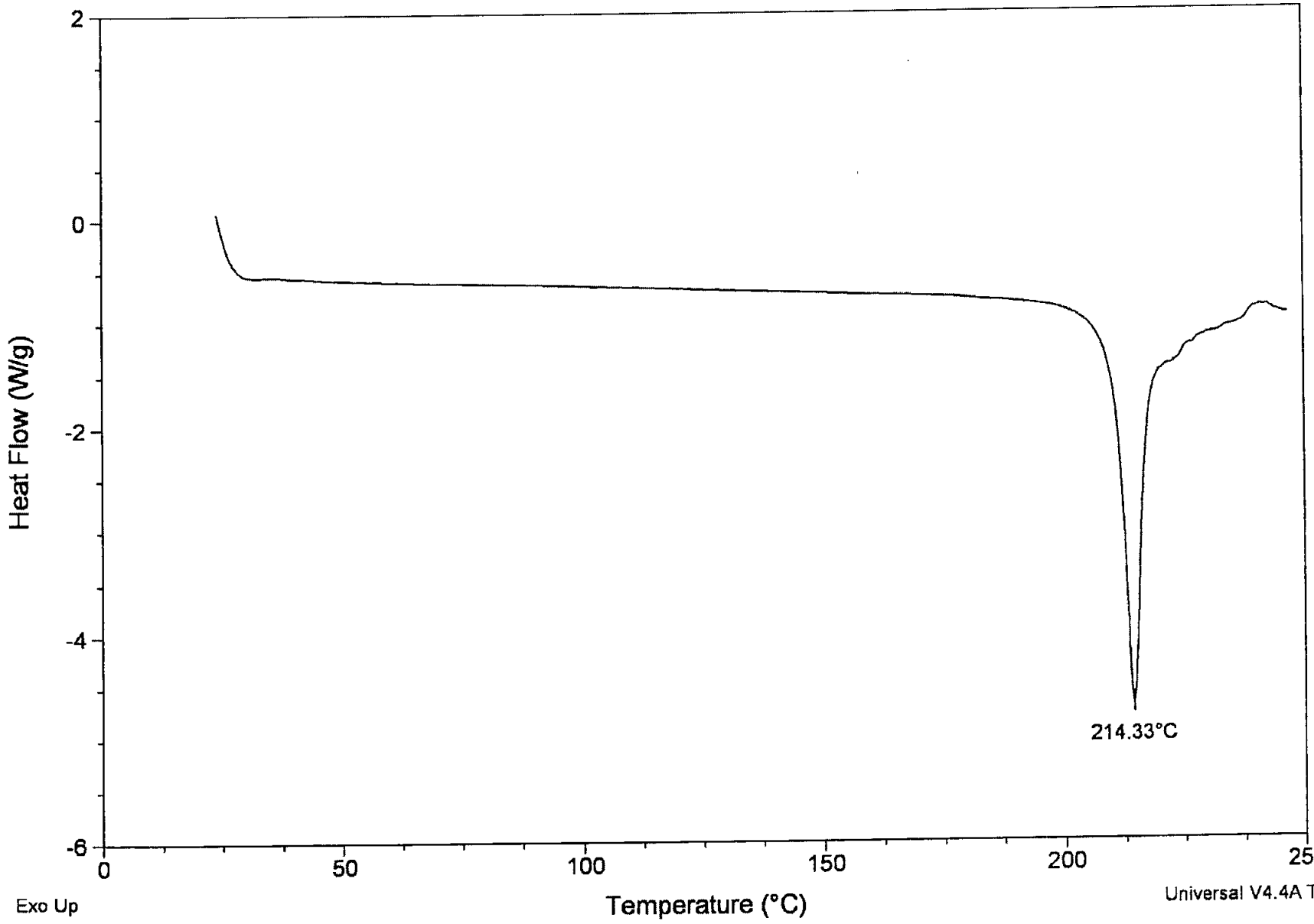
File: I:...\EL20100011\DSC\403330.DSC
Operator: DMP
Run Date: 04-Jun-2010 10:10
Instrument: 2920 MDSC V2.6A



Sample: Compound 184
Size: 1.9000 mg
Method: 25-250-10
Comment: 235848, 4063-56-01, 10°C/min, T0HSLP

DSC

File: I:...\EL20100011\DSC\403548.DSC
Operator: DMP
Run Date: 04-Jun-2010 16:08
Instrument: 2920 MDSC V2.6A



Sample: Compound 184
Size: 2.1000 mg
Method: 25-250-10
Comment: 235849, 4063-57-01, 10°C/min, T0HSLP

DSC

File: I:...\EL20100011\DSC\403550.DSC
Operator: DMP
Run Date: 04-Jun-2010 16:50
Instrument: 2920 MDSC V2.6A

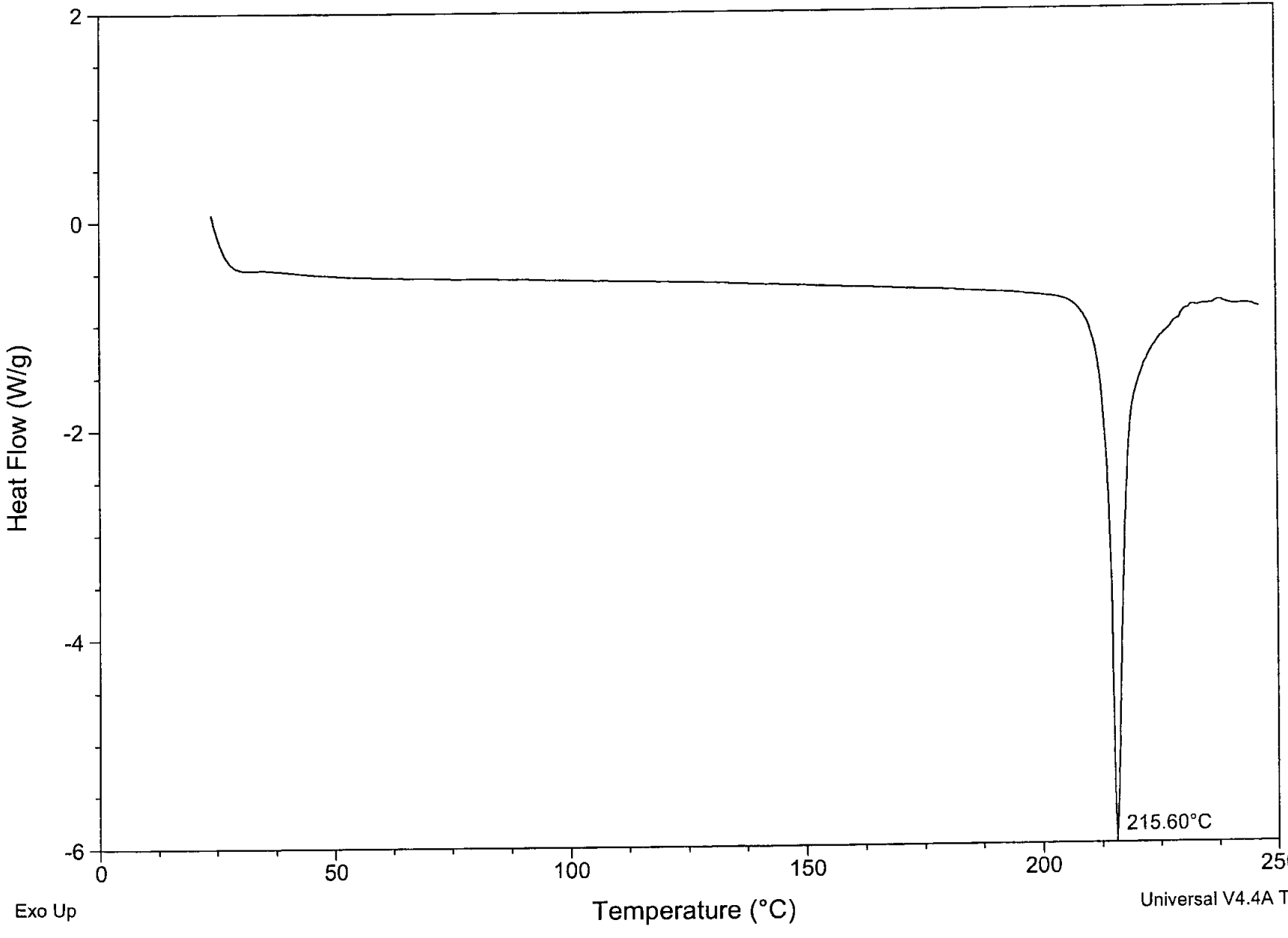


EXHIBIT L

Laboratory Report

Report prepared for:
Karen Gushurst
Aptuit West Lafayette
SSCI
3065 Kent Ave
W Lafayette, IN 47906
Phone: 765-463-0112 ext. 3231
Fax: 765-463-4722
Email: karen.gushurst@aptuit.com

Report prepared by:
Debbie S Robertson

Purchase Order:

For further assistance, contact:
Debbie S Robertson
Report Production Coordinator
PO Box 51610
Knoxville, TN 37950-1610
(865) 546-1335
debbierobertson@galbraith.com

Sample: LIMS 233141
Lab ID: 2010-K-8837 **Received:** 2010-05-11

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon	GLI Procedure ME-3	37.76 %	As Received	1.882 mg	2010-05-12
H : Hydrogen	GLI Procedure ME-3	3.47 %	As Received	1.882 mg	2010-05-12
N : Nitrogen	GLI Procedure ME-3	13.60 %	As Received	1.882 mg	2010-05-12
P : Phosphorus	GLI Procedure ME-70	5.97 %	As Received	110.15 mg	2010-05-11

Sample: LIMS 233142
Lab ID: 2010-K-8838 **Received:** 2010-05-11

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon	GLI Procedure ME-3	37.78 %	As Received	1.345 mg	2010-05-12
H : Hydrogen	GLI Procedure ME-3	3.41 %	As Received	1.345 mg	2010-05-12
N : Nitrogen	GLI Procedure ME-3	13.62 %	As Received	1.345 mg	2010-05-12
P : Phosphorus	GLI Procedure ME-70	5.91 %	As Received	94.28 mg	2010-05-11

Sample: LIMS 233140
Lab ID: 2010-K-8839 **Received:** 2010-05-11

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon	GLI Procedure ME-3	37.92 %	As Received	1.335 mg	2010-05-12
H : Hydrogen	GLI Procedure ME-3	3.43 %	As Received	1.335 mg	2010-05-12

N : Nitrogen					
	GLI Procedure ME-3	13.54 %	As Received	1.335 mg	2010-05-12
P : Phosphorus					
	GLI Procedure ME-70	6.15 %	As Received	135.85 mg	2010-05-11

Signatures:

Published By: Debbie.S.Robertson 2010-05-12T20:27:16.163-04:00

Laboratory Report

Report prepared for:
Karen Gushurst
Senior Research Investigator
SSCI/Aptuit
3065 Kent Ave
W. Lafayette, IN 47906
Phone: 765-463-0112 ext. 3231
Email: karen.gushurst@aptuit.com

Report prepared by:
Tony Pickett

Purchase Order:
APWLF-934

For further assistance, contact:
Tony Pickett
Technical Manager
PO Box 51610
Knoxville, TN 37950-1610
(865) 546-1335
tonypickett@galbraith.com

Sample: 4063-18-01 LIMS 234636
Lab ID: 2010-K-9866 **Received:** 2010-05-21

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	38.01 %	As Received	2.285 mg	2010-05-24
	GLI Procedure ME-2	37.89 %	As Received	2.132 mg	2010-05-24
	GLI Procedure ME-2	37.90 %	As Received	2.213 mg	2010-05-22
	GLI Procedure ME-2	37.75 %	As Received	2.251 mg	2010-05-22
H : Hydrogen					
	GLI Procedure ME-2	3.69 %	As Received	2.285 mg	2010-05-24
	GLI Procedure ME-2	3.67 %	As Received	2.132 mg	2010-05-24
	GLI Procedure ME-2	3.58 %	As Received	2.213 mg	2010-05-22
	GLI Procedure ME-2	3.62 %	As Received	2.251 mg	2010-05-22
N : Nitrogen					
	GLI Procedure ME-2	13.73 %	As Received	2.285 mg	2010-05-24
	GLI Procedure ME-2	13.68 %	As Received	2.132 mg	2010-05-24
P : Phosphorus					
	GLI Procedure ME-70	6.04 %	As Received	26.62 mg	2010-05-22
	GLI Procedure ME-70	6.19 %	As Received	25.64 mg	2010-05-22
	GLI Procedure ME-70	114 % Recovery	As Received	25.22 mg	2010-05-22

Sample: 4063-19-01 LIMS 234584
Lab ID: 2010-K-9867 **Received:** 2010-05-21

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.71 %	As Received	2.148 mg	2010-05-24
	GLI Procedure ME-2	37.64 %	As Received	2.044 mg	2010-05-24
	GLI Procedure ME-2	37.81 %	As Received	2.167 mg	2010-05-22
	GLI Procedure ME-2	37.71 %	As Received	2.157 mg	2010-05-22
H : Hydrogen					
	GLI Procedure ME-2	3.69 %	As Received	2.148 mg	2010-05-24
	GLI Procedure ME-2	3.70 %	As Received	2.044 mg	2010-05-24
	GLI Procedure ME-2	3.66 %	As Received	2.167 mg	2010-05-22

	GLI Procedure ME-2	3.62 %	As Received	2.157 mg	2010-05-22
N : Nitrogen					
	GLI Procedure ME-2	13.65 %	As Received	2.148 mg	2010-05-24
	GLI Procedure ME-2	13.63 %	As Received	2.044 mg	2010-05-24
P : Phosphorus					
	GLI Procedure ME-70	6.05 %	As Received	27.45 mg	2010-05-22
	GLI Procedure ME-70	6.00 %	As Received	25.92 mg	2010-05-22

For all samples on this report:

1. These analyses were performed in compliance with the requirements of 21 CFR 58 pertaining to an analytical chemistry laboratory supporting a study under Good Laboratory Practices, with the following exceptions;
   1. The protocol for the study is not available.
      A. The test article has not been identified.
      B. The sponsor has not been identified.
      C. The study director has not been identified.
      D. The date that the study was initiated has not been supplied.
      E. The current status of the study is not known.
   2. The completion date of the study is not known.
   3. A retention sample and sample container are not archived at our facility. They will be returned for your archival.
   4. The archival of the raw data will occur during the second quarter of 2011. Raw data will be retained for ten years. If data retention is required for a longer period of time, arrangements for a transfer of records must be made.

2. Matrix spike analyses were performed as part of our internal Quality Control Program. There is no additional charge for matrix spike values.

3. Additional duplicate values were generated for *Carbon* and *Hydrogen*. There will be no charge for the additional values.

Signatures:

Modified By:	tony.pickett	2010-05-24T21:02:17.35-04:00
Inspected By:	david.r.venner	2010-05-24T21:04:22.5-04:00
Published By:	david.r.venner	2010-05-24T21:04:30.767-04:00

Quality Assurance Statement

Please forward this document to the Study Director responsible for the chemical analyses enclosed.

Galbraith Sample Identification Number(s): K-9866-9867

Protocol/Study Title: unidentified

Regulations Applicable:21 CFR 58

Galbraith Laboratories, Inc. Project Manager(s): Tony Pickett

Offsite Study Director: unidentified

Sponsor: unidentified

The following are inspection dates and report dates of QA inspections of this study:

PHASES OF STUDY INSPECTED	DATE INSPECTED	REPORTED TO STUDY DIRECTOR	REPORTED TO STUDY DIRECTOR'S MANAGEMENT	REPORTED TO GALBRAITH MANAGEMENT
Raw data for all analyses	05/24/2010	05/24/2010	05/24/2010	05/24/2010
Final Report	05/24/2010	05/24/2010	05/24/2010	05/24/2010

David R. Venner, Inspector D R. Venner Date 05/24/10

Dear Study Director and Management Representative:

Please sign and date below to acknowledge receipt of this Quality Assurance Statement and attached findings/observations. Fax signed and dated acknowledgement to the Quality Assurance Unit at 865-546-7209.

Study Director _____ Date _____

Management Representative _____ Date _____

Mailing: P.O. Box 51610 | Knoxville, TN 37950-1610
Toll Free: 1.877.445.8797
Shipping: 2323 Sycamore Dr. | Knoxville, TN 37921-1700
www.galbraith.com
Fax: 865.546.7209

***** CONFIDENTIAL *** CONFIDENTIAL *** CONFIDENTIAL *****

The following findings proceeded from our inspection of your study under Galbraith sample identification number(s): K-9866-9867

Finding/Observation	Disposition of Finding
The project management sheet listed an incorrect method for the Phosphorus analysis.	Corrected
The Phosphorus prep analyst omitted the hot plate instrument number from the project management sheet and the data sheet	Corrected
The data verifier and Phosphorus prep analyst left blank spaces on the data sheet without marking them "NA"	Corrected
Valid Carbon and Hydrogen data from 05/22/10 was omitted from the analytical report.	Report amended prior to release.

David R. Venner, Inspector

05/24/10
Date

Mailing: P.O. Box 51610 | Knoxville, TN 37950-1610
Toll Free: 1.877.449.8797
Shipping: 2323 Sycamore Dr. | Knoxville, TN 37921-1700
www.galbraith.com
Fax: 865.546.7209

Quality Assurance Statement

Please forward this document to the Study Director responsible for the chemical analyses enclosed.

Galbraith Sample Identification Number(s): L-0077 thru L-0079

Protocol/Study Title: unidentified

Regulations Applicable: 21 CFR 58

Galbraith Laboratories, Inc. Project Manager(s): Tony Pickett

Offsite Study Director: unidentified

Sponsor: unidentified

The following are inspection dates and report dates of QA inspections of this study:

PHASES OF STUDY INSPECTED	DATE INSPECTED	REPORTED TO STUDY DIRECTOR	REPORTED TO STUDY DIRECTOR'S MANAGEMENT	REPORTED TO GALBRAITH MANAGEMENT
Raw Data	5/26/10	5/26/10	5/26/10	5/26/10
Report	5/26/10	5/26/10	5/26/10	5/26/10

Inspector D. R. Harris Date 05/26/10

Dear Study Director and Management Representative:

Please sign and date below to acknowledge receipt of this Quality Assurance Statement and attached findings/observations. Fax signed and dated acknowledgement to the Quality Assurance Unit at 865-546-7209.

Study Director _____ Date _____

Management Representative _____ Date _____

Mailing: P.O. Box 51610 | Knoxville, TN 37950-1610
Toll Free: 1.877.449.8797
www.galbraith.com
Shipping: 2323 Sycamore Dr. | Knoxville, TN 37921-1700
Fax: 865.546.7209

***** CONFIDENTIAL *** CONFIDENTIAL *** CONFIDENTIAL *****

The following findings proceeded from our inspection of your study under Galbraith sample identification number(s): L-0077 thru L-0079

Finding/Observation	Disposition of Finding
Comment box on datasheet not filled out	Corrected
Number of pages in datasheet packet was not indicated	Corrected
Units were missing for duplicate result on L0079	Corrected

David R. Vernon
Inspector

05/26/10
Date

Mailing: P.O. Box 51610 | Knoxville, TN 37950-1610
Toll Free: 1.877.449.8797
www.galbraith.com
Shipping: 2323 Sycamore Dr. | Knoxville, TN 37921-1700
Fax: 865.546.7209

Report Number: 22108

GALBRAITH LABORATORIES, INC.

Report Date: 2010-05-26

Laboratory Report

Report prepared for:
Karen Gushurst
Senior Research Investigator
SSCI/Aptuit
3065 Kent Ave
W Lafayette, IN 47906
Phone: 765-463-0112 ext. 3231
Email: karen.gushurst@aptuit.com

Report prepared by:
Tony Pickett

Purchase Order:
APWLF-945

For further assistance, contact:
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Technical Manager
PO Box 51610
Knoxville, TN 37950-1610
(865) 546-1335
tonypickett@galbraith.com

Sample: 234872
Lab ID: 2010-L-0077 **Received:** 2010-05-25

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.86 %	As Received	2.410 mg	2010-05-26
	GLI Procedure ME-2	37.78 %	As Received	2.230 mg	2010-05-26
H : Hydrogen					
	GLI Procedure ME-2	3.60 %	As Received	2.410 mg	2010-05-26
	GLI Procedure ME-2	3.66 %	As Received	2.230 mg	2010-05-26
N : Nitrogen					
	GLI Procedure ME-2	13.97 %	As Received	2.410 mg	2010-05-26
	GLI Procedure ME-2	13.94 %	As Received	2.230 mg	2010-05-26
P : Phosphorus					
	GLI Procedure ME-70	6.18 %	As Received	25.51 mg	2010-05-26
	GLI Procedure ME-70	6.26 %	As Received	25.61 mg	2010-05-26
	GLI Procedure ME-70	115 % Recovery	As Received	23.18 mg	2010-05-26

Sample: 234873
Lab ID: 2010-L-0078 **Received:** 2010-05-25

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.79 %	As Received	2.290 mg	2010-05-26
	GLI Procedure ME-2	37.71 %	As Received	2.053 mg	2010-05-26
H : Hydrogen					
	GLI Procedure ME-2	3.57 %	As Received	2.290 mg	2010-05-26
	GLI Procedure ME-2	3.59 %	As Received	2.053 mg	2010-05-26
N : Nitrogen					
	GLI Procedure ME-2	13.97 %	As Received	2.290 mg	2010-05-26
	GLI Procedure ME-2	13.97 %	As Received	2.053 mg	2010-05-26
P : Phosphorus					
	GLI Procedure ME-70	6.33 %	As Received	25.33 mg	2010-05-26
	GLI Procedure ME-70	6.25 %	As Received	26.15 mg	2010-05-26

Sample: 234874
Lab ID: 2010-L-0079 **Received:** 2010-05-25

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.85 %	As Received	2.029 mg	2010-05-26
	GLI Procedure ME-2	37.79 %	As Received	2.111 mg	2010-05-26
H : Hydrogen					
	GLI Procedure ME-2	3.60 %	As Received	2.029 mg	2010-05-26
	GLI Procedure ME-2	3.64 %	As Received	2.111 mg	2010-05-26
N : Nitrogen					
	GLI Procedure ME-2	13.92 %	As Received	2.029 mg	2010-05-26
	GLI Procedure ME-2	13.90 %	As Received	2.111 mg	2010-05-26
P : Phosphorus					
	GLI Procedure ME-70	6.03 %	As Received	27.98 mg	2010-05-26
	GLI Procedure ME-70	6.21 %	As Received	25.91 mg	2010-05-26

For all samples on this report:

1. These analyses were performed in compliance with the requirements of 21 CFR 58 pertaining to an analytical chemistry laboratory supporting a study under Good Laboratory Practices, with the following exceptions;
   1. The protocol for the study is not available.
      A. The test article has not been identified.
      B. The sponsor has not been identified.
      C. The study director has not been identified.
      D. The date that the study was initiated has not been supplied.
      E. The current status of the study is not known.
   2. The completion date of the study is not known.
   3. A retention sample and sample container are not archived at our facility. They will be returned for your archival.
   4. The archival of the raw data will occur during the second quarter of 2011. Raw data will be retained for ten years. If data retention is required for a longer period of time, arrangements for a transfer of records must be made.

2. Matrix spike analyses were performed as part of our internal Quality Control Program. There is no additional charge for matrix spike values.

Signatures:

Inspected By:	david.r.venner	2010-05-26T20:14:32.68-04:00
Published By:	david.r.venner	2010-05-26T20:14:38.68-04:00

Laboratory Report

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Technical Manager
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Knoxville, TN 37950-1610
(865) 546-1335
danlongnecker@galbraith.com

Sample: 235805
Lab ID: 2010-L-1100 **Received:** 2010-06-07

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	36.44 %	As Received	2.406 mg	2010-06-08
	GLI Procedure ME-2	36.57 %	As Received	2.099 mg	2010-06-08
H : Hydrogen					
	GLI Procedure ME-2	3.58 %	As Received	2.406 mg	2010-06-08
	GLI Procedure ME-2	3.65 %	As Received	2.099 mg	2010-06-08
N : Nitrogen					
	GLI Procedure ME-2	13.59 %	As Received	2.406 mg	2010-06-08
	GLI Procedure ME-2	13.53 %	As Received	2.099 mg	2010-06-08
P : Phosphorus					
	GLI Procedure ME-70	6.33 %	As Received	26.88 mg	2010-06-08
	GLI Procedure ME-70	6.29 %	As Received	31.59 mg	2010-06-08
	GLI Procedure ME-70 [1]	94 % Recovery	As Received	31.89 mg	2010-06-08

1. The matrix spike analysis was performed to satisfy method requirements. There is no additional charge for the matrix spike result.

Sample: 235806
Lab ID: 2010-L-1101 **Received:** 2010-06-07

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.97 %	As Received	2.066 mg	2010-06-08
	GLI Procedure ME-2	37.94 %	As Received	2.245 mg	2010-06-08
H : Hydrogen					
	GLI Procedure ME-2	3.58 %	As Received	2.066 mg	2010-06-08
	GLI Procedure ME-2	3.61 %	As Received	2.245 mg	2010-06-08
N : Nitrogen					
	GLI Procedure ME-2	14.05 %	As Received	2.066 mg	2010-06-08
	GLI Procedure ME-2	14.11 %	As Received	2.245 mg	2010-06-08
P : Phosphorus					
	GLI Procedure ME-70	5.72 %	As Received	26.90 mg	2010-06-08

	GLI Procedure ME-70	5.84 %	As Received	27.60 mg	2010-06-08

Sample: 235848
Lab ID: 2010-L-1102 **Received:** 2010-06-07

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.68 %	As Received	2.114 mg	2010-06-08
	GLI Procedure ME-2	37.59 %	As Received	2.072 mg	2010-06-08
H : Hydrogen					
	GLI Procedure ME-2	3.54 %	As Received	2.114 mg	2010-06-08
	GLI Procedure ME-2	3.58 %	As Received	2.072 mg	2010-06-08
N : Nitrogen					
	GLI Procedure ME-2	13.95 %	As Received	2.114 mg	2010-06-08
	GLI Procedure ME-2	14.00 %	As Received	2.072 mg	2010-06-08
P : Phosphorus					
	GLI Procedure ME-70	6.02 %	As Received	29.57 mg	2010-06-08
	GLI Procedure ME-70	5.81 %	As Received	27.39 mg	2010-06-08

Sample: 235849
Lab ID: 2010-L-1103 **Received:** 2010-06-07

Analysis	Method	Result	Basis	Amount	Date (Time)
C : Carbon					
	GLI Procedure ME-2	37.89 %	As Received	2.119 mg	2010-06-08
	GLI Procedure ME-2	37.85 %	As Received	2.088 mg	2010-06-08
H : Hydrogen					
	GLI Procedure ME-2	3.59 %	As Received	2.119 mg	2010-06-08
	GLI Procedure ME-2	3.57 %	As Received	2.088 mg	2010-06-08
N : Nitrogen					
	GLI Procedure ME-2	13.99 %	As Received	2.119 mg	2010-06-08
	GLI Procedure ME-2	13.96 %	As Received	2.088 mg	2010-06-08
P : Phosphorus					
	GLI Procedure ME-70	5.84 %	As Received	28.72 mg	2010-06-08
	GLI Procedure ME-70	5.85 %	As Received	28.14 mg	2010-06-08

For all samples on this report:

2. These analyses were performed in compliance with the requirements of 21 CFR 58 pertaining to an analytical chemistry laboratory supporting a study under Good Laboratory Practices, with the following exceptions;
   1. The protocol for the study is not available.
      A. The test article has not been identified.
      B. The sponsor has not been identified.
      C. The study director has not been identified.
      D. The date that the study was initiated has not been supplied.
      E. The current status of the study is not known.
   2. The completion date of the study is not known.
   3. A retention sample and sample container are not archived at our facility. They will be returned for your archival.
   4. The archival of the raw data will occur during the second quarter of 2011. Raw data will be retained for ten years. If data retention is required for a longer period of time, arrangements for a transfer of records must be made.

Signatures:

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Modified By: daniel.r.longnecker 2010-06-08T20:31:52.147-04:00

Quality Assurance Statement

Please forward this document to the Study Director responsible for the chemical analyses enclosed.

Galbraith Sample Identification Number(s): L-1100 thru L-1103

Protocol/Study Title: unidentified

Regulations Applicable: 21 CFR 58

Galbraith Laboratories, Inc. Project Manager(s): Dan R.Longnecker

Offsite Study Director: unidentified

Sponsor: unidentified

The following are inspection dates and report dates of QA inspections of this study:

PHASES OF STUDY INSPECTED	DATE INSPECTED	REPORTED TO STUDY DIRECTOR	REPORTED TO STUDY DIRECTOR'S MANAGEMENT	REPORTED TO GALBRAITH MANAGEMENT
Raw Data	6/8/10	6/8/10	6/8/10	6/8/10
Report	6/8/10	6/8/10	6/8/10	6/8/10

Inspector _____ Date 6/8/10

Dear Study Director and Management Representative:

Please sign and date below to acknowledge receipt of this Quality Assurance Statement and attached findings/observations. Fax signed and dated acknowledgement to the Quality Assurance Unit at 865-546-7209.

_____ _____
Study Director Date

_____ _____
Management Representative Date

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The following findings proceeded from our inspection of your study under Galbraith sample identification number(s): L-1100 thru L-1103

Finding/Observation	Disposition of Finding
Calculation error on first test for L-1102	Corrected

Inspector

6/8/10
Date

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EXHIBIT M

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of Pure and Applied Chemistry (IUPAC)

Handbook of

Pharmaceutical Salts

Properties, Selection, and Use

P. Heinrich Stahl, Camille G. Wermuth (Eds.)

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Chapter 2

Solubility and Dissolution of Weak Acids, Bases, and Salts

by **Madhu Pudipeddi*, Abu T. M. Serajuddin, David J. W. Grant,** and **P. Heinrich Stahl**

Contents

1. Introduction

Improvement of dissolution rate of weakly acidic or weakly basic drugs that are poorly soluble is one of the primary reasons for preparation of pharmaceutical salt forms [1–7]. Salt forms have also been used to control drug dissolution [8] [9]. This chapter examines the pH-dependencies of the solubility and dissolution rate of pharmaceutical acids, bases, and salts, and the relevance of these dependencies to the selection of the final form.

2. Solubility Behavior of Acids, Bases, and Their Salts

2.1. Measurement of Aqueous Solubility

The solubility of an ionizable compound as a function of pH, known as the pH–solubility profile, can be determined by phase-solubility techniques [10] [11]. Saturated solutions of a free acid, base, or salt are prepared by shaking an excess of the solid with an appropriate volume of deionized water at controlled temperature. Solubilities at various pH values are determined by stepwise titration of these suspensions with a relatively strong acid or base. After each addition, equilibrium is re-established by agitation. The pH of the suspension is measured, and the supernatant solution phase is analyzed for total solute concentration. The process is continued until the entire pH–solubility profile is obtained. The ionic strength of the system is not controlled in this method.

Alternatively, buffer solutions of suitable ionic strength may be used to maintain the desired pH conditions [12 – 14]. However, inadequate buffer capacity and suppression of solubility due to ionic-strength effects have been noticed when buffers are used for pH–solubility studies [12]. Salt formation with buffer species may also occur when buffers are used to control pH. Recently, an automated potentiometric method has been described for determination of pH–solubility profiles [15].

Establishment of equilibrium must be confirmed in all cases by verifying the constancy of solubility values at various times during equilibration. The solid phase in equilibrium with the saturated solution (referred to as 'excess solid') must be verified at selected pH values to identify potential solid-state transformations, such as polymorph/hydrate formation, or conversion of salt to free acid or base, or *vice versa*. In a routine preformulation operation, thermal analysis of the excess solid may provide a simple means to identify solid-state transformations, but powder X-ray diffractometry is usually more reliable. Elemental analysis may also be performed for further identification of the solid phase.

2.2. General Features of pH–Solubility Profiles

Fig. 1 shows a classical pH–solubility profile for a weakly acidic compound, *Flurbiprofen*, with the two key regions marked as *Region I* and *II* [2] [16]. In *Region I* ($pH < 7.3$ in *Fig. 1*), the excess solid phase in equilibrium with the saturated solution is the free acid. In *Region II* ($pH > 7.3$), the excess solid phase is the sodium salt. In *Region I*, the total solubility is described by the following equations:

$$S = [HA] + [A^-] \quad (1)$$

$$S = S_0\left(1 + \frac{K_a}{[H^+]}\right) \tag{2}$$

where, S is the total solubility at any given pH, S_0 is the intrinsic solubility of the free acid, [HA] and [A^-] represent concentrations of the undissociated and dissociated forms, respectively, in solution, and K_a is the acid dissociation constant defined as

$$K_a = \frac{[H^+]\cdot[A^-]}{[HA]} \tag{3}$$

The total solubility in *Region II* is described by:

$$S = \left(1 + \frac{[H^+]}{K_a}\right)\sqrt{K_{sp}} \tag{4}$$

$$K_{sp} = [Na^+]\cdot[A^-] \tag{5}$$

where, K_{sp} is the solubility product of the salt.

For a weakly acidic compound at pH $\ll$ pK_a (*e.g.*, by 2 units), the solubility is practically independent of pH and remains constant at S_0. At pH > pK_a, the solubility increases exponentially with pH (*i.e.*, log S increases linearly with pH). At a certain pH value, the log-linear relationship of solubility with pH abruptly ends, and the solubility plot enters *Region II*. The pH value where

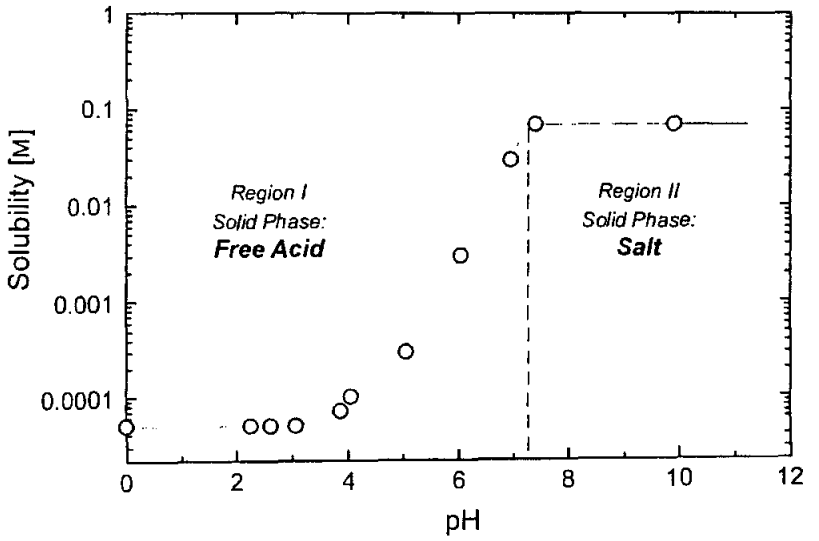


Fig. 1. *pH–Solubility profile of a weakly acidic drug,* Flurbiprofen, *using NaOH* (redrawn from [2])

the two regions intersect is the pH of maximum solubility, referred to as pH_{max}.

Thus, two equations (*Eqns. 2* and *4*) are required to describe the entire pH–solubility profile of a mono-protic acid. The activity coefficients were assumed to equal unity in the above equilibria. Application of activity corrections has been discussed in the literature [17–19]. Ionic equilibria for polyprotic and amphoteric compounds have been described by *Peck* and *Benet* [18].

Fig. 2 shows the more general features of pH–solubility behavior with reference to a weakly basic compound, 4-{4-[(6-chloronaphthalen-2-yl)carbonyl]piperidin-1-yl}-1-(4-fluorophenyl)butan-1-one [19]. The solubility as a function of pH, in the presence or absence of added NaCl, is shown in *Fig. 2*. From left to right, the initial increase in solubility is due to a decrease in the common-ion effect of $[Cl^-]$. The total chloride ion concentration is approximately equal to the concentration of the ionized species plus the hydrogen ion concentration. At higher pH values ($[H^+] \ll [BH^+]$), there is a minimal change in solubility with pH. However, a slight increase in solubility leading up to the maximum is due to an increase in the uncharged species concentration (see [20] for details). As the pH increases, a rapid decrease in solubility beyond the pH_{max} is due to a decrease in the extent of ionization. At higher pH values ($pH \gg pK_a$), the solubility is practically independent of pH and remains constant at the intrinsic solubility of the base. The precise

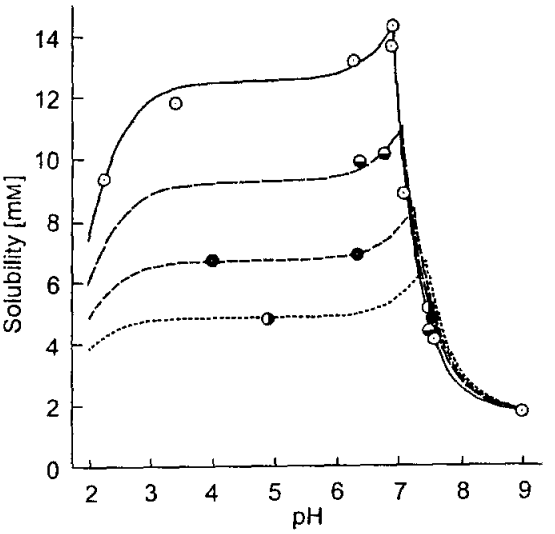


Fig. 2. *pH–Solubility profile of a weakly basic drug, 4-{4-[(6-chloronaphthalen-2-yl)carbonyl]-piperidin-1-yl}-1-(4-fluorophenyl)butan-1-one, with HCl.* Key: (○) no NaCl added; (◒) 0.01M NaCl; (●) 0.025M NaCl; (◑) 0.05M NaCl (redrawn from [19]).

shape of a pH–solubility profile may vary depending on the relative extent of these general features.

The common-ion effect of added NaCl on the solubility of the salt at $pH < pH_{max}$ is significant in *Fig. 2*. In the gastro-intestinal tract, hydrochloride salts are particularly sensitive to the common chloride ion effect. Any minor differences in the solubility at different salt concentrations at $pH > pH_{max}$ in *Fig. 2* are due to the effects of activity coefficients.

2.3. pH_{max}

Analogous to *Eqn. 1*, the total solubility S of a weak base is the sum of the concentrations of the unionized and the protonated fractions of a base:

$$S = [B] + [BH^+] \tag{6}$$

At pH_{max}, in the presence of excess solid, the solution is saturated with the free base ([B] equals its intrinsic solubility S_0) and the salt form, and the following equations are simultaneously valid.

$$S = S_0\left(1 + \frac{[H^+]}{K_a}\right) \tag{7}$$

$$S = \left(1 + \frac{K_a}{[H^+]}\right)\sqrt{K_{sp}} \tag{8}$$

Bogardus et al. [21] reported that the excess solid in equilibrium with the saturated solution of doxycycline at pH_{max} contained both the free base and the hydrochloride salt phases. By setting S equal in *Eqns. 7* and *8*, and solving the resulting quadratic equation, these authors derived an expression for pH_{max} of a base.

$$pH_{max} = pK_a + \log\frac{S_0}{\sqrt{K_{sp}}} \tag{9}$$

The above equation provides a quantitative means to understand the effect of various parameters on pH_{max}. For a weakly basic compound, for example,

a) an increase in the pK_a by one unit (*i.e.*, the strength of the base increases) results in an increase of the pH_{max} by one unit;
b) an order of magnitude increase in the intrinsic solubility of the free base increases the pH_{max} by one unit;
c) an order of magnitude increase in the solubility of the salt ($\sqrt{K_{sp}}$) results in a decrease in the pH_{max} by one unit.

These effects are illustrated in *Fig. 3*.

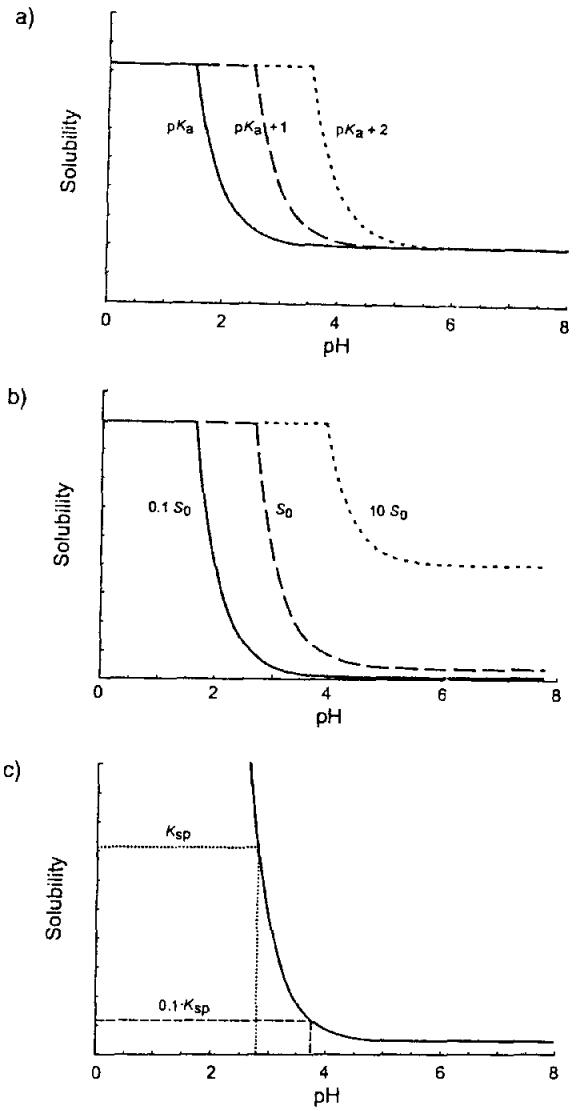
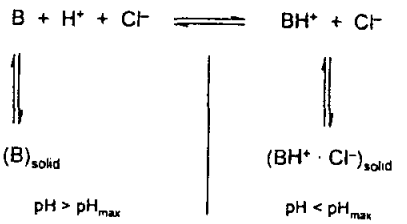


Fig. 3. *Effect of relevant parameters on* pH_{max}: a) *Effect of* pK_a, b) *effect of* S_0, c) *effect of* K_{sp}

As noted by *Bogardus* and *Blackwood* [21], the salt and the free form simultaneously exist in the solid state in equilibrium with the saturated solution as a thermodynamically invariant system at $pH = pH_{max}$ as shown in the *Scheme*.

Scheme



When a small amount of $[H^+]$ is added to the system at pH_{max}, the equilibrium shifts to the right, and conversion of the free base to the salt occurs along the equilibrium path shown. The reverse process occurs when a small amount of alkali is added. Both the pH *and* solubility remain invariant until one of the solid phases is completely converted to the other. The term '*Gibbs*' buffer' has been used to describe this phenomenon [15].

Streng et al. [19] reported a study where the pH–solubility profiles of terfenadine were determined using different acids. The maximum solubility (S_{max}) and pH_{max} depended on the acid used for pH adjustment as shown in *Fig. 4*. The dependence of S_{max} on the counter-ion arises from the differences in the solubility of different salts, *i.e.*, the ion pairs forming a salt. The solubility of a salt is affected by a number of factors including its melting point and counter-ion hydrophobicity [14]. Sometimes, a higher S_{max} with a particular counter-ion may be due to formation of a supersaturated solution as discussed below.

Formation of metastable, supersaturated solutions near pH_{max} has been observed in pH–solubility profiles [22–26]. *Fig. 5* shows a pH–solubility profile where supersaturation near pH_{max} is seen [27]. Metastable 'pseudo-equilibrium' solutions were formed only when the starting material used for the phase solubility study was the free base. Such an inconsistency in the solubility behavior may result from 'kinetic barriers' to phase transformation where nucleation of one phase occurs more readily than that of the other. It should be noted that the 'solubility' values in this region would not have a true thermodynamic meaning. *Fig. 6* shows the peculiar pH–solubility profile of papaverine [25]. Self-association of solute molecules or the metastable nature of the system due to inconsistent nucleation of the free base or the salt may have resulted in such a profile. If supersaturation is suspected in determination of pH–solubility profiles, it may be prudent to 'seed' the system with the expected salt form (or the free acid or base, as appropriate) to facilitate nucleation.

For practical purposes, pH_{max} must be considered as a 'range' of pH over which a change in the equilibrium solid phase occurs. Although the mathe-

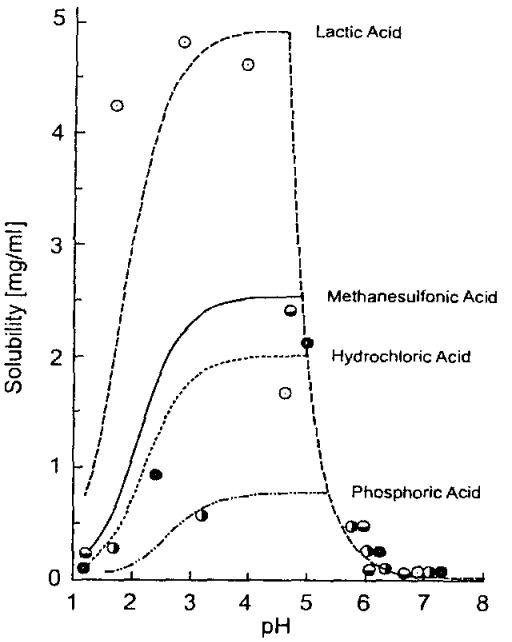


Fig. 4. *Dependence of S_{max} and pH_{max} of terfenadine on salt forming agent* (redrawn from [19])

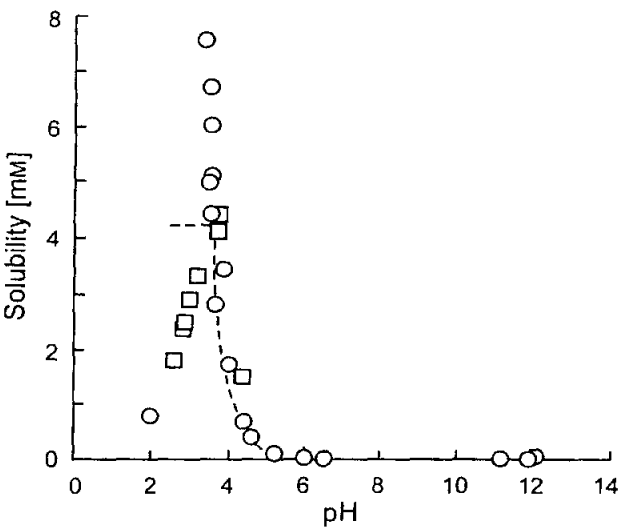


Fig. 5. *pH–Solubility profile of a weakly basic compound, CEL 50, using the free base* (circles) *and phosphate salt* (squares) *as the starting material.* Supersaturation was observed only when the free base was the starting material (redrawn from [27]).

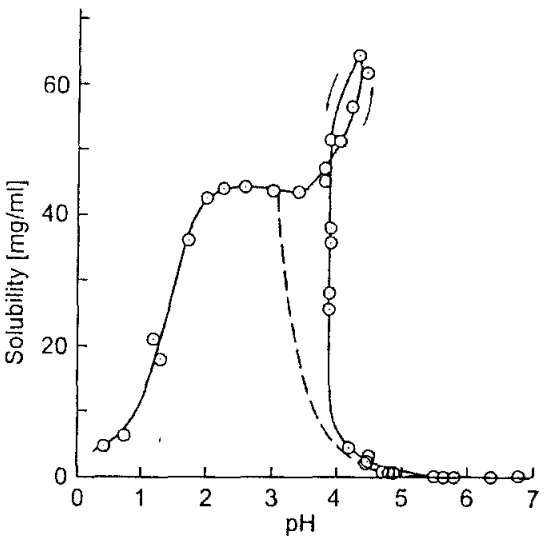


Fig. 6. *pH–Solubility profile of papaverine using HCl.* The broken line shows the theoretical solubility profile (redrawn from [25]).

matical formulation outlined in *Eqn. 9* is theoretically valid, experimental realization of precise pH_{max} may not be possible due to kinetic effects. *Carstensen* [28] discussed the general features of the pH–solubility profile of a divalent acid (H_2A) and the poorly reproducible nature of the pH_{max} region.

2.4. Difficulties in Determination of Salt Solubility

In a typical salt solubility experiment an amount of salt thought to be in excess of its solubility limit is equilibrated in H_2O, and the supernatant is assayed for drug content. The excess solid, however, may not be the salt due to the potential for its dissociation to the free form. In such a case the measured solubility does not represent that of the salt [2] [14]. It is simply the solubility of the free form at that particular pH. The pH of the suspension depends on the extent of conversion of the salt to the free form. The 'apparent solubility', therefore, varies with the ratio of the total amount of solid to that of the solvent used for the experiment until a critical ratio is reached. To ensure reliable measurement of salt solubility the solid phase must contain excess salt after equilibration. After isolation and air-drying of the excess solid phase, thermal analysis provides a preliminary indication of phase transfor-

mation. Further confirmation of the nature of the excess solid phase may be obtained by powder X-ray diffractometry or elemental analysis. If the excess solid phase is entirely in the free form, the experiment must be repeated with a higher solid to solvent (H_2O) ratio until the excess solid contains the salt. In some cases, complete conversion of the salt to the free form may occur at all solid-to-solution ratios, often leading to formation of a 'gel'.

As a result of the above phenomenon, in some cases, an attempt to make a solution of a 1:1 salt at a concentration well below the solubility of the salt (as estimated from the K_{sp} value) will be unsuccessful due to precipitation of the free unionized form. To further develop this point *Anderson* and *Flora* [2] defined the term 'stoichiometric solubility'. The stoichiometric solubility of a 1:1 salt is the concentration of salt which can be dissolved in pure H_2O with no formation of a precipitate of the unionized free form. The authors pointed out that the difficulties of low 'apparent solubility' of a salt due to free form precipitation may be encountered in the preclinical stages of development when concentrated solutions in H_2O are required for toxicology testing. However, appropriate pH adjustment should overcome this problem.

3. Dissolution Behavior of Acids, Bases, and Their Salts

The term *dissolution* refers to the overall process by which a solid compound dissolves in a liquid medium, while *dissolution rate* is the kinetic descriptor giving the rate at which the dissolution takes place. The concept of solubility, on the other hand, implies that the process of dissolution has reached equilibrium, and the solution is saturated. Dissolution rate of solids is of paramount importance in the development of pharmaceutical products and quality control. Salt formation is one of the most commonly employed techniques to improve dissolution of weakly acidic or basic drugs.

A number of theories of the dissolution of solids have been proposed [17] [29] [30]. However, the simple diffusion model may be adequate to describe the dissolution behavior of most pharmaceutical solids in aqueous and non-aqueous media. The diffusion layer model (*Fig. 7*) assumes that a thin film of saturated solution of concentration c_s exists at the interface of the dissolving solid and the dissolution medium. The dissolution rate is controlled by the diffusion rate of solute molecules from this thin saturated film into the bulk solution. The concentration of the solute in the bulk is denoted by c_b. As the distance x increases from $x=0$ (at the surface of the solid) to $x=h$ (at the beginning of the bulk solution), the concentration decreases from $c=c_s$ to $c=c_b$. Beyond $x=h$, the bulk concentration remains uniform at c_b. In the simplest form of the theory, following *Fick*'s First Law, the rate of dissolu-

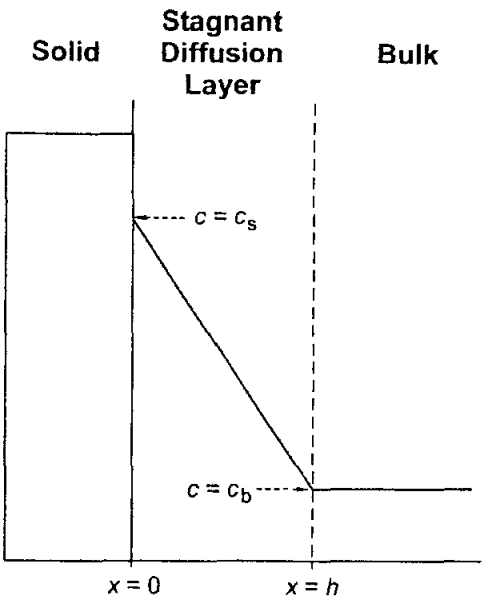


Fig. 7. Diffusion layer model of drug dissolution

tion is given by:

$$J = \frac{dm}{A \cdot dt} = \frac{D}{h}(c_s - c_b) \tag{10}$$

where, J is flux which is defined as the amount m of material dissolved, and, therefore, transported across the diffusion layer, in unit time per unit surface area A of the dissolving solid. D is the diffusion coefficient (diffusivity) of the solute. *Eqn. 10* is the *Nernst-Brunner* diffusion layer form of the *Noyes-Whitney* equation [31]. Under 'sink' conditions ($c_b < 10\%$ of c_s) the equation is reduced to:

$$J \approx \frac{D}{h} c_s \tag{11}$$

3.1. Measurement of Dissolution Rate

The rate of dissolution of pharmaceutical solids is often determined by one of two methods: *i*) powder dissolution or *ii*) disc dissolution. The experimental details of both methods have been well-described [32]. Because of the complexities of determination of powder surface area, the disc dissolution

method, which provides the intrinsic dissolution rate (IDR), is more commonly used. Briefly, discs are prepared by transferring *ca.* 100–500 mg of powder into a die-hole and compressing at a pressure of 14–35 MPa (2000–5000 psi). It is recommended that powder X-ray diffractometry or spectroscopic and/or thermal analysis of the compressed solid be performed to detect any physical transformations that may have occurred during compression [33][34]. The die-and-disc assembly is mounted on the rotatable shaft of the dissolution apparatus. The IDR die is often described as the '*Woods* die' [35]. Modifications of the method have been reported in [36]. A rotation speed of 50–200 rpm (8.3–33.3 Hz) is usually employed. Laminar flow conditions must be maintained in dissolution studies [17][37]. The hydrodynamics of the rotating disc method have been thoroughly discussed [17][38–40]. A detailed account of the theoretical and practical aspects of drug dissolution and its relevance to bioavailability and bioequivalence issues has been presented by *Abdou* [41].

3.2. Dissolution into Reactive Media

The *Noyes-Whitney* equation (*Nernst-Brunner* form, *Eqn. 10*) has been very successful in quantifying the dissolution of solids into nonreactive media where the diffusion coefficient does not change significantly, and the drug does not undergo any chemical changes such as ionization, complexation, and degradation. Dissolution of acids, bases, or salts into buffered or unbuffered solutions, on the other hand, is reactive if proton exchange reactions occur in solutions. For dissolution of weak acids, bases, and their salts, the pH of the diffusion layer is especially important. A number of models have been proposed to evaluate self-buffering capacity of acids, bases, and salts in controlling the diffusion layer pH (micro-environmental pH). The theoretical basis of the majority of these models has been discussed by *Grant* and *Higuchi* [17].

A common assumption of most dissolution models is that the diffusion layer theory is still applicable to reactive systems, and all acid-base proton exchange reactions occur instantaneously. Ionic equilibria are set up, and the second order differential equations for *Fick*'s Second Law of diffusion are solved with defined boundary conditions. Equations are derived for diffusion layer pH and mass transfer rates in terms of independently measurable parameters such as diffusion coefficients, pK_a values, and intrinsic solubility of the dissolving species. The complexity and accuracy of the models depend on the number of equilibria considered. The salient features of some of these models along with their applications are presented below. In the following discussion, the mathematical equations, which are often unwieldy, are presented to an extent only necessary to develop the practical implications of

theoretical concepts. Reference is made to the appropriate sources from which the exact equations can be obtained. However, if ionization is not instantaneous, as for carbon acids, such as phenylbutazone or 7-acetyltheophylline, rate equations for the relevant reaction enter into the differential equations represented by *Fick*'s Second Law of diffusion. These complexities are not considered here; they have been reviewed in [17].

Higuchi et al. [42] reported the simultaneous chemical reaction and diffusion (SCRD) model for dissolution of benzoic acid into a basic solution. The authors provided predictive equations for the intrinsic dissolution rate or flux, J, of the acid in terms of fundamental parameters such as diffusion coefficients of reactants and products, the intrinsic solubility of the acid, the strength of the base, and the apparent ionization constants. *Higuchi et al.* [43] also reported an equation for the initial dissolution rate of sodium salt of a weak acid dissolving in acidic medium.

By considering additional equilibria, *Mooney et al.* [44] extended the SCRD model to dissolution kinetics of carboxylic acids into unbuffered media whose pH was controlled by a pH-stat. The authors presented an expression for the concentration of hydrogen ion at the surface of solid ($x=0$):

$$[H^+]_0 = \frac{(D_H[H^+]_h - D_{OH}[OH^-]_h) + \sqrt{(D_H[H^+]_h - D_{OH}[OH^-]_h)^2 + 4D_H K_w (D_{OH} + D_A K_1 [\mathbf{HA}]_0)}}{2D_H} \quad (12)$$

where, the subscripts h and 0 indicate the bulk and surface concentrations, respectively. D denotes the diffusion coefficient of the species indicated, the experimental determination of which was detailed by the authors. K_1 is the dissociation constant of the test acid, and $[HA]_0$ is its intrinsic solubility. It can be seen that, as K_1 and $[HA]_0$ (bold faced) increase, the value of $[H^+]_0$ increases, meaning that the micro-environmental pH becomes more acidic. In other words, the self-buffering capacity of the dissolving acid increases with its acid strength, K_1, and intrinsic solubility, but decreases with increasing concentration of OH^- ion in the bulk solution, $[OH^-]$. *Fig. 8* shows the agreement between the theoretically predicted and the experimentally determined micro-environmental pH (pH_0) values for benzoic acid dissolving into unbuffered medium. At very low bulk pH (pH 2), the self-buffering capacity of benzoic acid is overcome by the bulk pH, so that the micro-environmental pH is almost equal to that of the bulk pH. In the pH range of 4–10, the self-buffering action of benzoic acid was most significant, and the pH of the diffusion layer remained fairly constant. At bulk pH greater than 10, the diffusion layer pH showed a gradual increase.

Mooney et al. [44] extended the SCRD model and developed equations to predict the initial dissolution rates of benzoic acid, 2-naphthoic acid, and

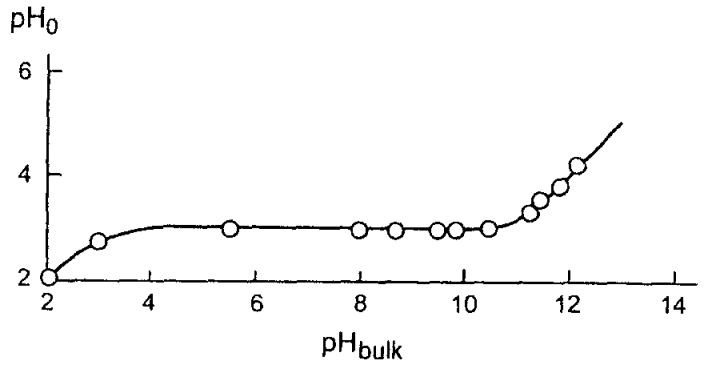


Fig. 8. *Experimental and theoretical pH_0 values of benzoic acid for various bulk pH values.* The data points are pH values of unbuffered aqueous media in the presence of excess benzoic acid; the solid line represents the predicted values (redrawn from [24]).

indomethacin dissolving into unbuffered media whose pH was controlled by a pH-stat. The relative dissolution ratio, R, was defined as the ratio of the dissolution rate (J) at any pH to the rate (J_0) at pH 2.0, where ionization was suppressed and the free acid species was the only diffusant.

$$R = \frac{J}{J_0} = \left[\frac{D_{HA}[HA]_0 - D_H([H^+]_0 - [H^+]_h) + D_{OH}([OH^-]_h - [OH^-]_0)}{D_{HA}[HA]_0} \right] \quad (13)$$

Fig. 9 shows good agreement between the experimental flux and that predicted by the extended SCRD model. The flat regions in the plots are the result of self-buffering capacity of the acids. Benzoic acid, with highest acid dissociation constant and intrinsic solubility, shows largest buffering-capacity. These models were later extended to dissolution of carboxylic acids into buffered systems [45] [46]. In a buffered medium, the buffer capacity of the medium influences the pH of the micro-environment of the solid. The higher the buffer strength the closer will be the surface pH to the bulk pH. The authors, therefore, recommended that the exact buffer conditions be specified when reporting dissolution rates.

Al-Janabi [47] reported on the prediction of intrinsic dissolution rates of ephedrine and diproteverine hydrochloride. *French* and *Mauger* [48] developed equations for the diffusion layer pH and intrinsic dissolution rates of mesalamine in a triprotic buffer systems. The authors discussed the relevance of pH–dissolution profiles to development of a controlled release formulation. The concept of micro-environmental pH has been utilized in a number of additional reports to understand dissolution of pharmaceutical salts and their behavior in formulations [9] [26] [49–51].

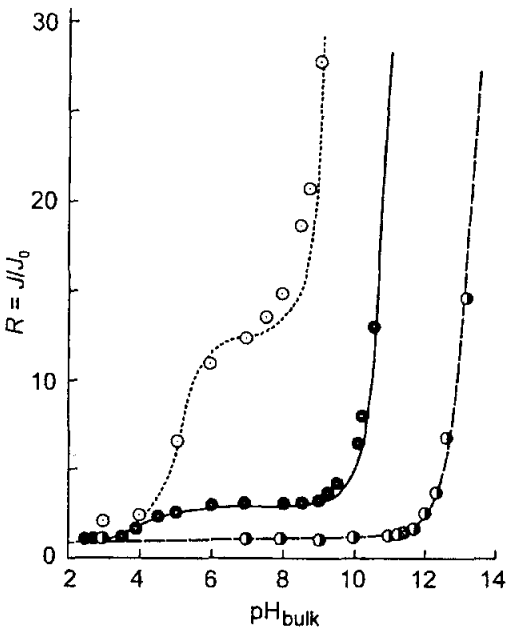


Fig. 9. *Relative dissolution rate*, R, vs. pH_{bulk} *for several carboxylic acids at 25 °C*, $\mu = 0.5$M (KCl) *using a pH-stat to maintain constant* pH_{bulk}. The solid lines are those predicted by the equation and the data points are those experimentally determined; J_0 refers to the dissolution rate at pH 2.00. $\mu = 0.5$. (⊙): indomethacin; (●): 2-naphthoic acid, and (◑): benzoic acid (redrawn from [44]).

The SCRD model and the *Nernst-Brunner* total solubility model assume that mass transfer occurs primarily by diffusion. Mass transfer due to liquid movement, or convection, is generally ignored. Models with increased complexity that include both convective and diffusive transport have been reported [52] [53].

Bogardus and *Blackwood* [21] [54] investigated the dissolution of doxycycline free base and its hydrochloride salt. At pH 4 (0.1M acetate buffer) and 7 (0.1M phosphate buffer), the dissolution rate of the hydrochloride salt was significantly higher than that of the free base. The dissolution rate of the hydrochloride salt was, however, significantly lower than the free base in 0.1M HCl medium. This reduced dissolution rate was attributed to the common-ion effect in chloride-containing media. In the micro-environment of the diffusion layer, the free base did not readily convert to the HCl salt during the experimental time scale and was not sensitive to the common-ion effect of chloride.

Higuchi et al. [55] reported experimental dissolution profiles of 1,1-hexamethylene-*p*-tolylsulfonylsemicarbazide and its sodium salt, and those pre-

dicted by SCRD model. When the bulk buffer concentration was high and the pH was low, the dissolution from the salt was essentially equal to that of the free acid due to formation of a coat of the free acid (acid-coat) on the surface of the dissolving solid. At intermediate pH conditions a 'stepwise' dissolution occurred due to the formation of an initial acid-coat followed by its dissolution, and then the dissolution of the sodium salt before a second acid-coat was formed. At higher pH conditions, no conversion to the free acid was observed and the dissolution of the salt was faster. However, such stepwise profiles are uncommon.

Serajuddin and *Jarowski* [24] reported the effect of diffusion layer pH on the dissolution rate of theophylline, salicylic acid, and benzoic acid. The dissolution rate of sodium salicylate was significantly higher than that of salicylic acid at all pHs investigated (pH 1.1, 2.1, and 7). The authors measured the pH of the diffusion layer and showed that wide differences existed between the bulk pH and the diffusion layer pH. The dissolution data conformed well to the *Nernst-Brunner* form of the *Noyes-Whitney* equation (*Eqns. 10* and *11*), when saturation solubility in the diffusion layer was used (for c_s in *Eqn. 11*) rather than the solubility at the pH of the bulk solution. This result follows from the fact that the SCRD model and the total solubility model would yield comparable results, when the diffusion coefficients of all dissolving or reacting species are set equal [43]. In most cases, the diffusion coefficients of the free form and its salt do not differ widely [42].

Serajuddin and *Jarowski* [24] pointed out that the above principles of diffusion layer effects may be used for estimation of the dissolution rate of a drug or its salt under various pH conditions. The flux, J_1, in a particular bulk medium under sink conditions is given by:

$$J_1 = \frac{D \cdot c_{s,0-1}}{h} \tag{14}$$

where, D and h have the conventional meaning, and $c_{s,0-1}$ is the saturation solubility at the diffusion layer pH for the chosen dissolution medium (*Medium 1*). The value of $c_{s,0-1}$ is approximated from the pH–solubility profile with a knowledge of the diffusion layer pH. To measure the diffusion layer pH, a small volume of the dissolution medium is added to a large excess of the solid, mixed on a vortex, and the pH is measured by a pH electrode. Alternatively, a micro-electrode may be inserted directly into the dissolution medium [36]. Assuming that D and h are unchanged under identical hydrodynamic conditions, the flux, J_2, in a second medium (*Medium 2*) is:

$$J_2 = \frac{D \cdot c_{s,0-2}}{h} \tag{15}$$

$$\frac{J_1}{J_2} = \frac{c_{s,0-1}}{c_{s,0-2}} \tag{16}$$

Thus, with a knowledge of J_1, $c_{s,0-1}$ and $c_{s,0-2}$, the value of J_2 may be estimated. Although deviations from these predictions may be observed in practice, this method may be of value when only limited amounts of drug substance are available.

4. Relevance of pH Relations of Solubility and Dissolution to Salt Selection and Formulation

4.1. Solution Formulations

For formulation of a stable solution dosage form (oral or parenteral), the equilibrium solubility is more important rather than the rate of dissolution. '*In-situ*' salt formation with the free form and an appropriate counter-ion to adjust pH may provide the same advantage as using a salt. A preliminary investigation of pH–solubility profile with different counter-ions provides an indication of the counter-ion best suited to maximize solubility (or optimize pH) as illustrated in *Fig. 4*. Further utilization of this principle was reported by *Marra-Feil* and *Anderson* [56]. The authors demonstrated that multiple counter-ions, added in predetermined amounts so as not to exceed the solubility product (K_{sp}) of any salt, provided significantly higher solubility than any single counter-ion. The relevance of pH_{max} to solution formulations with acceptable pH for intravenous administration has been reported [20]. If pH adjustment alone does not provide sufficient solubility enhancement, combination approaches such as addition of a co-solvent along with pH adjustment may be used [20] [57]. The equilibrium solubility of a salt and its free form are expected to be the same under identical conditions of pH, counter-ion, and ionic strength. However, salt forms may still be considered for liquid formulations for processing convenience such as rapid dissolution. Additional factors that may influence the choice of the final form include crystallinity and solid state stability and will be discussed in detail in *Chapt. 6*.

4.2. Solid Formulations

The equilibrium solubility and the rate of dissolution are equally important for salt forms intended for oral dosage forms. While a detailed account of salt selection strategies is outlined in *Chapt. 6*, a brief discussion of pH–solubility principles related to salt selection will be presented here.

Biopharmaceutical evaluation in the early development stage can identify if drug absorption is dissolution-rate-limited. As a first step in evaluating salt formation to improve dissolution rate, the pH–solubility behavior is in-

vestigated using pure, crystalline, well-characterized acid, base, or a salt form. Generally speaking, pH–solubility profiles generated using a free form or its salt are comparable, if not identical, when the same acid or base is used for pH adjustment in both cases. Examples of this observation and deviations thereof have been discussed by *Ledwidge* and *Corrigan* [27].

In determining aqueous solubility of salts, the possible effect of solid to solvent ratio on solubility must be kept in mind. It is beneficial to understand the factors that effect aqueous solubility of salt forms, although attempts to predict salt solubility have not been fully successful except for establishing general trends [14] [58].

Comparison of the aqueous solubility of various salts with the free form alone may not provide an indication of which form would result in optimal dissolution behavior. The dissolution of a salt can be equal, higher, or lower than that of the free form depending on the micro-environmental pH at the surface of the dissolving solid. Attempts have been made by several workers to use intrinsic dissolution rates at multiple pH conditions to evaluate relative performance of salts and free forms. If buffer solutions are used, the buffer strength and composition must be chosen with due consideration to physiological conditions because the diffusion layer pH depends on the strength of the buffer among other factors [44]. It is advisable to include simulated gastric and intestinal fluids described in the U.S.P. or in [59] as dissolution test media.

Although intrinsic dissolution rate studies are frequently used as one of the criteria for salt selection, it has been reported that disc dissolution rates may tend to exaggerate the real differences in the dissolution rates that would be obtained from capsule formulations [9]. Powder dissolution may be valuable but lack of reliable particle size or surface area in the early development phase may limit extensive powder dissolution studies.

The relative superiority of a single salt or the free form may not always be obvious from the results of intrinsic dissolution rates, because of the differences in the self-buffering capacity of the salt and the free form. This is illustrated in *Fig. 10* where the relative performance of the free form or the salt depended on the pH. In such cases, a judicious choice of the final form is required based on a balance of physiological (*e.g.*, pH of the gastro-intestinal tract) and physico-chemical (*e.g.*, pK_a, pH_{max}) factors. *In-vivo* bioavailability studies in animals using the free form and carefully characterized salt forms can guide the final form selection [60]. The complex nature of physiological variables may not always allow prediction of the relative *in-vivo* performance of a salt form and its free form [61] [62]. However, a careful physico-chemical characterization of salt forms prior to *in-vivo* testing provides a rational basis for selection and minimizes the number of *in-vivo* trials.

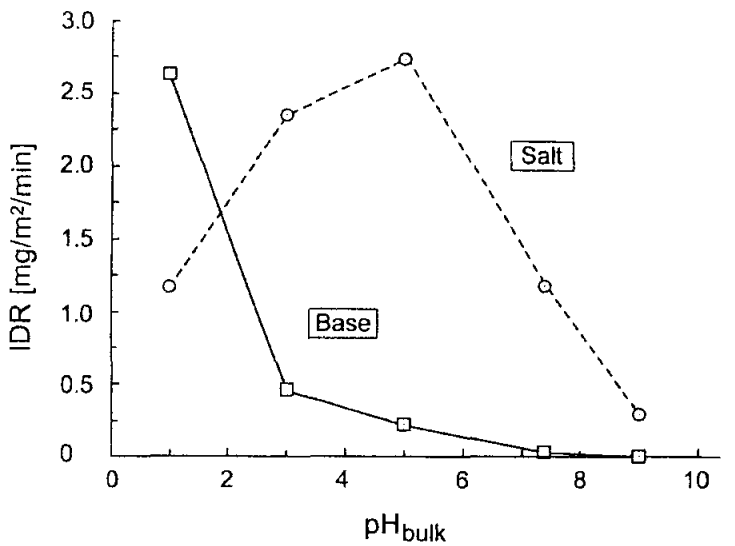


Fig. 10. *Intrinsic dissolution rate of bupivacaine and its hydrochloride salt as a function pH of the dissolution medium.* The faster dissolution of the salt at pH values greater than 1 is due to greater self-buffering capacity (redrawn from [26])

In addition to general solubility characteristics, a pH–solubility profile (*e.g.*, pH_{max}) also provides insight into potential conversion of a weak salt to the free form upon formulation or storage. For example, when the microenvironmental pH of a salt of a weakly acidic drug is less than the pH_{max}, conversion of the salt to the free acid may occur upon storage or formulation [63]. The influence of pH_{max} on salt stability is further discussed in *Chapt. 6*.

5. Non-Ideal Solubility Behavior

Deviations have been observed from *Henderson-Hasselbalch* equation due to self-association of solute molecules. Self-association of solute molecules can effect the apparent pK_a and solubility. *Bogardus* and *Blackwood* reported on the non-ideal solubility behavior of doxycycline in aqueous solutions [21]. *Surakitbanharn et al.* described the solution equilibria of a self-associating drug, dexverapamil [64]. *Ledwidge* and *Corrigan* [27] reported an interesting observation of non-ideality in the pH–solubility profile of diclofenac. The solid form in equilibrium with diclofenac *N*-(2-hydroxyethyl)-pyrrolidine (HEP) aqueous solution ($pH > pH_{max}$) was diclofenac HEP dihy-

drate in agreement with theory. Lowering the suspension pH with HCl caused conversion of the HEP salt to the free acid below the pH_{max} (*ca.* 8.9) and a reduction in solubility. Adjusting the pH of the suspension with HEP, however, resulted in a sharp deviation of solubility from the theoretical pH–solubility profile. Analysis of the solid phase at pH 8.05 (less than the pH_{max}, 8.9) showed conversion of the solid phase to a monohydrate form of crystalline $(diclofenac)_2 \cdot HEP$ (2:1 salt). Under normal conditions, the expected solid phase would be the free acid. While a complete discussion of non-idealities in pH–solubility behavior is beyond the scope of this chapter, the above examples are provided to illustrate the complexities one may encounter with pharmaceutical acids, bases, and their salts.

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EXHIBIT N

MERCK & CO., INC.

Rahway New Jersey USA

European Patent Department

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Hertfordshire
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jocelyn_man@merck.com

19 February 2007

Our ref: 21409Y

European Patent Office,
Erhardstrasse 27,
D-80298 Munich 2,
GERMANY

Dear Sirs,

European Patent Application No. 04755691.5 - 2117
in the name of Merck & Co., Inc.

I am writing in response to the Communication pursuant to Article 96(2) EPC dated 28 November 2006, the term for response having been extended by the communication dated 28 December 2006.

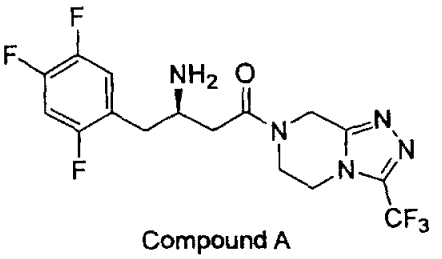
Amendment: Article 123(2) EPC
Please find enclosed a new set of Claims 1 to 22 to replace the claims currently on file. For the convenience of the Examiner, a manuscript-amended set of claims is also enclosed.

Specifically, Claims 8, 11, 14-17, 24 and 29 have been deleted. The remaining claims have been renumbered accordingly. Also, references to the "monohydrate" in former Claims 5-7, 9, 10, 12 and 13 (new Claims 5 to 11 respectively) have been amended to refer to the "salt". Furthermore, former Claim 18 and dependent Claims 19 to 23 (new Claims 12 to 17 respectively) have been reformatted to refer to "a drug substance" that comprises the crystalline monohydrate of Claim 4. Finally, former Claim 25 (new Claim 18) has been amended to delete the term "prophylactically".

As requested by the Examiner, documents D2 and D3 have been identified and briefly discussed in the background of the description. Also, the description has been amended to conform with the new set of claims enclosed herewith. Manuscript-amended pages 1, 5, 6 and 9 are enclosed herewith.

It is submitted that the amendments made herein are fully in accordance with the requirements of Article 123(2) EPC.

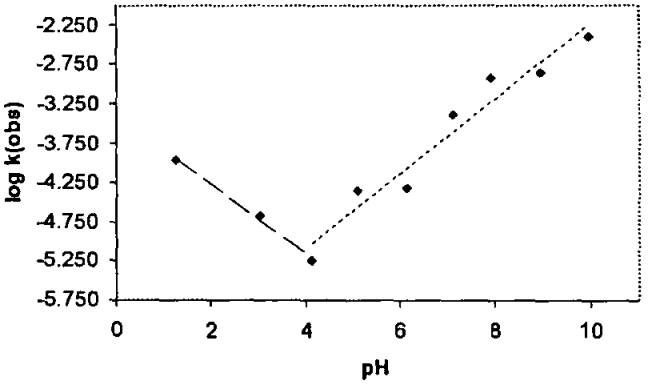
Inventive step: Article 56 EPC
Document D1 (WO 03/004498) teaches the hydrochloride salt of Compound A, whereas the instant application discloses the dihydrogenphosphate salt of Compound A:



Compound A

The dihydrogenphosphate salt of Compound A has remarkable advantages over the hydrochloride salt with respect to chemical stability. The pH-rate profile for the degradation of Compound A was determined in solution at 40 °C and indicated maximum stability at around pH 4 as shown in the figure below. Degradation is acid-catalysed at pH lower than 4 (primarily hydrolysis observed as shown in Scheme 1) and both, thermal and base-catalysed degradation at pH above 4 (hydrolysis and deamination observed as shown in Scheme 1).

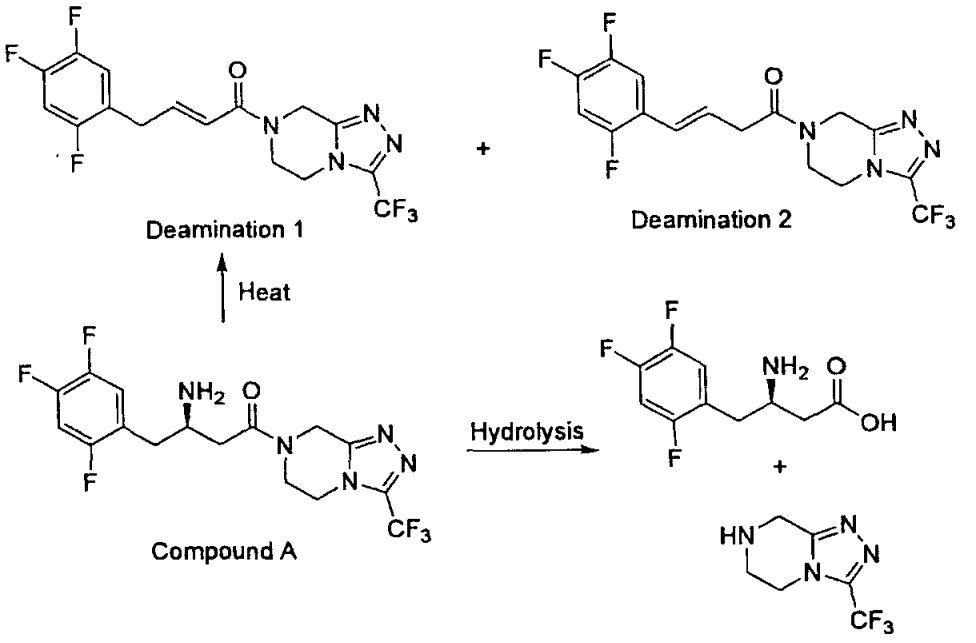
Rate of Degradation of Compound A versus pH at 40°C



The native pH of the dihydrogenphosphate salt in aqueous solution is approximately 4.5. However, the native pH of the hydrochloride salt is approximately 3.2. As seen from the rate of degradation vs. pH figure above, the dihydrogenphosphate salt of the instant invention possesses superior properties over the hydrochloride salt with respect to chemical stability.

Main Degradation Pathways for Compound A

Scheme 1



Compound A is a stable and highly soluble crystalline solid. The crystalline free base and various salts of Compound A were evaluated as possible candidates for clinical development. The formulation design effectively sought to maintain the amino group in the protonated state to minimise the reactivity of the molecule. The dihydrogenphosphate salt was selected over the other salts based on a combination of factors, particularly in view of the observation above that it was the most stable in aqueous solution. The native pH of the dihydrogenphosphate salt in aqueous solution is approximately 4.5, which is close to the pH of maximum stability as shown in the figure above. The pKa of Compound A was determined to be 7.7. Both the anhydrous dihydrogenphosphate and monohydrate dihydrogenphosphate salt forms were evaluated during development. Selection of the final dihydrogenphosphate salt form considered both physicochemical properties and performance in the formulation. The thermodynamically and chemically more stable monohydrate form of the dihydrogenphosphate salt also provided superior performance over the anhydrous dihydrogenphosphate in the direct compression tablet formulation.

The Applicant therefore submits the claimed subject-matter is inventive and fully meets the requirements of Article 56 EPC.

It is believed that the application is now in order for allowance and the Applicant looks forward to receipt of the Rule 51(4) EPC communication in due course. However, should the Examiner have any further objections, a further written communication is respectfully requested. In the unlikely event the Examiner is minded to reject the present application, merely as a precaution, Oral Proceedings under Article 116 EPC are hereby requested.

Kindly acknowledge receipt of this letter and its enclosures by stamping and returning the enclosed Form 1037.

Yours faithfully,

Jocelyn Man
Authorised Representative

EXHIBIT O

BM161

DECLARATION

I, Robert M. Wenslow, Jr., Ph.D., hereby swear:

1. I am currently an Associate Scientific Director in the Center for Materials Science and Engineering of the Merck Manufacturing Division of Merck & Co., Inc.. I have been in this position since 2005. I received a Ph. D. degree in Analytical Chemistry from the Pennsylvania State University in 1997. I am an author or co-author on over 20 publications, patents, and presentations in the field of chemistry.

2. I am a co-inventor on European patent 1654263. I understand that this patent has been opposed by Teva Pharmaceutical Industries Ltd, and I already provided a written declaration (dated January 23rd, 2009) for use in defending the patent against the opposition. I understand that this new declaration will also be filed as evidence for use in the defense.

3. Various experiments concerning different chemical and physical forms of sitagliptin [(2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine] have been performed either by me or under my direction. This declaration gives details of such experiments.

4. Sitagliptin is a base and can form salts with various organic and inorganic acids. Clinical development of sitagliptin has focused on the dihydrogenphosphate salt (referred to hereafter simply as the "phosphate" salt). This salt has been shown to have various advantages when compared to other salts (*e.g.* the hydrochloride salt). These advantages are seen with both amorphous and crystalline forms of the phosphate salt but are more apparent in the crystalline form. The phosphate salt's advantages in the crystalline form are seen both with anhydrous and monohydrate salts but are more apparent in the crystalline monohydrate form.

5. The anhydrous crystalline phosphate salts are chemically and physically stable. Details of these anhydrous salts can be seen, for example, in WO2005/020920 (another patent application for which I am a co-inventor). For instance, we have studied the solid-state stability of the anhydrous phosphate salt. The results in Annexe A show that this salt is stable for at least 36 months at 25°C.

6. The crystalline monohydrate phosphate salt is also chemically and physically stable. For instance, we have compared the solid-state stability of the crystalline monohydrate phosphate salt to the amorphous hydrochloride (HCl) salt. The results in Annexe B show that the crystalline phosphate monohydrate salt is more suitable for pharmaceutical development into a stable drug product for therapeutic use. Its greater chemical stability reduces development costs by allowing for less stringent storage conditions for bulk material. Furthermore, it makes sitagliptin more amenable to formulation of a robust, chemically stable drug product and provides increased flexibility in formulation development as it does not require stabilization of the active ingredient from degradation.

7. An amorphous non-crystalline form of the phosphate salt has also been tested. Details of the amorphous phosphate salt can be seen, for example, in WO2006/033848 (another patent application for which I am a co-inventor). We have found that the amorphous phosphate salt is thermodynamically less stable than the crystalline monohydrate salt

8. In addition to studying hydrated phosphate salts we have also tested crystalline forms with other solvents *e.g.* ethanol and isopropyl alcohol. Such solvates are disclosed in WO2005/020920. In practice these solvates have been very difficult to isolate and maintain. For instance, they readily lose solvent at ambient conditions and convert to an undefined mixture of anhydrous crystal forms.

9. We have also tested other salts of sitagliptin. These results were publicly presented at the "Polymorphism & Crystallization Forum 2003" in Philadelphia, PA, in November 2003 (Annexe C). As shown in the presentation, the phosphate was superior in several respects. Other salts, such as the tartarate (called "Salt A" in Annexe C), were very poor in terms of both chemical stability and their physical suitability for pharmaceutical use.

10. I also reiterate the points from my January declaration. In brief, the amorphous hydrochloride salt of sitagliptin was tested but rejected for pharmaceutical development due to *inter alia* its hygroscopic and morphological properties. In contrast, the crystalline phosphate salt phases of sitagliptin are non-hygroscopic and are also highly and stably crystalline. Among the crystalline forms of the phosphate salt the monohydrate form is thermodynamically superior to the anhydrous form.

11. In addition, we have also tested the free base form of sitagliptin. At room temperature the water solubility of the free base form is about 10 times lower than for the phosphate salt (~7mg/ml, compared to >70mg/ml). Bulk crystalline free base degrades by thermal de-amination after 2 weeks at 80°C (accelerated degradation studies) and hydrolysis of the amide bond is observed in solution at all pHs even under relatively mild conditions (25°C). Solutions of the free base also undergo de-amination at elevated temperature. Thus the free base form was not suitable for pharmaceutical development.

12. Finally, I have been given a copy of a document which is labelled "BM 2" from the Opposition. It shows a graph of the predicted degradation rates of sitagliptin salts at different pH values. Data for two different salts are shown as dotted or dashed lines. I understand that the opponent has used this graph to argue that the degradation rate of the hydrochloride salt at pH 3.2 is the same as the degradation rate of the phosphate salt at pH 4.5. The opponent's view is wrong. The vertical axis in this graph is logarithmic and so a small difference on this scale can mean a large difference in absolute stability. The opponent argues that the graph shows no difference in stability but, in fact, the phosphate salt is about 3-fold more stable. Even so, for solid pharmaceutical formulation it is more important to consider a salt's stability in the solid phase, rather than in solution, and by this measure the phosphate salt is much more stable than the hydrochloride salt.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

SIGNED: _____

Robert M. Wenslow, Jr.

DATE: 21 Dec 2009

ANNEXE A: Solid-state stability of the anhydrous crystalline phosphate salt.

Sample Preparation

The anhydrous crystalline phosphate salt of sitagliptin was prepared as described in Example 1 of WO2005/020920.

Stability

To measure stability of the anhydrous phosphate salt, several glass vials each containing about 5 mg of solid were placed in a stability chamber at either (i) 25°C and 60% relative humidity or (ii) 40°C and 75% relative humidity.

Samples were removed from the chamber at various time points and were stored at -20°C until analyzed by HPLC for degradation. For this chromatographic analysis the samples were dissolved in 1:1 acetonitrile-water and HPLC conditions were as follows.

Column	Eclipse Plus C18 (50 x 4.6 mm) 1.8 µm
Mobile Phase	pH = 2.0 (A) = 0.1 % H_3PO_4/H_2O (B) = acetonitrile
Linear gradient	10-90% B
Flow rate	1.5 mL/min
Gradient Time	15 min (+ 2 min re-equilibration)
Temperature	40°C
Injection volume	10 µL
Sample concentration	~0.5 mg/mL solution
Wavelength	250 nm

Stability data for the anhydrous phosphate salt was as follows:

Storage time	**HPLC assay (%)***	**Degradate area (%)***
25°C and 60% RH		
Time 0	99.63	0.32
3 months	99.68	0.26
6 months	99.67	0.25
9 months	99.67	0.30
12 months	99.72	0.26
18 months	99.68	0.29
24 months	99.70	0.25
36 months	99.63	0.27
40°C and 75% RH		
Time 0	99.63	0.32
1 month	99.70	0.26
2 months	99.69	0.26
3 months	99.68	0.26
6 months	99.67	0.26

* error is ±0.05%

Thus the anhydrous crystalline salt is stable for at least 6 months at 40°C and 75% relative humidity, and for at least 3 years at 25°C and 60% relative humidity.

ANNEXE B: A comparison of the solid-state stability of the crystalline monohydrate phosphate salt and the amorphous hydrochloride salt of sitagliptin.

Sample Preparation

The amorphous HCl salt of sitagliptin was prepared by precipitation. Four lots of the crystalline phosphate monohydrate were prepared by a seeded crystallization in IPA/water as described in the patent.

Stability

To measure stability of the amorphous HCl salt, several glass vials each containing about 5 mg of solid were placed in a stability chamber at 40°C and 75% relative humidity. These are standard industry conditions for accelerated small-scale stability testing of drug substances.

Crystalline phosphate monohydrate stability data were obtained from samples (four lots) packaged in a plastic bag and placed in a fiber drum which was placed in the stability chamber under the same conditions. This was done in accordance with International Conference on Harmonisation (ICH) guidelines for large-scale stability testing. These stability data were used in the WMA.

Samples were removed from the chamber after 6 months and were stored at -20°C until analyzed by HPLC for degradation. For this chromatographic analysis the samples were dissolved in 1:1 acetonitrile-water and HPLC conditions were as described in Annexe A.

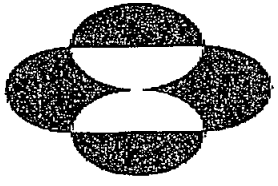
Stability data for the HCl and phosphate salts were as follows:

Salt form	HPLC assay (%)*	Degradate area (%)*
Amorphous HCl	99.2	0.8
Crystalline phosphate, lot 1	99.7	<0.05
Crystalline phosphate, lot 2	100.0	<0.05
Crystalline phosphate, lot 3	99.8	<0.05
Crystalline phosphate, lot 4	99.9	<0.05

* error is ±0.05%

Thus four different lots of the crystalline phosphate salt were stable from degradation for at least up to 6 months at 40°C/75% RH, whereas the amorphous HCl salt showed significant degradation under these storage conditions. Based on these results, the crystalline phosphate salt was preferred over the amorphous HCl salt from the standpoint of chemical stability for further development into a drug product.

ANNEXE C : November 2003 presentation



MERCK

Parallel Development of Multiple Crystal Forms for a New Drug Candidate: Selection of the Final Form via Integrated Chemical and Pharmaceutical Process Evaluation

Speakers

Cindy Starbuck, Patricia Hurter, Robert Wenslow

Co-authors

Joseph Armstrong, Alex Chen, Stephen Cypes,

Russell Ferlita, Karl Hansen, Mahmoud Kaba, Ivan Lee, Dina Zhang

Contributors

Danielle Euler, Tom Gandek, Jeff Givand, Brad Holstine, Feng Li, Yun Liu, Ernestina Luna, Kari Lynn, Robert Meyers, James Ney, Saurabh Palkar, Leigh Shultz, Iris Xie

Outline

Salt Selection

Desired particle properties for early formulation work

- Crystallization studies
- Polymorph characterization
- Development targets

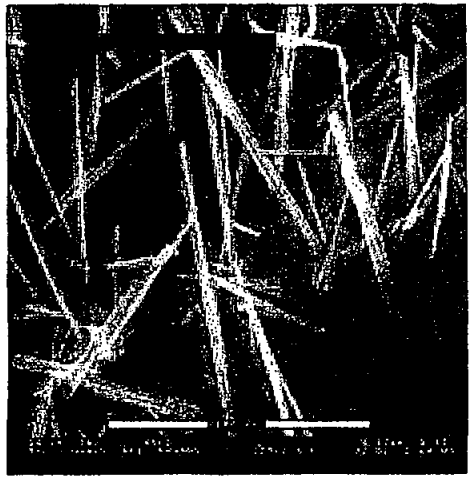
Emergence of a new crystal form during late stage development

- Chemical/Physical characterization
- Formulation characterization
- Biocomparability

Early Salt Selection Activities

- For rapid entry into Phase I, wanted dry filled capsule
- Salt selection focused on morphology as well as stability (v. soluble drug, bioavailability good)
- Targeted DC process for market formulation

Salt A



Salt B

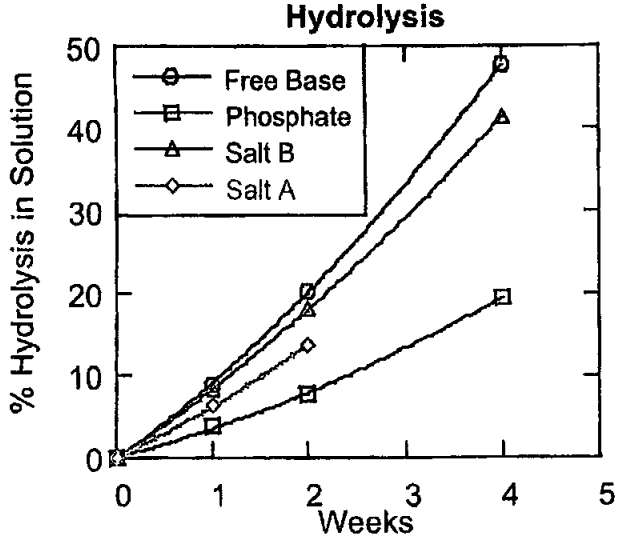
Phosphate

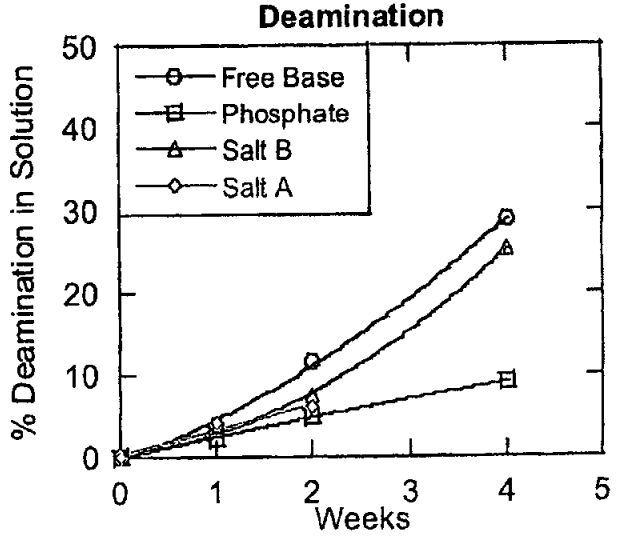
SEMs from Dina Zhang

Early Salt Selection - Stability

All salts stable in bulk at 80°C/amb and 40°C/75%RH

- Hydrolysis and deamination occur in solution





- Phosphate salt chosen for Phase I, on the basis of morphology and stability

Market Formulation Development

Initial studies

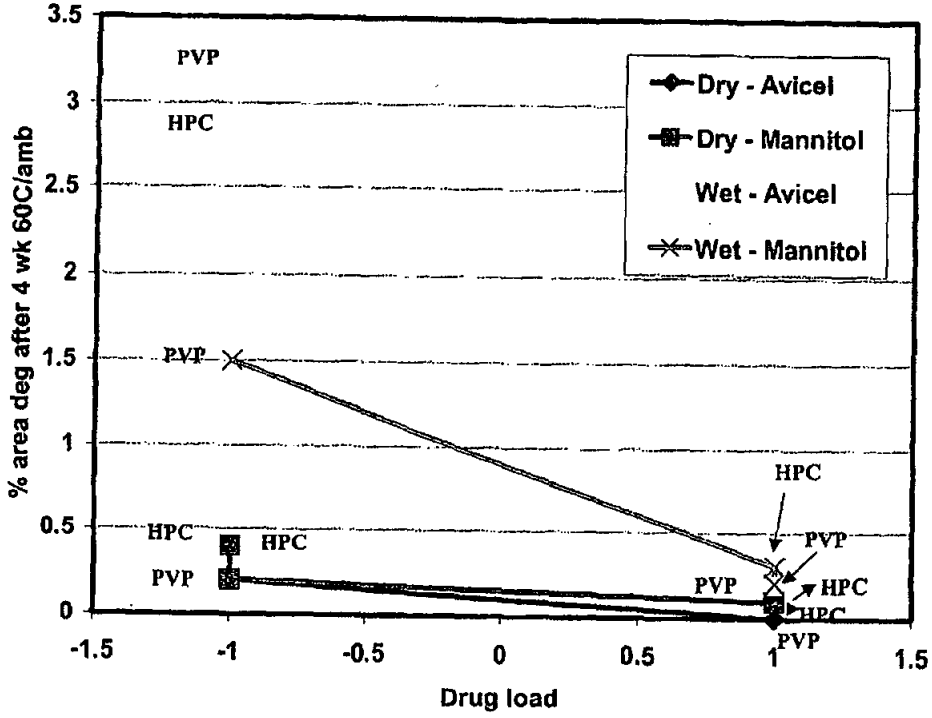
- Probe formulations for excipient compatibility
- Investigation of physical stability, with wet granulation and compression
- Compaction simulator studies

Investigation of feasible drug loading range (1% to max.)

Process selection

- Wet granulation, roller compaction, direct compression options
- DC most desirable if particle characteristics amenable
- If DC promising, two processes in parallel until delivery of API from pilot plant lot

Excipient/Process Choices



- Dry process more stable
- API properties (flow, compactibility, bulk density) suitable for DC processing
- Blend uniformity not an issue, drug loading ~ 25%

⇨ **DC selected as the lead process and RC developed as a back-up**

Desired Particle Characteristics for Early Development

Particle size distribution

- Mean of 20-200μ, unimodal distribution, low fines

Particle shape

- Not needles (rods, plates, cubic, spherical)

Stickiness

- Hard to quantify, affected by size and morphology, residual solvent (?), polymorph (?)

Bulk density

- High enough for processing

Flow

- Acceptable flow for processing

Compactability and compressibility

- Forms integral compacts with acceptable hardness at required drug loading

Public disclosed

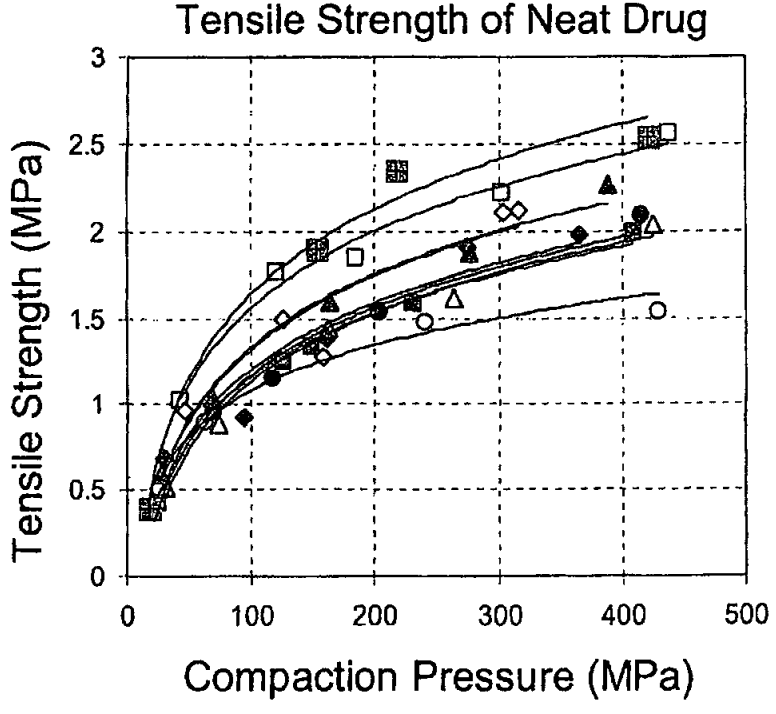
Small-Scale Evaluation of API Characteristics

Prior to first pilot plant campaign, extensive evaluation of effect of crystallization conditions on API characteristics and pharmaceutical processability

- SEM examination (morphology, agglomeration)
- Particle size distribution (mean, fines, unimodal, width of distribution)
- Compaction simulator
  - Neat drug vs formulated
  - Measure tensile strength, ejection force (binding)
- Sticking evaluation on manual single tablet press
- Flow evaluation
  - Bulk/tap density, calculate Carr's Index

Compaction Simulator Results
Tensile Strength of Compacts

Varying crystallization conditions affected tensile strength of neat drug and formulated compacts



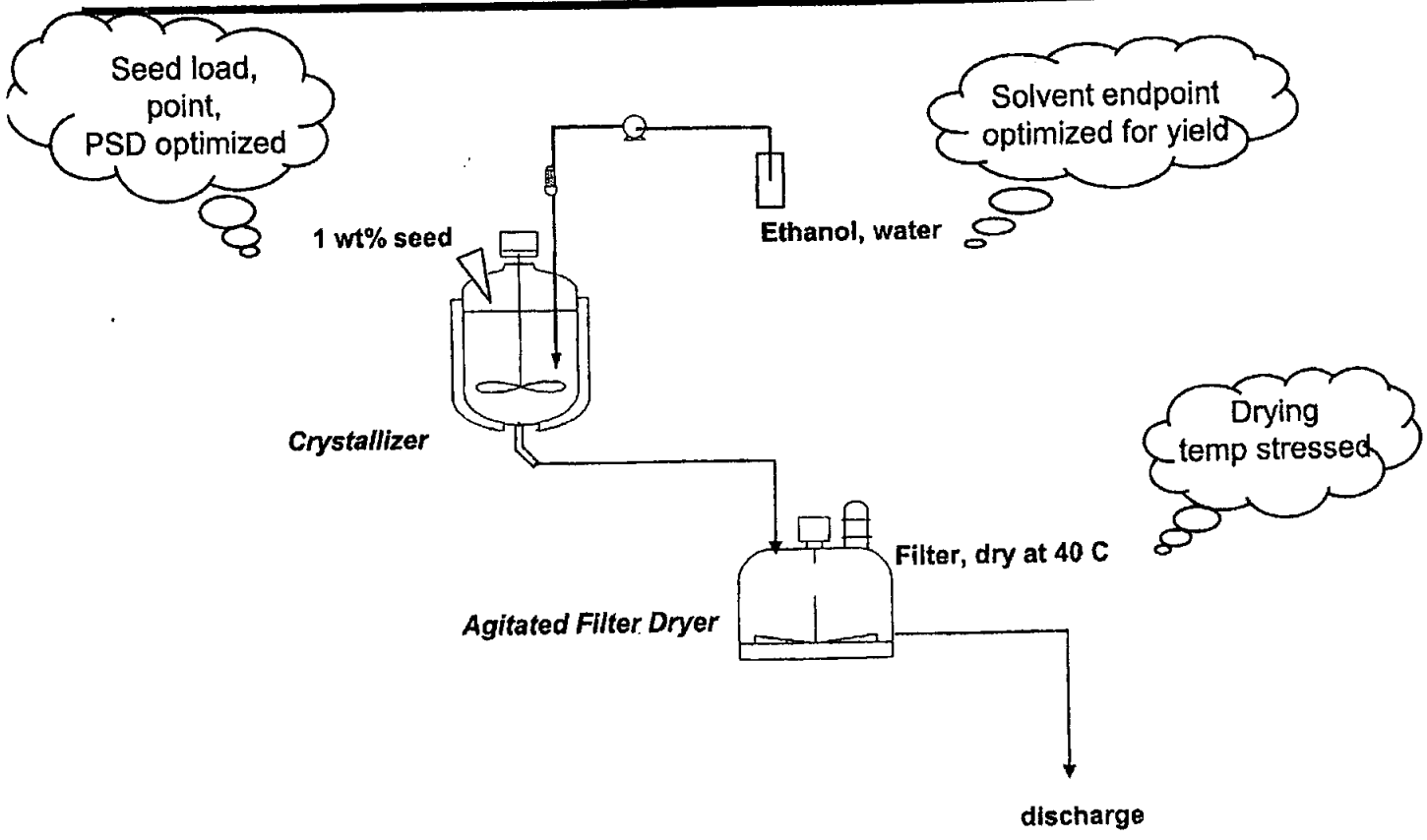
Small-Scale Evaluation of Particles

Lot#	Median Size (μm)*	Agg ?	Density (g/cc)		Carr's Index	Tensile Strength	
			Bulk	Tap		Neat	Form.
A	99	Y	0.27	0.37	27	2.27	3.50
B	65	Y	0.26	0.40	35	1.76	3.50
C	20	Less	0.25	0.44	43	1.81	3.75
D	39	Less	0.25	0.44	43	2.00	3.64
E	50	Y	0.25	0.43	42	2.00	2.80
F	64	Y	0.24	0.45	46	1.78	2.94
G	50	N	0.23	0.42	45	1.80	3.40
H	62	Y	0.31	0.46	33	1.50	3.06

*30s sonication

Crystallization conditions that would generate material similar to Lot A were chosen for the first pilot plant batch

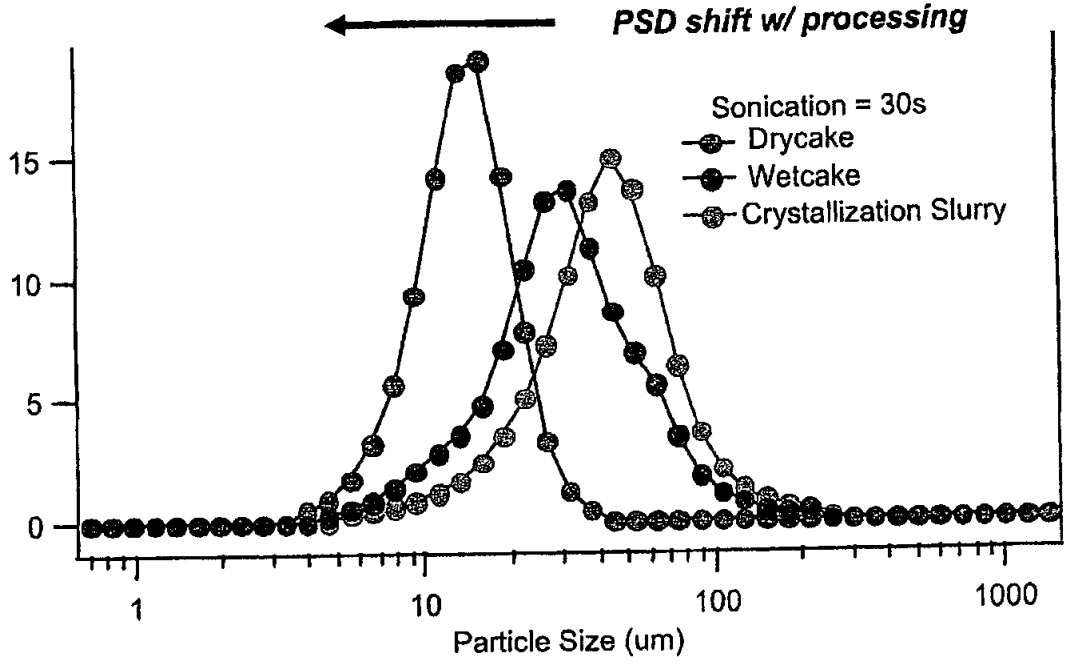
First pilot plant batch - process flow diagram



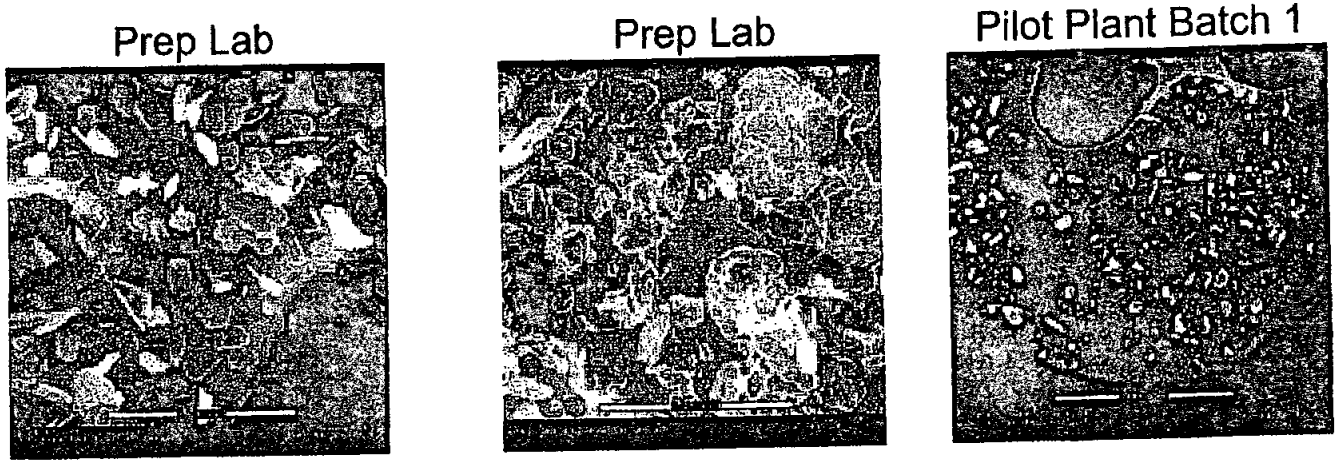
First pilot plant batch - results

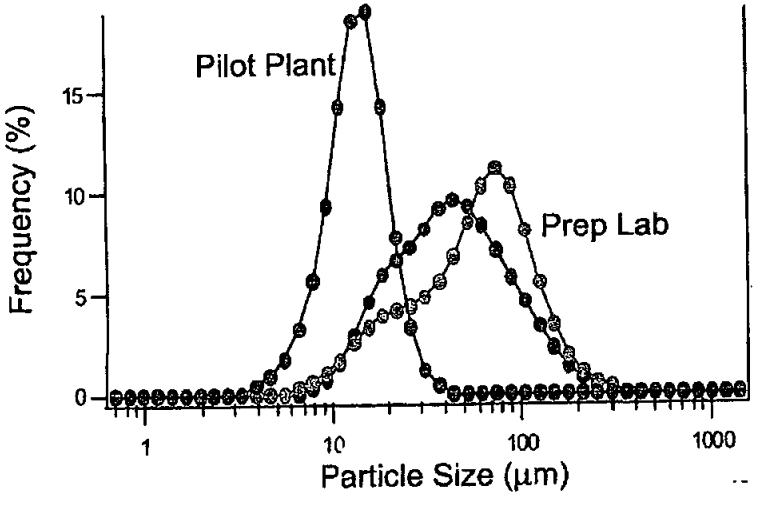
Wetcake found to be friable during filtration/drying

- Agitated filter dryer implemented in pilot plant (scaleable)
- Particle size decrease from 43μm (slurry) to 34 μm (wetcake) to 14 μm (dry cake)



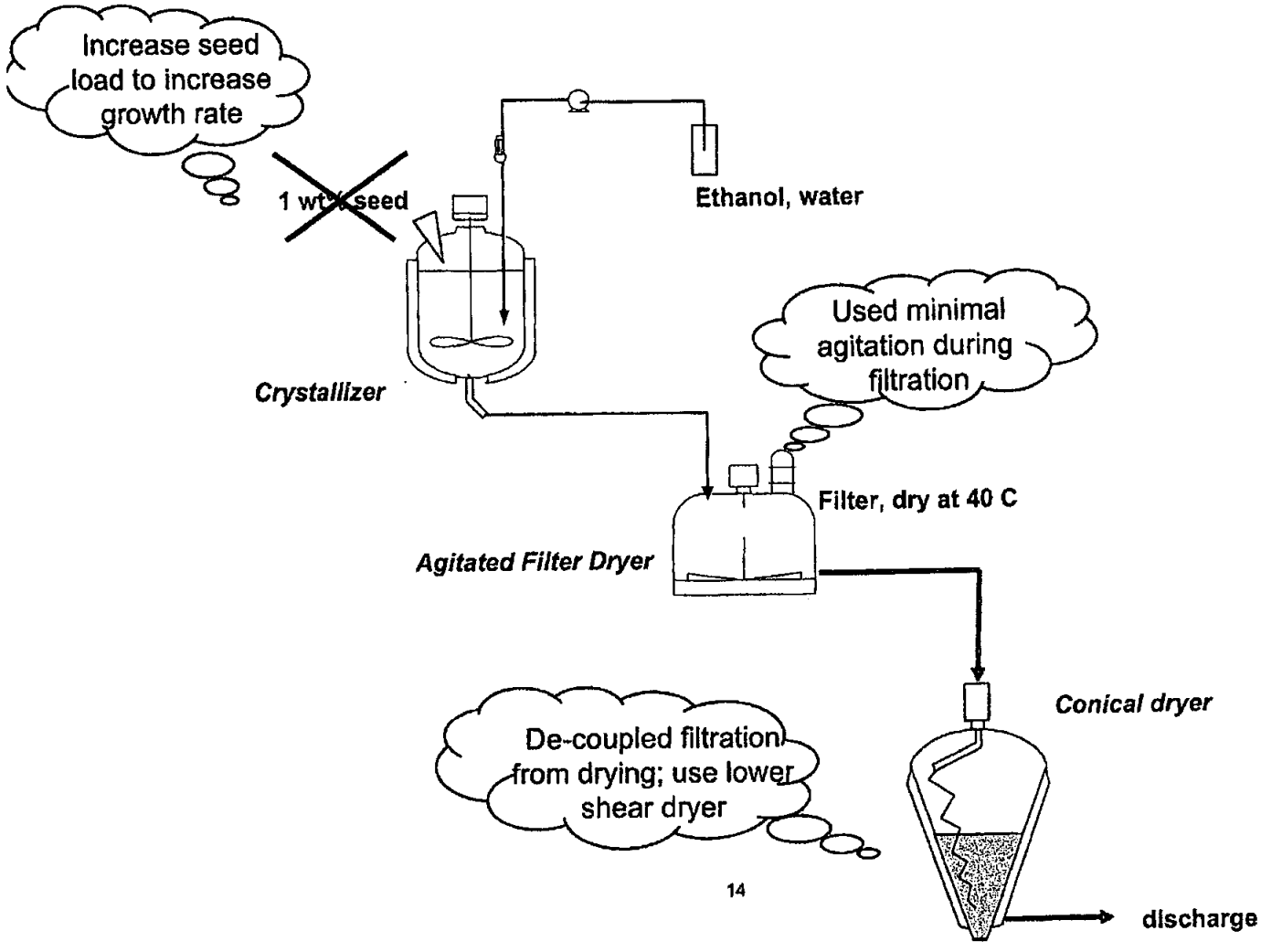
Early deliveries vs First Pilot Plant Batch





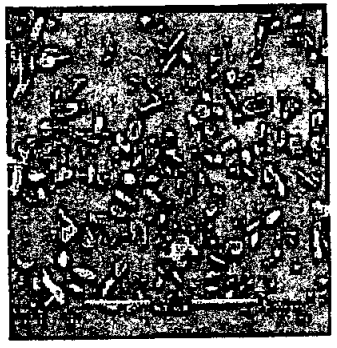
- Measured size = agglomerate size
- Agglomerates (+ single crystals) found to be shear sensitive at large scale

Second pilot plant batch - process flow diagram



Second pilot plant batch - results

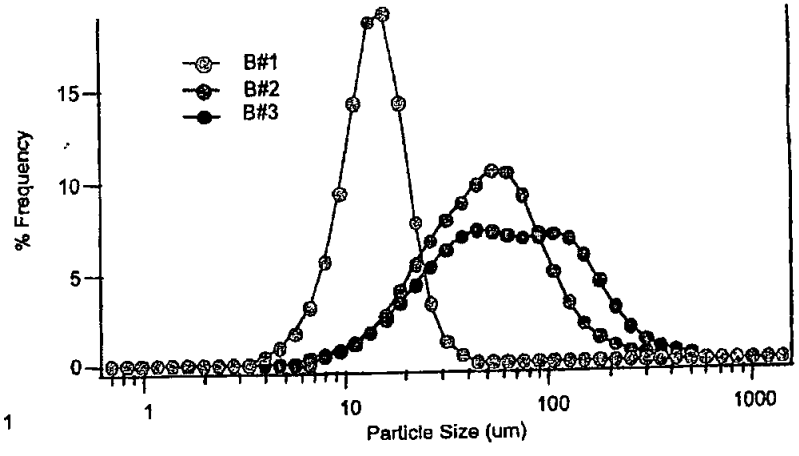
Batch #1



Batch #2

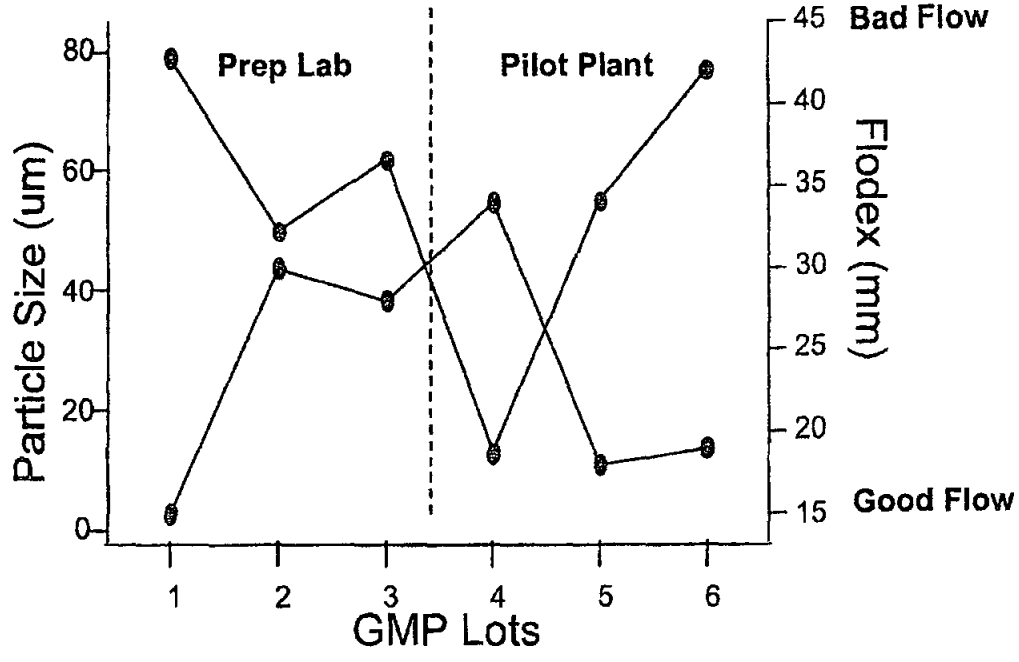
Batch #3

Lot	Bulk Density		Mean	Flowdex
	Loose	Tapped	PSD	(<25 good)
Batch#1	0.30	0.56	14	34
Batch#2	0.53	0.76	55	18
Batch#3	0.55	0.81	70	16



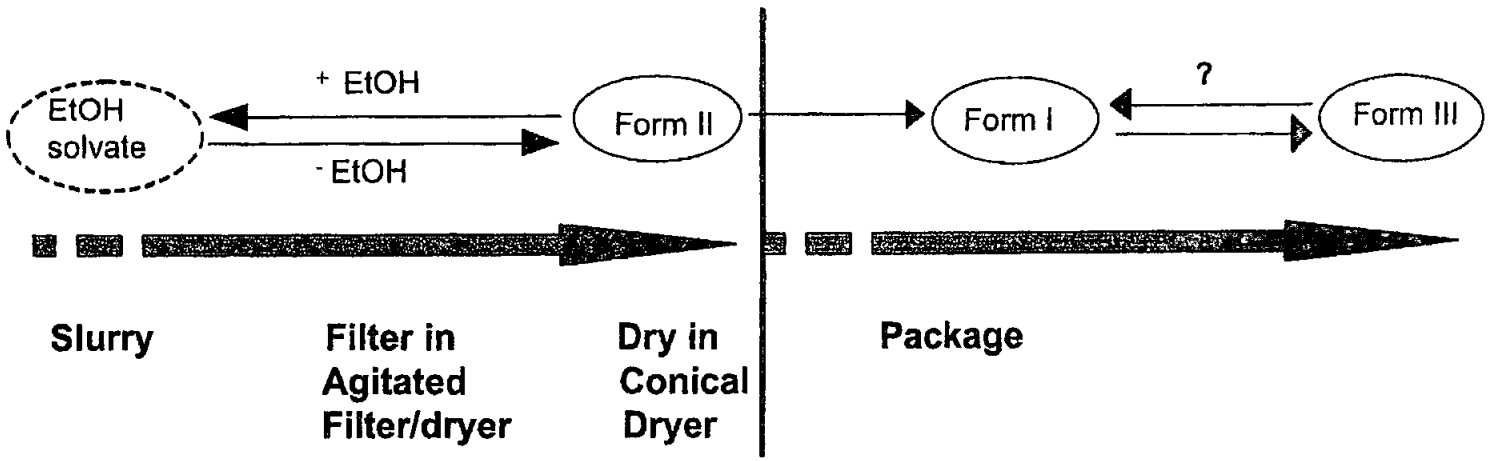
Flowability

- Larger PSD API from subsequent pilot plant campaigns improved flow and pharmaceutical processability

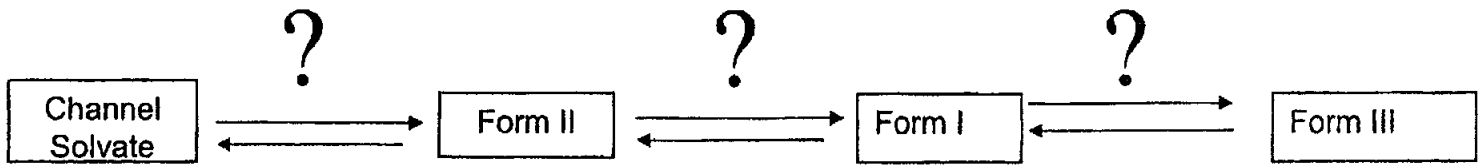


A new problem emerges: multiple polymorphs!

Batch#	Crystal Form Packaged from Dryer	Crystal Form upon Storage
B#1	I + III	I + III
B#2	II	I + III
B#3	II	I + III



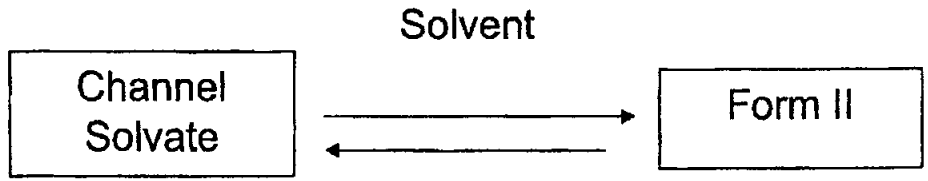
Anhydrous Phases



Critical Issues

- Which are the ***MOST*** relevant phases
- Thermodynamic Stability/Kinetics of Transformation
- Water Solubility (Biopharm Performance)
- Chemical/Physical Stability (API and Tablets)
- Conversion upon drying/grinding/compaction (Pharm Processing)
- ***CRYSTALLIZATION OF PURE PHASE***

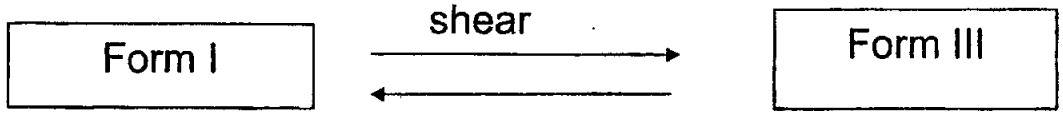
Solvate/Form II



Conversion Conditions

- Isomorphic non-stoichiometric channel solvates with many solvents
- Solvate Formation at all solvent/H_2O ratios
- Form II is de-solvate
  - Identical XRPD/Raman compared to solvate
  - SSNMR only method for Form II identification
- Early crystallizations formed solvate then dried to ***anhydrous Form***
- Form II converts to Form I (<10 min) in water slurry

Form I/Form III



Conversion Conditions (From Experience)

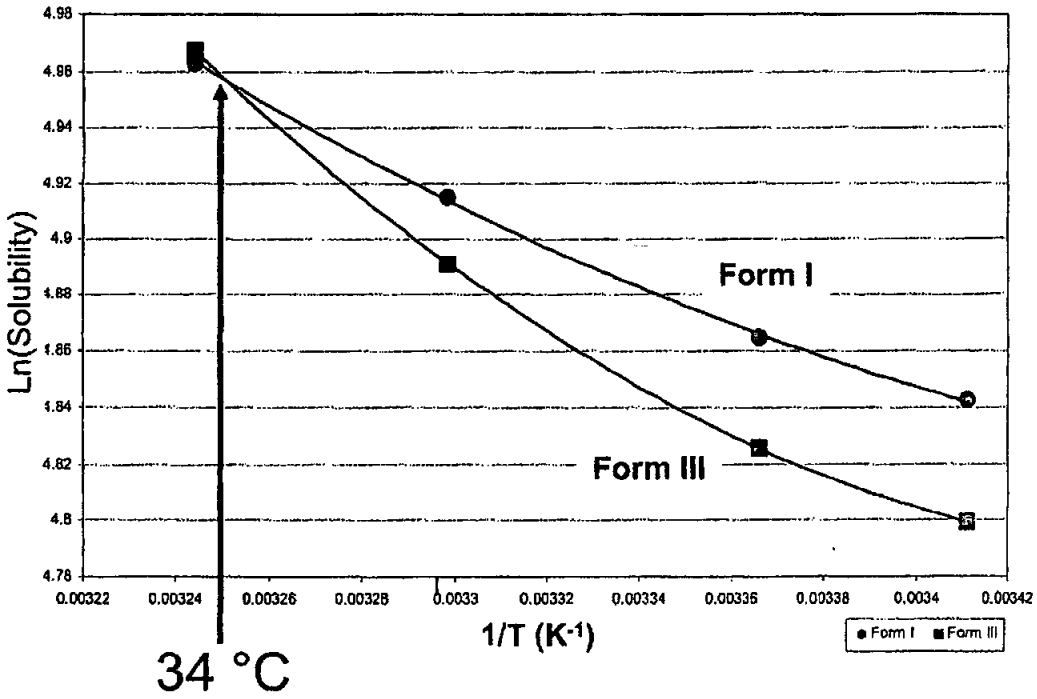
- Form III formed during drying/grinding/compaction
- Mixtures of Form I/III in API and formulations

CRYSTALLIZE PURE, STABLE POLYMORPH

Critical Experiments

- Determine thermodynamically stable polymorph at RT
- Identify kinetics of solid-solid conversion as well as solvent mediated conversion

Thermodynamic Stability

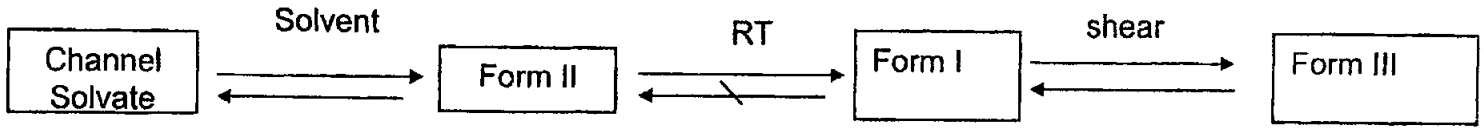


Forms I and III are very close in energy (10^{-2} kcal/mol)

Likely to get mixtures

- solid state turnover during drying - morphology set by Form II template
- solid state turnover during compaction

Anhydrous Phases - characterized



T_{trans} = 34 °C

Water Solubility vs. Temperature of "pure" Forms

Drying induces polymorph conversion

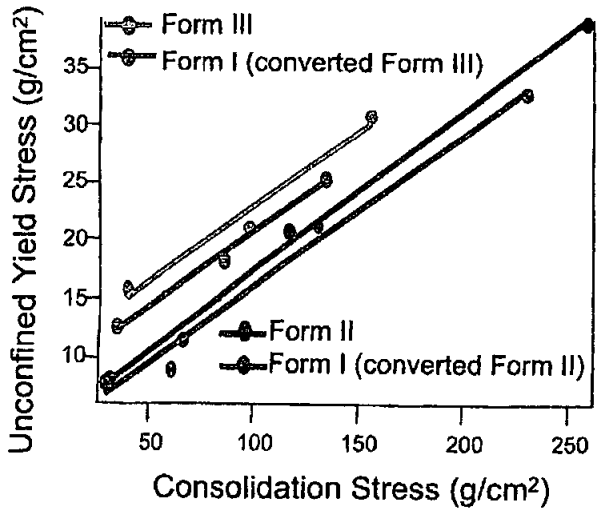
Compaction induces polymorph conversion

} MIXTURES

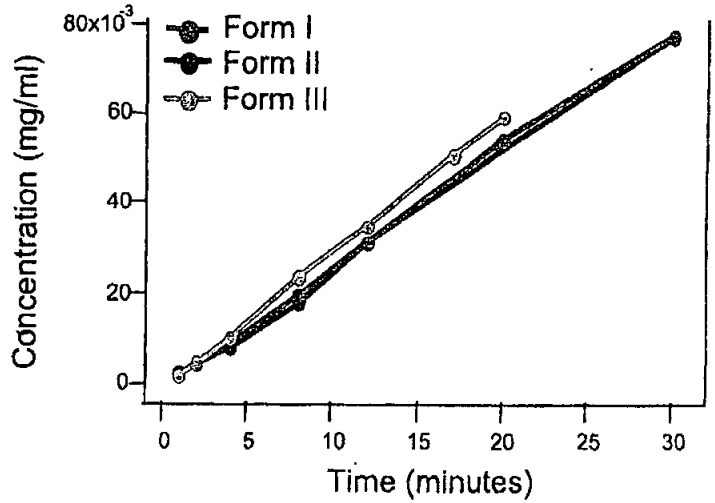
Polymorphism - Anhydrous Polymorphs

- API process produced mixtures of Form I/III
- Experiments were conducted to determine effect of polymorph conversion on pharmaceutical processability
- Particle size/fines had a larger effect on processability than form

Flow (shear cell)



Intrinsic Dissolution



Pure Step Development Goals for Campaign II

Reduce drying time cycle

- Projected factory time cycle for drying > 24 hours

Understand filtration/drying-mediated attrition

- Minimize fines production (=> associated with sticking?)

Understand/eliminate drying-mediated polymorph turnover

- Search for alternate (non-solvating) solvent?

Deliver samples to Pharm R&D for comparative testing

Solvent screening performed to find non-solvating solvent

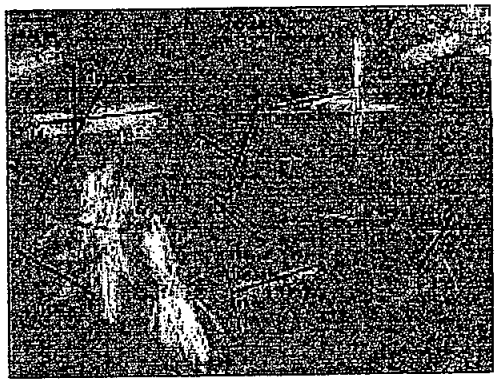
Solvent	Form a Solvate?	Becomes Amorphous?	Free Base Soluble?	Phosphate Salt Soluble?	Degrades Product?	Miscible with Phosphoric Acid?
Water*	N	N	N	Y	N	Y
Methanol	Y	N	Y			
Ethanol	Y	N	Y	N	N	Y
1-Propanol	Y	N				
2-Propanol	Y	N				
t-Butanol	N	Y	Y			
Cyclohexanol	N	Y				
Ethyl Acetate	Y	N				
Acetone	Y	N				
MIBK	N	N	Y	N	Y	Y
Butyl Ether	Y	N				
THF	Y	N	Y	N	N	Y
n-Hexane	N	N	N	N		
Cyclohexane	N	N	N	N		
n-Heptane	N	N	N	N		
Toluene	N	Y	Y	Gels		
Acetonitrile	Y	N				
DMF	Y	N				
DMAC	Y	N				
DMSO	Y	N	Y	Y		Y
MTBE	N	N	Y	N		N
Methylene Chloride	Y	N	Y	N		N
Diethoxymethane	Y	N				
Methyl Benzoate	N			Sparingly		
Isoamyl Alcohol	N	N	Y	N	N	Y

* = As of Feb 2003

Lab work with non-solvating solvent: enter the Monohydrate!

Public Disclosed

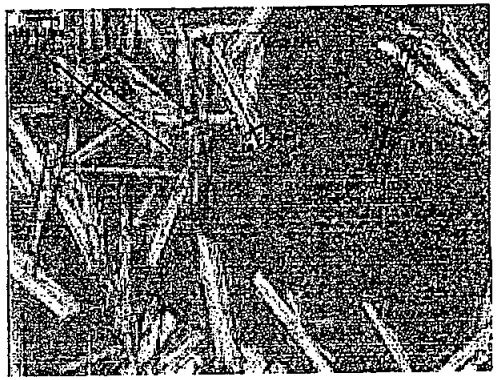
Successfully generated Form I directly from isoamyl alcohol → needle morphology



Incorporated 5% water to improve particle size and morphology

new crystal form emerged !!

(Monohydrate!)



→ **An aside. . .van't Hoff plot for Forms I/III: solubilities measured in water!**

Properties of monohydrate

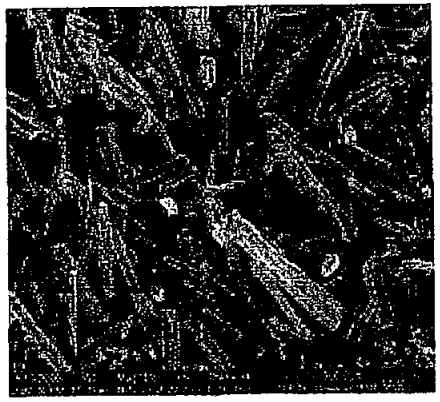
Water Solubility (free base eq) = 68 mg/ml (Anhydrous = 108 mg/ml)

Morphology is rod-like

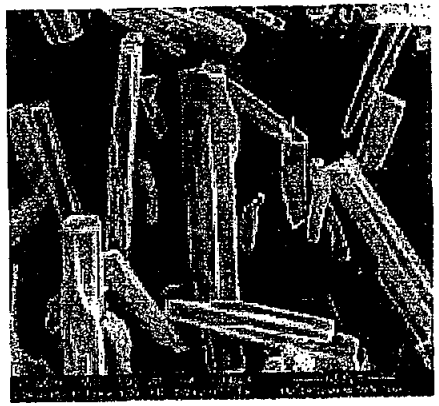
Non-hygroscopic, picks up <1% at 75%RH (4.5% at 95%RH)

All anhydrous forms, when put in water, now turn over to monohydrate (previously, Form I)

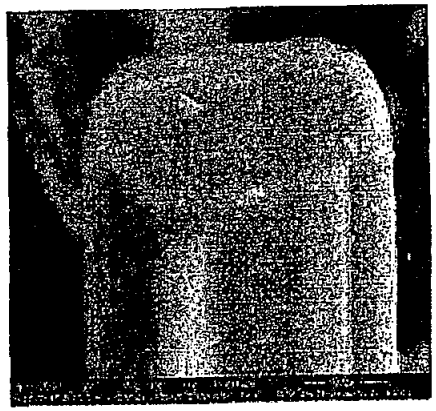
300 µm



100 µm



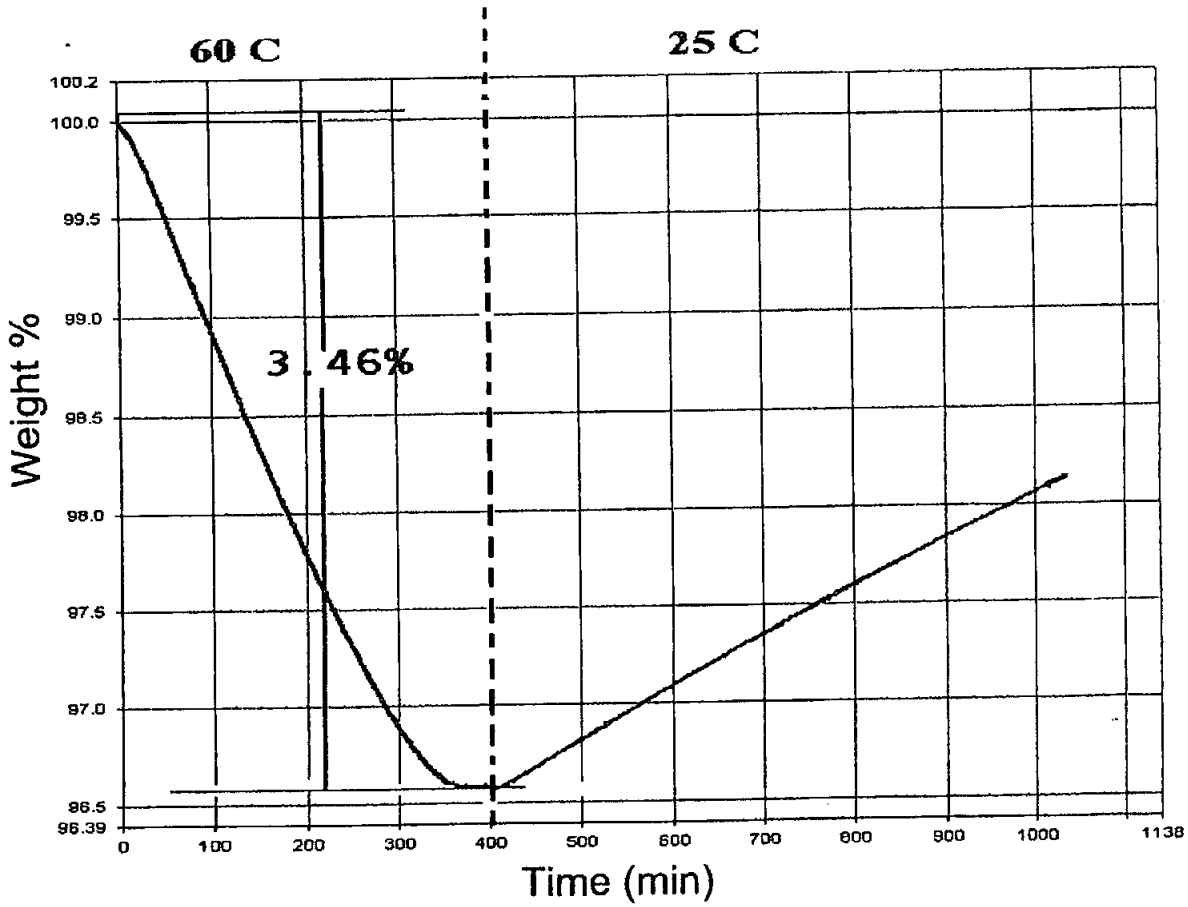
50 µm



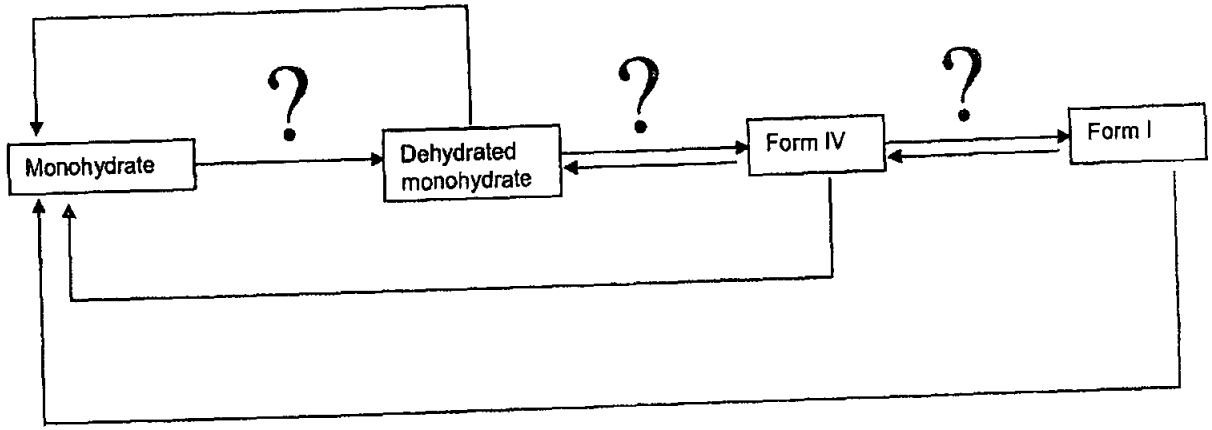
New Physical Property Action Plan

- Water solubility/tablet dissolution
- *Dehydration potential*
- *Higher hydrates?*
- Chemical/Physical API stability
- Physical stability during Milling/Compaction

Kinetics of Water Loss for Monohydrate



Form Conversion Starting with Monohydrate



Hot stage XRPD

- RT (monohydrate)
- 90°C 4 hours (dehydrated monohydrate)
- 110°C new spectrum (Form IV confirmed by SSNMR)
- 130°C (Form I)
- Back to Ambient (Form I)

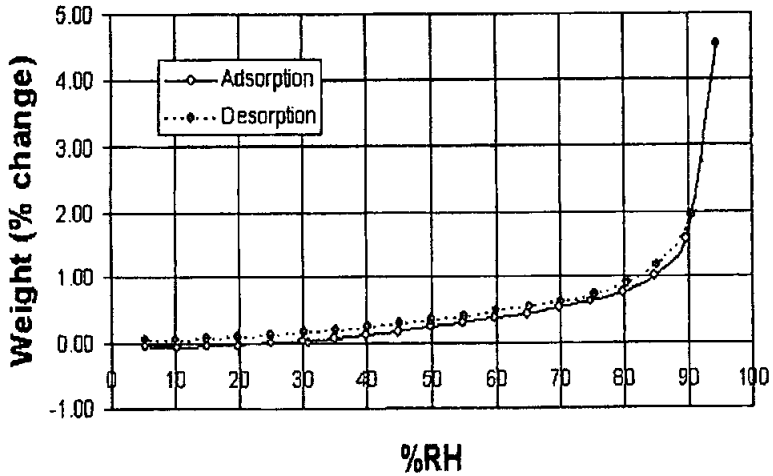
INTERCONVERSION

Hygroscopicity vs. Temperature

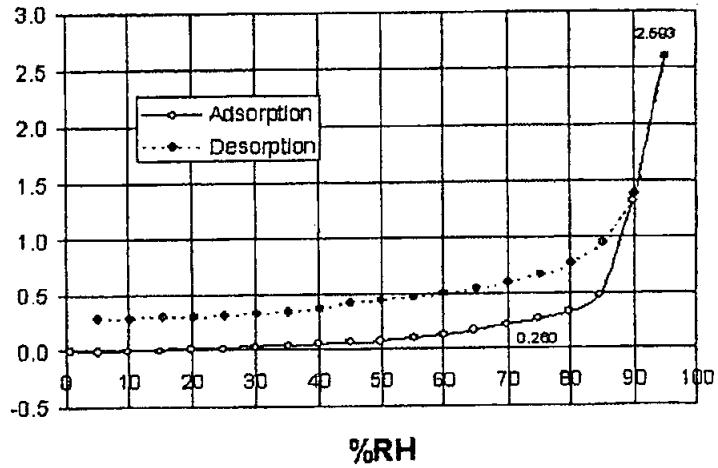
Pure Monohydrate

No Dryer Step, Long Equilibration Time, Lowest RH = 0.8%

25°C



40°C



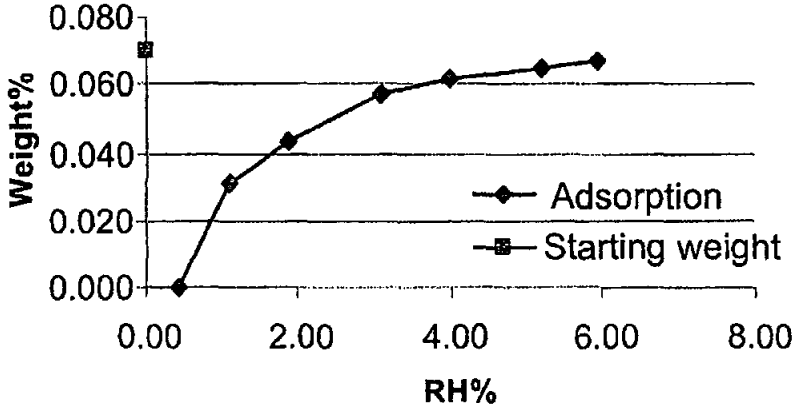
Monohydrate NEVER loses water at 25°C and 40°C >0.8%RH

Material remains Monohydrate

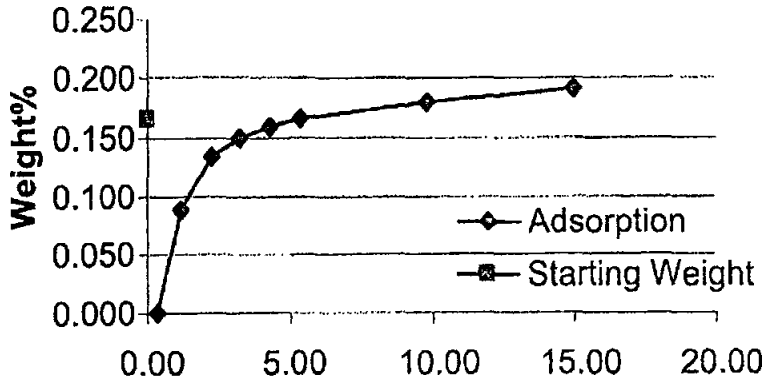
Extended Temperature Hygroscopicity Data

Pure Monohydrate

60°C



80°C

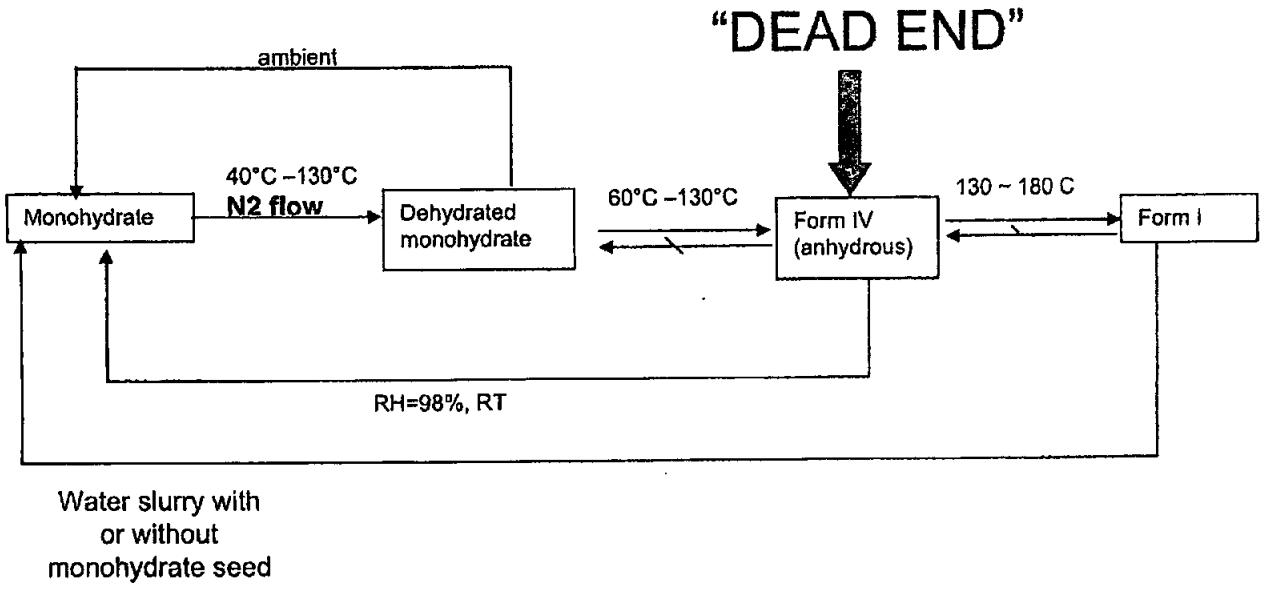


60°C Returns to original water content at ~6% RH

80°C Returns to original water content at ~10% RH

Material remains Monohydrate

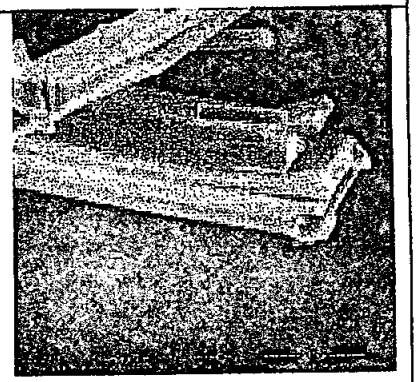
Solid Phases in Monohydrate System



API perspective - the case for monohydrate

	Monohydrate	Anhydrous (I/III)
Crystallization	IPA/Water or IAA/Water Thermo stab regions mapped vs. H2O content Monohydrate crystallized directly	IPA/Water or EtOH/Water Thermo stab regions mapped vs. water content Agglomerated morphology; Form II template
Drying	No potential for dehydration if dried without sweep	Polymorph conversion Form II to I/III mixtures
Bulk Chem Stab	4 wk no degradation -20C, 40C, 40C/75RH, 80C	12 month no degradation -20C, 40C, 40C/75RH, 60C
Physical Stab	No Physical change on stability Bulk and Tablets	Mixtures going into stab No change in mixture content on stab (bulk and tablets)
Regulatory	Single crystal form	Mixtures; must be controlled

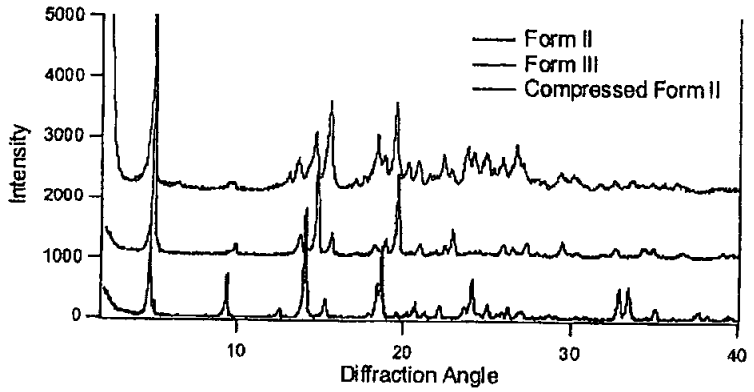
Pharm processing: evaluation of monohydrate

	Anhydrous	Monohydrate
Morphology/Shape		
Density [g/cm^3] *Loose* *Tapped*	 0.55 0.81	 0.28 0.55
Flodex	19	32
Sticking (mg/ml)	0.124	0.0043
Mean Particle Size (μm)	77	143

Effect of Compaction on Form Conversion

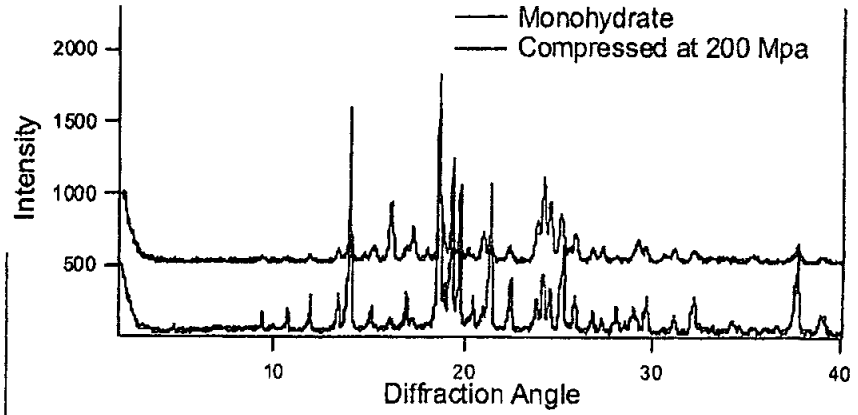
Anhydrous:

- Compaction:
  - Form II ⇨ Form I/III
  - Form I ⇨ Form I/III
  - Form III ⇨ Form I/III
- Compaction always results in Form I/III mixture



Monohydrate

- No form conversion



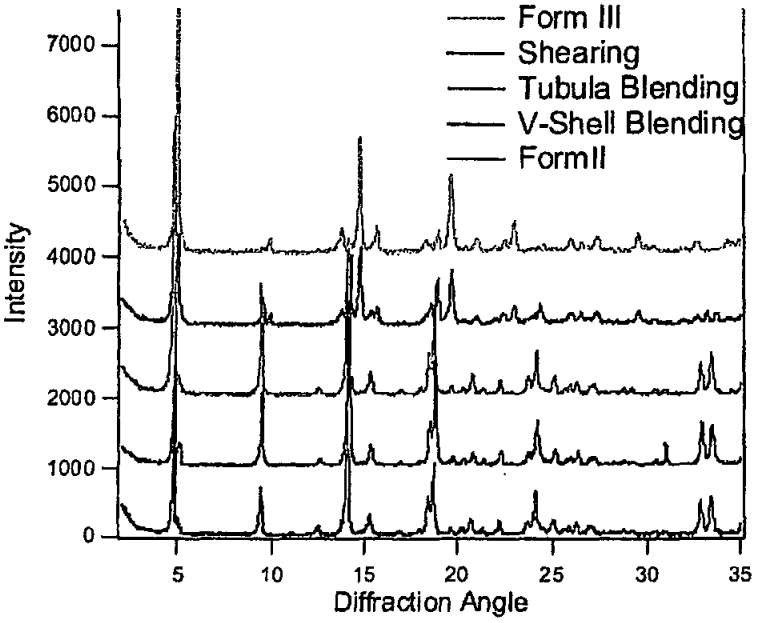
Drug Product Processing Effect

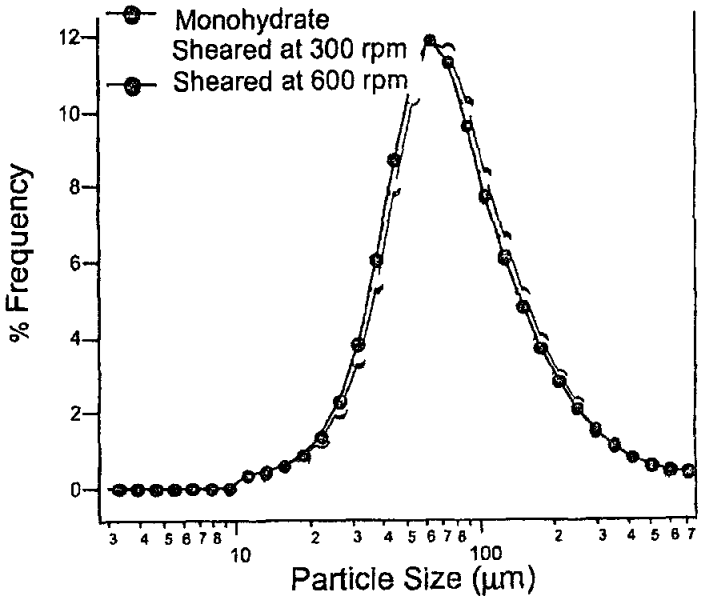
Anhydrous

- After Shearing
  - All forms (I, II, III) ⇨ I/III mix
  - Particle breakage

Monohydrate

- No form conversion
- No particle breakage

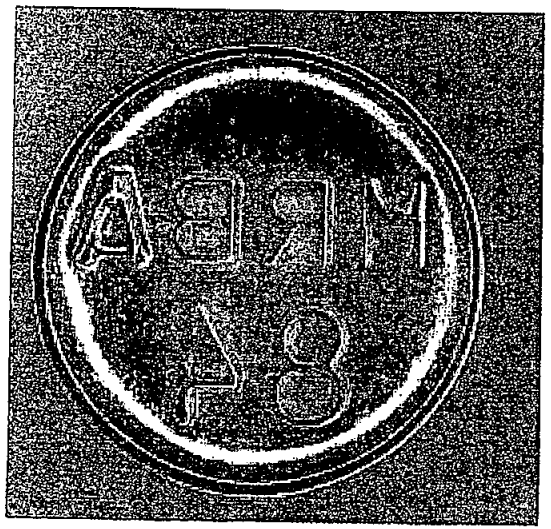




Tablet Sticking reduced with Monohydrate

- 5 minute compression run on Korsch press (9kN force)
  - Comparable formulations with monohydrate exhibited less punch sticking than those with anhydrous polymorph

Anhydrous API Formulation

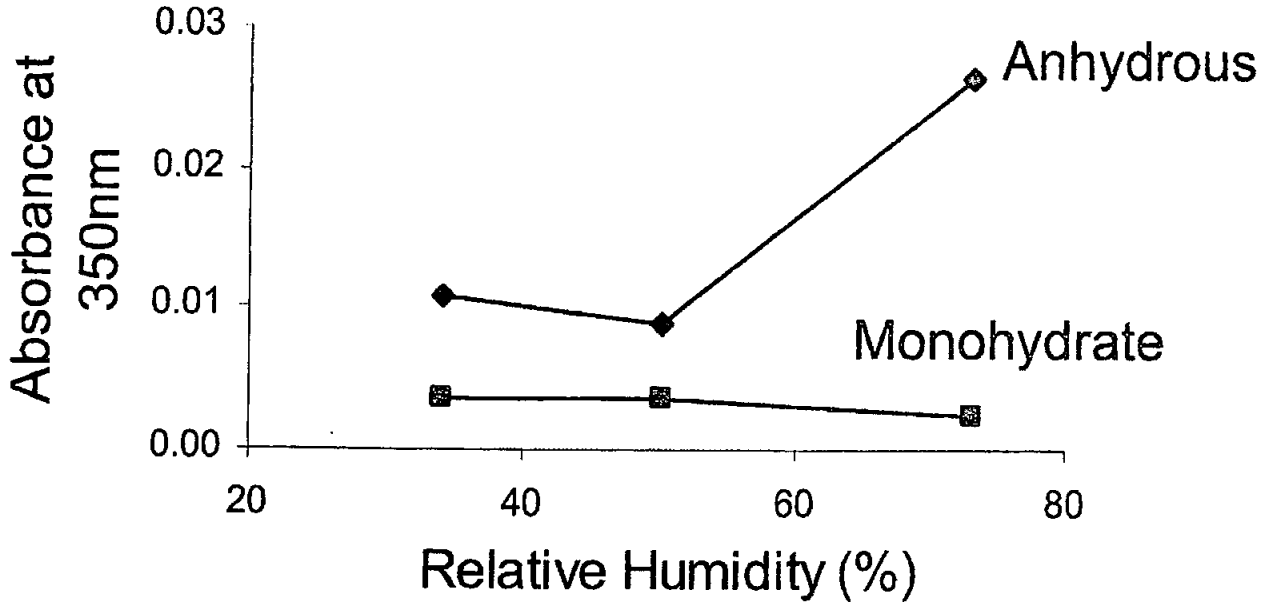


Monohydrate API Formulation

Color formation in formulations

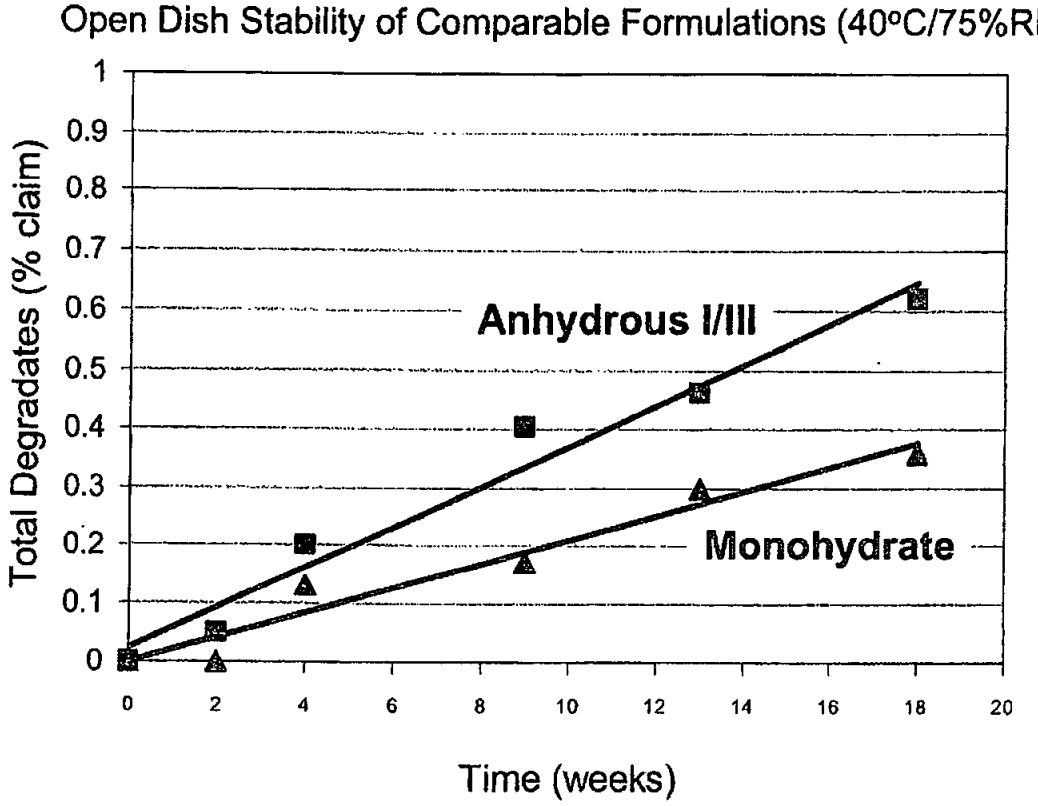
- Stressed formulations discolor, linked to low levels of impurities in excipients
  - Monohydrate API less susceptible to discoloration

Drug substance stressed with impurities at various RH, 50°C



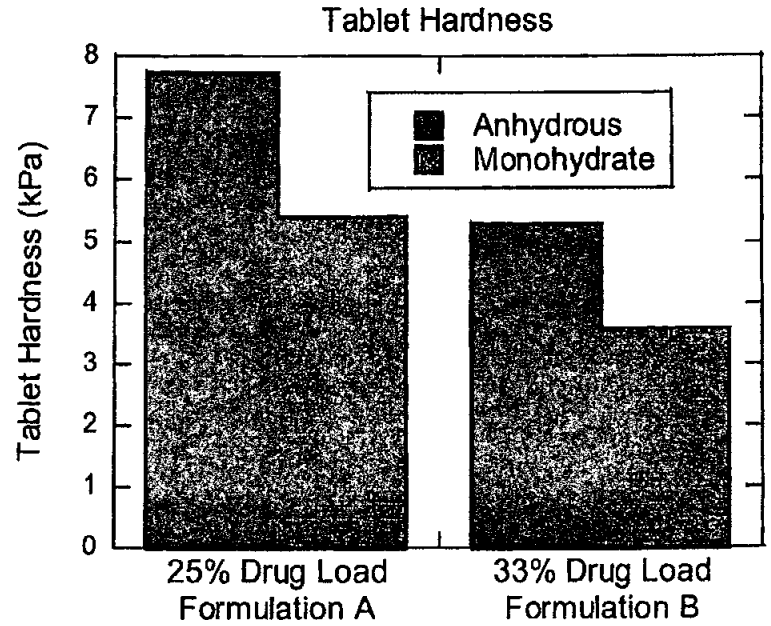
Chemical Stability of Monohydrate vs Anhydrous

- Chemical stability of monohydrate formulations superior to anhydrous



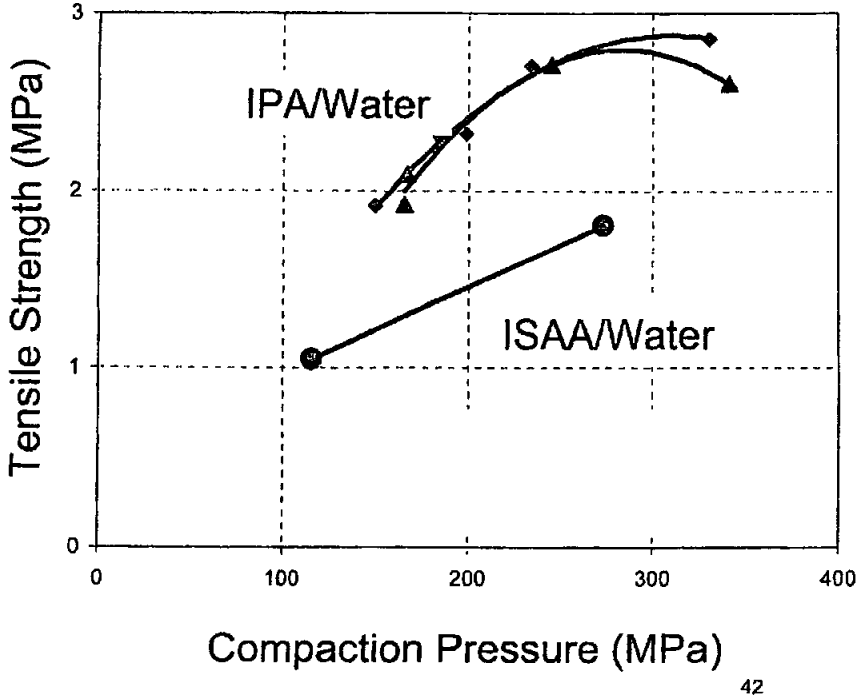
Compactability of Monohydrate vs Anhydrous

- Initially, monohydrate formulations appeared to have lower tensile strength than anhydrous formulations



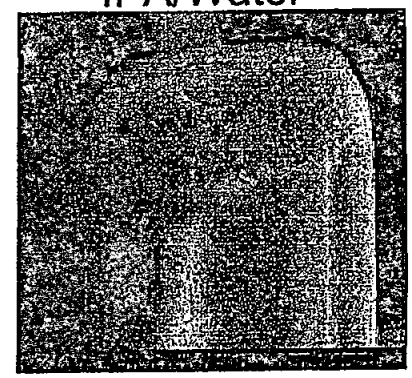
Effect of Crystallization Solvent on Monohydrate Properties

- Comparing monohydrate from IPA/water vs ISAA/water
  - Higher tensile strength
  - Stronger crystal strength
  - More difficult to lose crystalline water



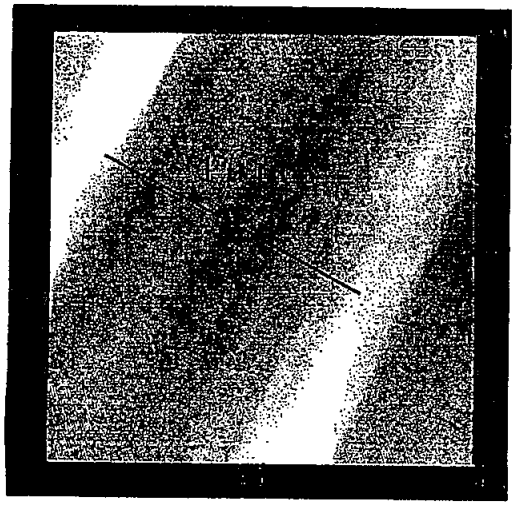
ISAA/Water

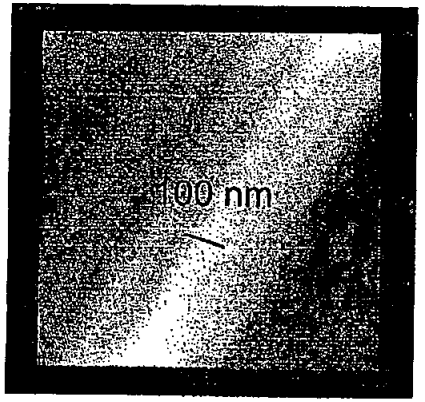
IPA/Water



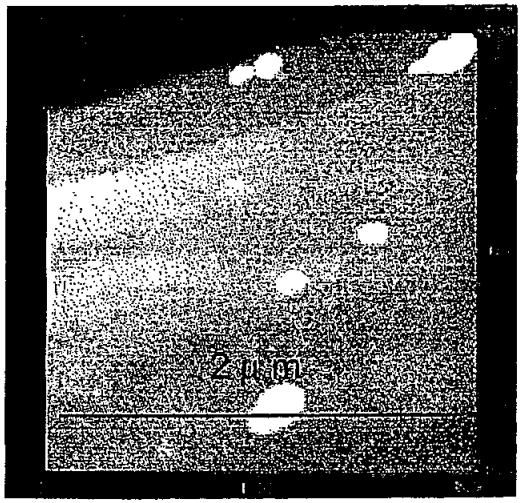
Crystal Structure by Atomic Force Microscopy

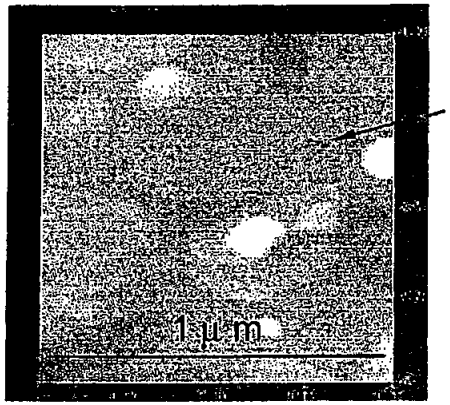
ISAA/Water





IPA/Water



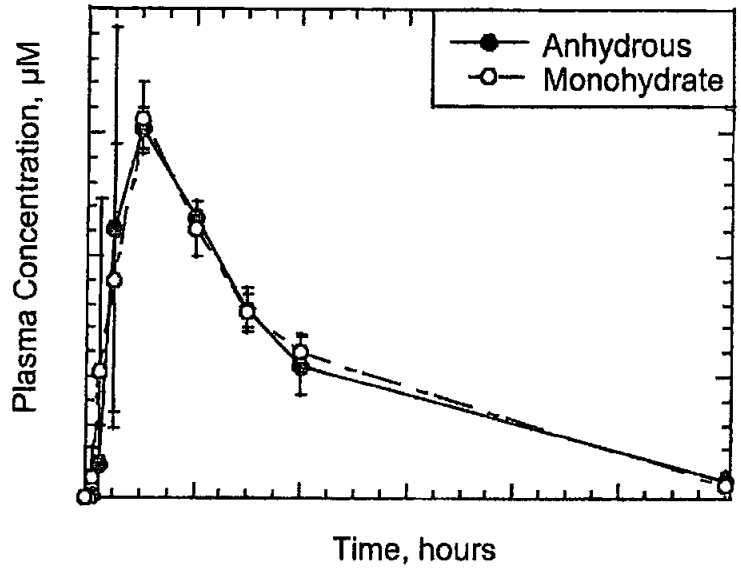


Layered Structure with a thickness of 3.5-4.2 nm

Biopharmaceutical Comparison

- High solubility API (68 mg/ml), no effect on absorption expected
- Dog study "disaster check" confirms no effect on dog PK

Anhydrous and Monohydrate Forms in an HPMC Capsule Dosed Orally to Beagle Dogs (n=4)



Decision to Switch to Monohydrate Form

Physical stability

- Monohydrate showed improved physical stability over anhydrous forms
- Anhydrous Forms undergo form conversion; loss of crystallinity during drying and compaction

Chemical Stability

- Less color development with monohydrate-based tablets
- Lower levels of degradation with monohydrate vs anhydrous
- Dessicant not required for monohydrate packaging

Biopharmaceutical equivalence

- Animal studies (mice, rats, dogs) showed exposure levels (PK) equivalent

Chem Processability

- Reduced concern over Form change and particle breakage during drying. Improved yield; streamlined process.

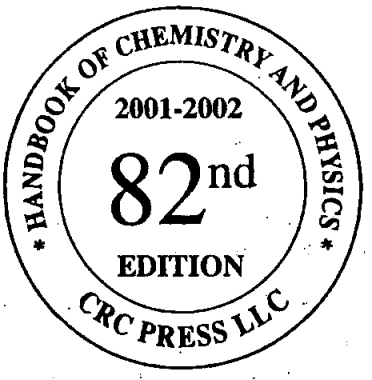
Pharm Processability

- Reduced sticking on tablet punch.

EXHIBIT P

CRC Handbook of Chemistry and Physics

A Ready-Reference Book of Chemical and Physical Data



Editor-in-Chief

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National Institute of Standards and Technology

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DISSOCIATION CONSTANTS OF INORGANIC ACIDS AND BASES (continued)

Name	Formula	Step	t/°C	pK_a
Hypochlorous acid	HClO		25	7.40
Hypoiodous acid	HIO		25	10.5
Iodic acid	HIO_3		25	0.78
Lithium ion	Li^+		25	13.8
Magnesium(II) ion	Mg^{+2}		25	11.4
Nitrous acid	HNO_2		25	3.25
Perchloric acid	$HClO_4$		20	-1.6
Periodic acid	HIO_4		25	1.64
Phosphoric acid	H_3PO_4	1	25	2.16
		2	25	7.21
		3	25	12.32
Phosphorous acid	H_3PO_3	1	20	1.3
		2	20	6.70
Pyrophosphoric acid	$H_4P_2O_7$	1	25	0.91
		2	25	2.10
		3	25	6.70
		4	25	9.32
Selenic acid	H_2SeO_4	2	25	1.7
Selenious acid	H_2SeO_3	1	25	2.62
		2	25	8.32
Silicic acid	H_4SiO_4	1	30	9.9
		2	30	11.8
		3	30	12
		4	30	12
Sodium ion	Na^+		25	14.8
Strontium(II) ion	Sr^{+2}		25	13.2
Sulfamic acid	NH_2SO_3H		25	1.05
Sulfuric acid	H_2SO_4	2	25	1.99
Sulfurous acid	H_2SO_3	1	25	1.85
		2	25	7.2
Telluric acid	H_2TeO_4	1	18	7.68
		2	18	11.0
Tellurous acid	H_2TeO_3	1	25	6.27
		2	25	8.43
Tetrafluoroboric acid	HBF_4		25	0.5
Thiocyanic acid	HSCN		25	-1.8
Water	H_2O		25	13.995

EXHIBIT Q

Organic Process Research & Development 2000, 4, 427–435

Salt Selection and Optimisation Procedures for Pharmaceutical New Chemical Entities

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Abstract:

Selection of an appropriate salt form for a new chemical entity provides the pharmaceutical chemist and formulation scientist with the opportunity to modify the characteristics of the potential drug substance and to permit the development of dosage forms with good bioavailability, stability, manufacturability, and patient compliance. Salts are most commonly employed for modifying aqueous solubility, however the salt form selected will influence a range of other properties such as melting point, hygroscopicity, chemical stability, dissolution rate, solution pH, crystal form, and mechanical properties. Where possible, a range of salts should be prepared for each new substance and their properties compared during a suitable preformulation program. Since it is normally possible to fully develop only one salt form, its properties should be appropriate to the primary route of administration and dosage form. An understanding of the influence of drug and salt properties on the finished product is essential to ensure selection of the best salt. The drug properties required for one dosage form may be quite different from those required for another. A well designed salt selection and optimisation study provides a sound base on which to build a rapid and economic product development programme.

Introduction

Modern drug discovery processes involve the screening of vast numbers of compounds that may have been made by the Company's research laboratories over many years. Added to these may be the many thousands of compounds that have been manufactured as libraries of structurally related series by "combinatorial chemistry" techniques. All of these compounds are generally dissolved in dimethylsulphoxide (DMSO) solution and screened in an enzyme- or receptor-based assay system. If the number of "hits" produced is large, the numbers are usually refined by further screening and selection until a manageable number of "leads" is available. Many of these leads will show only weak or moderate activity and further refinement and optimisation is invariably necessary. These optimisation procedures usually involve numerous structural modifications, aided by computational techniques, until a small number (usually 1–5) of highly active "candidates" remain.

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These candidates are usually free bases, free acids, or neutral molecules, rather than their salts. Also, because of the generally higher molecular weights of modern drug substances and the increased use of DMSO solutions in the screening processes, it is becoming apparent that there is a tendency towards ever more lipophilic candidates being presented. Frequently, when first proposed as potential development candidates, they are often amorphous or partially crystalline as little effort has been made to investigate formal crystallisation procedures. The need for water-soluble candidates has been recognised[1–4] for many years before the advent of 'combinatorial chemistry.

Investigations into the Possibilities of Salt Formation

When first presented for initial preformulation investigations, normally the amount of drug substance available from Discovery Chemistry rarely exceeds 1 g. To maximize the amount of data gained from such small quantities, semi-micro techniques have been developed and are used regularly within our groups. Invariably, the first information generated for each candidate is the calculated pK_a value of each ionisable group in the molecule.[5–8] This is quickly checked against the value determined experimentally on 1–2 mg of sample by potentiometric titration (e.g., Sirius Model GLpKa apparatus, Sirius Analytical Instruments Ltd.). Knowledge of the pK_a value enables potential salt forming agents (counterions) to be selected, for each candidate, based on lists that are available in the literature.[2,9–11] For the formation of a stable salt, it is widely accepted that there should be a minimum difference of about 3 units between the pK_a value

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Table 1. **Classification of common pharmaceutical salts**

salt class	examples
	Anions
inorganic acids	hydrochloride, hydrobromide, sulfate, nitrate, phosphate
sulfonic acids	mesylate,[b] esylate,[c] isethionate,[d] tosylate,[e] napsylate,[f] besylate[g]
carboxylic acids	acetate, propionate, maleate, benzoate, salicylate, fumarate
anionic amino acids	glutamate, aspartate
hydroxyacids	Citrate, lactate, succinate, tartrate, glycollate
fatty acids	hexanoate, octanoate, decanoate, oleate, stearate
insoluble salts	pamoate (embonate), polystyrene sulfonate (resinate)
	Cations
organic amines	triethylamine, ethanolamine, triethanolamine, meglumine, ethylenediamine, choline
insoluble salts	procaine, benzathine
metallic	sodium, potassium, calcium, magnesium, zinc
cationic amino acids	arginine, lysine, histidine

[a] Based on data from various sources.[9-11] [b] Methane sulfonate. [c] Ethane sulfonate. [d] 2-Hydroxyethane sulfonate. [e] Toluene sulfonate. [f] Naphthalene sulfonate. [g] Benzene sulfonate.

of the group and that of its counterion, especially when the drug substance is a particularly weak acid or base. Occasionally, exceptions may be found where a salt has an acceptable stability, despite there being a smaller difference in the pK_a values.

A microplate technique has been developed for the screening of salts; this involves dissolving approximately 50 mg of sample in a suitable, volatile solvent and adding a fixed volume of this solution, containing about 0.5 mg of sample, into each microplate well. Concentrated solutions of each potential counterion in equimolar proportion, or other appropriate stoichiometric ratio, are prepared and a few microlitres of each is added sequentially to each well. Thus, all of the wells in line 1 (x−direction) will contain the same combination of sample and counterion 1; all of the wells in line 2 contain the same combination of sample and counterion 2, etc. Different, potential crystallising solvents can be investigated methodically in the y−direction. The wells are inspected using an inverted microscope (Leica, Model DMIRB) at regular intervals for the appearance of crystals. Occasionally, crystallisation can be promoted by evaporation of any excess solvent in some wells using a slow stream of dry nitrogen gas.

Once the combinations of counterion and solvent(s) are identified, studies at a slightly larger scale (usually 10−50 mg, occasionally up to 500 mg) can be initiated to confirm the suitability and viability of the crystals. These studies can help identify problems with low melting points, determined by hot-stage microscopy, and hygroscopicity, if processed on a suitable apparatus (e.g., Dynamic Vapour Sorption Analyser, model DVS-1, Surface Measurement Systems Ltd.). Frequently these studies can also give preliminary information on the existence of solvates and hydrates, especially if differential scanning calorimetry (DSC, Mettler Toledo DSC, model 820), thermal gravimetric analysis (TGA, Mettler Toledo TGA, model 850) and hot-stage microscopy are also used in the evaluation process.

In parallel with these studies, a preliminary high performance liquid chromatographic (HPLC) method is quickly developed to give an estimate of the purity of the sample, whilst infrared and other spectroscopic techniques may be used to define the salt and the stoichiometry. Knowledge of the approximate purity is important at this stage as the presence of high levels of some impurities can often hinder crystallisation or alter the polymorphic form obtained.

Therefore, from these preliminary, small-scale studies, a range of potential salt formers and recrysallisation solvents can be quickly identified. Following further scale-up to gram quantities, more comprehensive data can be obtained to evaluate their suitability for use in formulations.

Choice of the Salt Former

Although the choice of salt is governed largely by the acidity or basicity of the ionisable group, safety of the counterion, drug indications, route of administration and the intended dosage form must also be considered. Toxicological and pharmacological implications of the selected salt former must be considered as well as the effects of the parent drug. Salt formers can be subdivided into a number of categories, depending upon their functionality and purpose. Some of the most frequently used examples are listed in Table 1.

The vast majority of salts are developed to enhance the aqueous solubility of drug substances. For weakly basic drug substances, salts of an inorganic acid (e.g., hydrochloride, sulphate, or phosphate), a sulphonic acid (mesylate or isethionate), a carboxylic acid (acetate, maleate or fumarate), a hydroxyacid (citrate or tartrate), or possibly an amino acid (arginine or lysine) could be considered. Hydrochloride salts have often been the first choice for weakly basic drugs, since as a consequence of the low counterion pK_a, salts can nearly always be formed, and recrystallisation from organic solvents is normally straightforward. However, the potential disadvantages of hydrochloride salts may include unacceptably high acidity in formulations (e.g., parenteral products), the risk of corrosion, less than optimal solubility due to the risk of salting out and the potential for poor stability if the drug is acid labile and hygroscopic.[2]

Occasionally, salts may be also prepared to decrease drug substance solubility for use in suspension formulations where very low solubility is necessary to prevent "Ostwald ripening", for taste-masking, or to prepare an extended release product. Embonate salts have been used in suspension

Table 2.[a] **Preformulation studies that are normally considered for comparison of salt forms and parent compound for oral dosage forms**

test	suitable techniques	comments
dissociation constant and basic physico−chemical properties	potentiometry, solubility, UV spectroscopy	determine pK_a for parent drug
melting point	capillary m.pt., hot stage microscopy, differential scanning calorimetry	perform on each salt and compare to parent
aqueous solubility	overnight equilibration at 25 °C; analysis by UV spectroscopy or HPLC	Perform on each salt and compare to parent
pH of solution		Examine pH of saturated solution if quantities permit.
cosolvent solubility	overnight equilibration at 25 °C, analysis by UV spectroscopy or HPLC	Determine solubilities in ethanol, poly(ethylene glycol), propylene glycol and glycerol and compare to parent.
common ion effect on solubility	overnight equilibration at 25 °C in suitable media and analysis by UV spectroscopy or HPLC	compare solubility in demineralized water with 1.2% NaCl for salts and parent
hygroscopicity	use DVS apparatus or expose to various RH values and measure weight gain after 1 week	perform at 53, 93, and 97% RH, and other values of interest; assign hygroscopicity classification to each salt[13]
intrinsic dissolution rate	use Wood's apparatus[14]	compare dissolution rates at various pHs (can provide data on wettability)
crystal shape and appearance	SEM or optical microscopy	Compare crystal habits and levels of agglomeration
particle size	SEM and laser diffraction	Examine particle size distributions.
polymorphism/pseudopolymorphism	recrystallizations, HSM, DSC, TGA	preliminary exploration
powder properties	bulk density measurement	determine Carr's compressibility index
stability	various	perform on parent drug and undertake preliminary tests on appropriate salts

formulations to increase the duration of action (e.g., chlorpromazine embonate). On some occasions, the selection of a salt with only modest aqueous solubility may be more suitable for use in tablet products prepared by wet granulation since the use of highly soluble salts can be detrimental to the granulation process. Depending on the dose required, aqueous solubilities in the range 0.1−1.0 mg/mL will normally be sufficient to satisfy the dissolution requirements for standard, solid, oral dosage forms of drugs with good to moderate potency. However, for parenteral solution products, higher solubilities, perhaps 10 mg/mL or greater, depending on the required dose and dose volume, may be required. For parenteral formulations, the pH of solution (normally within an acceptable range of 3−10 for intravenous solution) should be monitored to help ensure that the formulation will be well tolerated.

Salts are also frequently prepared for the reasons other than solubility modification; it is frequently necessary to prepare a specific salt to either achieve adequate physical stability or for taste masking (e.g., dextropropoxyphene napsylate suspension). Manipulation of drug substance solubility by selection of salts may also be employed to modify the pharmacokinetic profile of the drug (e.g., benzathine penicillin and insulin zinc complexes used in parenteral formulations). Salt formation may be also advantageous where the melting point of the active moiety is low, and it is necessary to mill or micronise the active ingredient to achieve adequate homogeneity. A suitably stable salt may have a melting point that is 50−100 °C higher than the free acid or free base. Also, being more ionic, the crystals are likely to be less plastic and more easily deformed by brittle fracture.

Scale-up of the Formation of Salts

The information from the preliminary crystallisation studies is communicated to the Process Chemistry group, who by this time will have started their investigations into possible manufacturing routes for each of the candidates remaining. At this stage in the development process, Process Chemistry usually aim to quickly manufacture 50−200 g of the one or two candidates that may remain to progress them towards initial clinical evaluation. The manufacturing route may be the same as used by the Discovery Chemistry group but usually is significantly different. The aims of both the Process Chemistry and Preformulation groups for the following 12−18 months is to collaborate extensively to ensure that, for the chosen candidate, there will be a viable synthetic route to the chosen form of the drug substance.

A significant portion of this batch is destined for the preparation of 3−4 g of each of the salts that were thought to be viable from the smaller-scale studies. A similar sized portion of the free base/acid is also taken for comparison purposes. The combination of individual studies undertaken on each of these 3−4 g portions varies depending on the type(s) of dosage form ultimately required for marketing. Occasionally, it may be necessary to undertake a pharmacokinetic evaluation of each salt in comparison with the free acid/base. The dosage forms most commonly used for the drug substances encountered during preliminary clinical investigations are tablets/capsules, inhalation dosage forms and injections.

Table 3. **Tests to be considered for the evaluation of candidate salts**

test to be considered	amount required, mg
Structural Analysis	
mass spectroscopy[a]	1
^{1}H NMR[a]	5
^{13}C NMR[a]	25
Ir spectrum	1
UV spectrum	1
fluorescence spectrum[a]	1
elemental analysis	10
Physicochemical Properties	
melting range	2
pK_a[a]	5
C log *P*/log *P*[a]	5
preliminary polymorphism study	200–500
X-ray diffraction	20
aqueous solubility[b]	100
pH – solubility profile	500
cosolvent solubilities[c]	300
propellant solubility[d]	500
Physical Properties	
hygroscopicity	20
microscopy (SEM/optical)	10
particle size (Malvern)	100
size reduction (sonication)	300
Impurities (hplc)	
related substances[a]	10
degradation products[a]	10
chiral purity[a]	10
Stability Studies	
stability to hydrolysis (pH 2, 7, 10)[a]	15
stability to oxidation (peroxide/peracid)[a]	15
stability to photolysis[a]	15

[a] Determined on free acid/base only. [b] Would include solubility in saline, 5% dextrose and some buffers [c] Also solubilities in complexing agents/surfactant systems where appropriate [d] Propellants and propellant/cosolvent systems for inhalation dosage forms.

Tables 2 and 3 show the types of tests normally chosen, the information that they can produce and the amount of sample normally required for these common dosage forms.

What to Develop: Salt or Free Acid/Base?

The results obtained from each of these tests are tabulated for the free acid/base, together with each of the salts, and discussed in detail between the Formulation Scientists, Preformulation Analysts, Physical Chemists, Process Chemists, and occasionally Pharmacokineticists. The Preformulation Scientists assess the relative merits of each form for use in the proposed clinical formulations and whether the properties such as solubility are adequate to give the high concentrations required in the various pre-clinical formulations. Process Chemistry need to assess the likely yield of each salt, as salt formation creates an additional step in the manufacturing process. Usually, the decision-making process results in the proposal of a single salt for further study, although occasionally it is seen that none of the salts have optimum properties, and two different salts can be proposed for in-depth study. Also, it is occasionally found that the overall properties of the free acid/base are much better than any of the salts. This occurs more frequently where the candidate has a low pK_a value and the resulting salts are less stable than required or when the salts are particularly hygroscopic or when they exhibit complex polymorphism/pseudopolymorphism (hydration or solvation).

These relatively simple investigations give much useful information very quickly; it should be noted, however, that the preliminary polymorphism study is far from the in-depth study that is always undertaken later. This preliminary study uses a range of protic and aprotic solvents of widely differing polarity and will normally show the presence of a stable hydrate or solvate.

Once a decision is agreed upon within the group, a document that gives a précis of the discussions and the basis for the proposal is normally drafted for agreement by senior management. Examples of these salt selection studies are given below:

Example No. 1 (RPR 111423)

RPR 111423 is a candidate drug substance that has been evaluated for the treatment of symptoms related to infection by AIDS. It is a crystalline, very weak base with a pK_a at 4.25. A comprehensive screening of possible salts demonstrated only a monohydrochloride (RPR 111423A) and a mesylate (RPR 111423B) could be isolated as crystalline solids.

It was decided that the free base should be taken through the simple evaluation process in comparison with these two salts. It was expected that the drug substance could be required in the form of tablets or capsules, with an injection form needed for some pre-clinical studies and for the determination of absolute bioavailability in man. Because of its high activity in screening studies, there was a possibility that very low dose oral formulations might be needed. This may require micronised drug substance to enable content uniformity requirements to be met; this micronised material would also be expected to enhance dissolution.

The results from the relatively simple studies undertaken are given in Table 4. The two salts clearly demonstrated the predictable problems associated with a relatively low pK_a value; the salts were quite weak and dissociated to liberate the free base in media with pH values below the pK_a. The very low solubility of the free base resulted in immediate precipitation following dissociation. There was clear evidence for multiple polymorphism for each of the salts, and establishing the existence of a stable polymorph, or a suitable pseudopolymorph, may have been necessary before a decision could be made on which of the two salts could be developed further.

The corresponding results for the free base indicated that it appeared to be the better candidate; it showed no evidence of polymorphism, and it was not hygroscopic. The two major areas that required further investigation were whether it had sufficient solubility in gastrointestinal media and whether it could be micronised. Studies performed on samples of drug substance and on simple capsule formulations demonstrated that the dissolution rates of micronised free base were equivalent or superior to those of the salts under the same conditions.

Table 4. **Comparison of some simple properties of RPR111423 and its two salts**

test	result for RPR 111423 (base)		result for RPR 111423A (hydrochloride)		result for RPR 111423B (mesylate)	
appearance	off-white to cream, crystalline powder		pale yellow, highly agglomerated powder		cream to pale yellow, highly agglomerated powder	
particle size by microscopy, μm	10−100 (large rhombic crystals)		2 × 1 (microcrystalline laths)		7 × 1 (microcrystalline laths)	
melting range, °C	241−244		242		210	
preliminary polymorphism study	no other form detected		at least four polymorphs detected; metastable forms revert to original on standing		at least six polymorphs detected; phase changes detected on grinding or micronisation; reverts to original form on heating	
other thermal behavior	nothing detected		loss of HCl detected at 110−120 °C		nothing detected	
aqueous solubility, mg/mL	at 25 °C	at 37 °C	at 25 °C	at 37 °C	at 25 °C	at 37 °C
- at pH 1	11.6	14.7	25.7	28.2	131.4	204.1
- at pH 2	0.71	0.89	2.51	4.58	6.11	8.91
- at pH 4	0.03	0.05	0.05	0.13	0.01	0.02
- at pH 6	0.01	0.02	0.01	0.02	0.03	0.34
- at pH 6,8	0.01	0.02	0.01	0.02	0.01	0.02
- in demineralized water	0.01	0.02	0.36	0.99	0.33	0.50
pH of saturated solution, at 20 °C, in water	6.50		2.43		2.74	
addition of water to concentrate						
- at pH 2	no changes detected		some precipitation of free base		some precipitation of free base	
- at pH 4	no changes detected		extensive precipitation of free base		extensive precipitation of free base	
hygroscopicity (hygrostat for 14 days)	non-hygroscopic <0.2%w/w water uptake at any RH		slightly hygroscopic 2.3% w/w uptake at 53% RH 22% w/w uptake at 97% RH		moderately hygroscopic 3.7% w/w uptake at 53% RH 32% w/w uptake at 97% RH	

Example No. 2 (RPR 127963)

RPR 127963 is a candidate drug substance that has been evaluated for the treatment of cardiovascular diseases; it is a crystalline, very weak base with a pK_a at 4.10. In common with most similar drug substances intended for the treatment of cardiovascular disease, it was considered that a high-dose (up to 250 mg) solid, oral dosage form and a correspondingly high-dose (up to 50 mg/mL) injection would be ultimately required. In line with our standard protocol, a comprehensive evaluation of possible salts was undertaken, and this demonstrated that five crystalline salts (a hydrochloride, a mesylate, a citrate, a tartrate, and a sulphate) could be readily produced. It was decided to quickly profile each of these salts in comparison with the free base. The results of these studies are given in Table 5.

When the anhydrous free base was evaluated, the existence of an additional mono-, di-, and trihydrate was found quite rapidly. It was shown that all four of these forms could be interconverted under conditions that might be expected to be found in granulation processing. The other potential problem with the anhydrate was the low melting point. In considering the results obtained for the various salts, the solubilities of the citrate and the tartrate were much lower than required for an injectable form and lower than ideal for high dosage formulations. An additional problem for the tartrate salt was the high hygroscopicity. Both of these salts were rejected before completion of the full evaluation. The hydrochloride salt was also shown to have several problems such as lower than ideal solubility, probable multiple polymorphism, and the formation of hydrates.

Thus, the mesylate and the sulphate were the two salts that remained; both had high melting points, excellent aqueous solubility, and were non-hygroscopic. The free base still remained a possible candidate, if a stable hydrate could be found. It was therefore decided to undertake some additional evaluations on these three forms; the results from these are presented in Table 6.

These additional results demonstrate a slight advantage in favour of the sulphate salt because of its greater solubility in cosolvents. This would give the formulator a better chance of achieving a higher dose in an injectable formulation. It was considered that the sulphate salt (RPR 127963E) could be studied further in the more detailed evaluations that would follow over the next few months. The mesylate or the free base (if a suitably stable hydrate could be found) would provide a possible back-up, should unforeseen problems arise.

Example No. 3 (RPR 200765)

RPR200765 is a candidate drug substance proposed for the treatment of rheumatoid arthritis. It is another crystalline, weak base with a pK_a of 5.3 which formed salts with a wide selection of counterions. It was expected that doses of 100−125 mg of RPR200765 in capsules would be required for clinical studies.

Early studies suggested that RPR200765 free base was unacceptable for use in solid, oral dosage forms due to a very poor aqueous solubility of approximately 10 μg/mL and poor bioavailability in animal models. However, RPR200765 would form stable salts with hydrochloride, hydrobromide,

Table 5. **Comparison of some simple properties of RPR127963 and its five salts**

test	result for free base (RPR 127963)	result for HCl salt (RPR 127963A)	result for mesylate salt (RPR 127963B)	result for citrate salt (RPR 127963C)	result for tartrate salt (RPR 127963D)	result for sulfate salt (RPR 127963E)
appearance	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder	yellow, crystalline powder
particle size (microscopy), μm	1−3 μm (agglomerates of microcrystals)	1−3 μm (agglomerates of microcrystals)	tightly packed spherulites of agglomerated microcrystals 18 μm diameter.	microcrystals (2−3 μm) with some aggregates (70 μm)	rounded agglomerates of microcrystals in domains (70 μm)	aggregates of microcrystals (10−15 μm)
melting range, °C	119−123	166−191 (re-grows at about 166, recrystallizes at 191, then melts at about 275	280.9−282.2	130.2−134.3	198.5−201.6	305.7−308.9
preliminary polymorphism study	several hydrates detected	two monohydrates and one anhydrate	no evidence of polymorphs	stable hemihydrate detected	unstable anhydrate	no evidence of polymorphs
aqueous solubility (25 °C), mg/mL						
- in demineralized water	n.d.[a]	3.92	108	0.83	0.89	~50
- in 0.1 M HCl	n.d.	5.2	50.4	n.d.	n.d.	5.9
- in 0.1 M NaOH	0.020	0.019	0.022	n.d.	n.d.	0.018
- in dextrose 5%w/v	n.d.	2.84	90	n.d.	n.d.	~40
pH of saturated solution	n.d.	2.33	1.76	2.49	2.56	1.32
hygroscopicity	n.d.	non-hygroscopic	non-hygroscopic	non-hygroscopic	very hygroscopic	non-hygroscopic

[a] n.d. = Not determined.

Table 6. **Comparison of additional properties of RPR127963 (anhydrate), its mesylate (RPR 127963B) and sulfate (RPR 12963E) salts**

test	result for free base anhydrate (RPR 127963)	result for mesylate salt (RPR 127963)	result for sulfate salt (RPR 127963)
solubility in cosolvents at 25 °C, mg/mL			
ethanol	190	0.6	0.2
propylene glycol	35.4	0.7	1.7
poly(ethylene glycol) 400	188	0.2	0.2
dimethylsulphoxide	> 500	14	110
N-methylpyrrolidone	> 400	4.4	8.5
glycerol	42	n.d.[a]	2.7
peanut oil	0.18	none detected	none detected
intrinsic dissolution rate, mg·min^{-1}·cm^{-2}			
- in water	0.01	n.d.	n.d.
- in 0.01 M HCl	0.35	7.3	7.7
powder flow properties	n.d.	Good, but becomes much worse with increasing humidity	Sticks slightly

[a] n.d. = Not determined.

methanesulfonate, and camphorsulfonate counterions. Aqueous solubility, particle size and shape, powder properties, and polymorphism profile were considered to be the key properties to permit a choice of salt to be made. In addition, it was recognised that the use of some counterions with high molecular weights, would require a large excess of drug substance to achieve the required doses.

Studies demonstrated that the solubility of RPR200765 depended on the amount of drug substance used for the study. This occurred because the counterion reduced the pH of solution and enhanced solubility of the drug base. The mesylate salt consistently produced a higher solubility than any of the other salt forms. The higher solubility resulted in an enhanced dissolution rate of the mesylate salt compared to the other salt forms. The solubility and dissolution rate of the hydrobromide salt was particularly poor. Intrinsic dissolution rate studies on compressed disks could not be carried out because a good compact could not be obtained for most of the salts, and the studies were carried out using drug powder (equivalent to 50 mg free base) in capsules.

Hygroscopicity studies demonstrated that the hydrochloride and hydrobromide salts adsorbed large amounts of moisture on exposure to humidity, resulting in the formation of multiple hydrated forms. The methanesulfonate salt however, was a stable monohydrate form which lost moisture at very low humidity (<10% relative humidity (RH)) but

Table 7. **Comparison of the physicochemical properties of RPR200765 salt forms**

test	result for mesylate salt (RPR 200765A)	result for camphorsulfonate salt (RPR 200765C)	result for hydrochloride salt (RPR 200765D)	result for hydrobromide (RPR200765E)
appearance	off-white to cream, free-flowing powder	white to off-white, crystalline, free-flowing powder	white, free-flowing powder	white to off-white, crystalline, free-flowing powder
MW	566.61	684.79	524.98	569.43
melting range, °C	214	265−267	245−248	276−277
maximum aqueous solubility at 25 °C, mg/mL	39	19.95	16.68	3.29
pH of saturated solution in demineralized water at 20 °C, mg/mL	1.93	2.22	2.16	2.63
hygroscopicity (by DVS)	non-hygroscopic with a stable, monohydrate form	non-hygroscopic	hygroscopic with multiple hydrated forms	hygroscopic with multiple hydrated forms
crystal habit and appearance	individual platelike crystals with some agglomeration.	clusters of highly aggregated, platelike crystals	platelike crystals; individual crystals contain stress lines	loosely agglomerated, flaky material
particle size by microscopy	~45−200 μm in agglomerates of 200−350 μm	crystals ~20−50 μm, clusters ~80−200μm -some larger clusters up to 500 μm	~30−100 μm particles	10−40 μm particles
dissolution studies, drug substance in capsule ($T_{80\%}$, min)				
at pH 2				
at pH 4 (in citrate buffer)	2.0	6.0	7.4	3.9
	>60% release	>60% release	>60% release	14% release

rapidly re-equilibrated to form the monohydrate form when the humidity was raised. These findings suggested that this salt would be amenable to solid dose formulation and there was little risk of changes in the hydration state on processing or storage under normal conditions. The camphorsulfonate was non-hygroscopic. The results of these studies are outlined in Table 7; in this case very little comparative work was undertaken on the free base due to the poor solubility and bioavailability.

Overall, the studies suggested that the mesylate salt was the favoured form on the basis of its low hygroscopicity, clean polymorphic profile in the preliminary screen, high solubility, and rapid dissolution rates. Another favourable factor supporting the selection of the mesylate salt proved to be the good flow properties which allowed very satisfactory capsule and tablet formulations to be developed.

The Next Steps?

Having evaluated several possible alternatives in the three cases above, using a relatively simple range of tests, a form of the drug substance has been chosen that should be possible to develop further. These simple studies have required 3−4 g of the free base and a similar quantity of each of the salts; the data for all forms normally can be generated in one month, or less. The next steps involve confirmation of the choice, by employing a further range of tests, followed by the optimisation of the form of the salt. A series of tests, analogous to those in Table 2, are used in this evaluation; these tests are given in Table 8.

Optimisation of the Drug Substance Form for Development

Having chosen what should be a reasonably stable form of the salt, free acid, or free base, one of the key activities is to start investigations into which other polymorphic or pseudopolymorphic forms exist. In the short development phase where preclinical administration occurs, only a preliminary screening of these different forms is considered necessary, as it is possible that the compound can be found too toxic for further study. Our team undertakes this on about 500 mg of sample; small portions are recrystallised from anhydrous and hydrated solvents of differing polarity. Any crystalline product recovered is examined by a variety of techniques to determine how many different forms are produced and whether any are hydrates or solvates. Preliminary information on the inter-relationships between the different forms can often be found, even at this early stage.

The remainder of the tests are designed for two main purposes:

To define the various preclinical formulations that are required, to devise analytical methods for these, to determine their stability, and establish shelf lives.

To establish a database for the chosen form and to give an indication of the possibilities for clinical formulations.

To accomplish this, it is normal to request a minimum of 25 g of drug substance, although occasionally more may needed if the drug substance is intended for inhalation and there are difficulties with micronisation.

Table 8. **Tests to be considered for "preclinical phase" (column 2) and in preparation for initial clinical investigation (column 3) for compounds intended for use in oral, injection and inhalation products**

test to be considered	amount required, mg or g	amount required, mg or g
	Physicochemical Properties	
melting range	50 mg	50 mg
optical rotation		1 g
polymorphism	500 mg	25−50 g
X- ray diffraction	20 mg	20 mg
intrinsic aqueous solubility	400 mg	
cosolvent solubilities[a]	500 mg	2 g
propellant solubility[b]		2 g
	Physical Properties	
hygroscopicity	800 mg	-
microscopy (SEM/optical)	100 mg	100 mg
particle size (Laser)	200 mg	200 mg
micronisation	5 g	
specific surface area	2 g (R)	4 g (R)
true density	200 mg (R)	200 mg (R)
bulk powder density	2.5 g (R)	10 g (R)
wettability		1 g
	Impurities (HPLC)	
related substances	10 mg	10 mg
degradation products	10 mg	10 mg
chiral purity	10 mg	10 mg
electrophoresis/TLC	10 mg	
	Stability Studies	
hydrolytic profile (identify degradants)	100 mg	
bulk drug powder		2 g
	Excipient Compatibility	
HPLC, XRPD, and DSC	50 mg	250 mg
	Compression Properties	
for dry powder inhaler only		5 g
	Preclinical Formulation Development	
intra-tracheal suspensions	2 g	
oral solutions/suspensions	2 g	
solutions for nebulization	2 g	
IV solutions	2 g	
other routes (ip/sc)	1 g	-
	Clinical Formulation Development	
	predict suitable dosage forms	Phase I−IIa[c] 250−1200 g
	3 g	
microbiological controls		*d*
total substance requirements	20−25 g	depends on form and dose

[a] Also solubilities in complexing agents/surfactant systems where appropriate. [b] Propellants and propellant/cosolvent systems for inhalation dosage forms. [c] Develop and specify Phase I formulation − commence stability/compatibility studies. [d] Dependent on drug availability; (R) possible to recycle drug substance for certain other tests.

Once the drug substance is shown to be nontoxic, studies leading to the definition of a suitable series of clinical formulations can begin. For this, we normally expect to have a reasonably clear picture of the inter-relationships between the different forms of the drug substance and should have started to define the most stable form. As more batches are manufactured at increasing scale by Process Chemistry, they are examined using some of the key tests to add information to the database. Also, with the increased availability of drug substance, it is possible to initiate maturation studies as an additional technique to assist in the definition of polymorphism. If the structure of the drug substance has been determined by single crystal X-ray, under certain circumstances it may be possible at this stage to initiate the theoretical search for other polymorphic forms. This is achieved using the Polymorph Predictor software (Molecular Simulations Inc.). This software has been used successfully on several small molecules (molecular weight <500) and predicts theoretical crystal structures and their relative energies. The most stable form has the lowest energy; increasing energy signifies lower stabilities.

As larger quantities of drug substance and samples from different batches become available it is imperative that the variation in basic physical properties (e.g., crystal size and shape, specific surface area, powder flow properties, bulk and tapped density etc.) are studied for each batch. By close liaison with Process Chemistry, it is normally possible to modify the recrystallisation conditions such that greater batch-to-batch uniformity of these physical characteristics can be achieved. Also, these characteristics can often be modified such that they are closer to ideal.

The Negative Aspects of Salt Formation

One of the negative aspects of salt formation is that the percentage active content decreases markedly as higher-molecular weight counterions are used. If the free acid or base has only moderate or low activity, it may be necessary for the patient to have a relatively high dose for a clinical effect. If 20−50% of the weight of the drug substance is due to inactive counterion, the addition of suitable excipients for encapsulation or tableting may result in a powder volume that is too great, even after granulation, to fit successfully into even the largest acceptable capsule shell. This forces the formulator towards a tablet. Even with these formulations, a large tablet (or even multiple, smaller tablets) may be necessary; these do not aid patient compliance.

Other problems that are frequently created, or exacerbated, by salt formation are an increased tendency for the existence or formation of hydrates and polymorphs. Hydrates may be produced in formulations by interaction with water bound to excipients, water in capsule shells, etc.

Final Definition of the Form

As the candidate passes through initial clinical evaluation (Phases Ia and Ib), additional characterisation and refinement of the drug substance form continues in parallel with the finalisation of studies on the drug substance manufacturing process. Close liaison and teamwork between the Process Chemist and the Preformulation Scientist in an exploration of the various possible recrystallisation solvents can often result in further refinement of the crystal properties. An excellent scheme for the final characterisation of solid drug substances, prior to the final regulatory submission, has been described recently.[12] The aim of both the Preformulation and Process Chemistry teams is to finalise the definition of all of the characteristics of the drug substance in readiness for the initiation of Phase IIa clinical trials

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EXHIBIT R

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P. Heinrich Stahl, Camille G. Wermuth (Eds.)

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Chapter 6

Salt-Selection Strategies

by **Abu T. M. Serajuddin*** and **Madhu Pudipeddi**

Contents

1. Introduction

Because of the introduction of combinatorial chemistry and high-throughput screening (HTS) during the past ten years, the pharmaceutical in-

dustry is going through a revolutionary change in the way it has been discovering and developing drugs [1]. Larger, more lipophilic, and less water-soluble leads are being selected as a result of the quest for more potent and highly specific molecules. The widespread use of dimethyl sulfoxide (DMSO) in HTS also favors the selection of lipophilic, water-insoluble compounds, which are easily solubilized in this solvent. Since some of the attributes of newer drug molecules are unfavorable to their development as dosage forms, the 'developability' is becoming a critical consideration for the transition of a chemical entity from the discovery phase to the development phase [2] [3]. There is now a greater collaboration between discovery and development scientists in evaluating such developability criteria as solubility, dissolution rate, stability, permeability, and so forth, for the selection of optimal-development candidates. Since, as mentioned in *Chapt. 2*, salt formation can improve solubility and dissolution rate of basic and acidic drugs, thus increasing their absorption rate and bioavailability, we will present in this chapter various strategies for the selection of optimal salt forms for new drug candidates. The physicochemical principles to be described in this chapter will also be helpful in identifying acidic or basic drug candidates that can form more developable salts.

The salt selection should be viewed as a part of the overall objective of selecting the 'optimal form' of a drug candidate for development. When one refers to the optimal form, it involves both chemical and physical forms. A new chemical entity can be an acid, a base, or a neutral species. If it is a neutral species, there are no options for chemical manipulation to make it more developable other than possibly preparing prodrugs. On the other hand, if it is an acid or a base, one can select the free acid or base form, or, alternatively, one can select a salt form. In the selection of free *vs.* salt form, questions that need to be answered are: Is the acid or base form preferred because of biopharmaceutical considerations? Is the salt form more suitable? Is the preparation of stable salt forms feasible? Among various potential salt forms of a particular drug candidate, which has the most desirable physicochemical and biopharmaceutical properties?

Along with the evaluation of chemical form, the strategy for the selection of physical form must also be considered. One needs to determine whether the compound exists in crystalline or amorphous form, and, if crystalline, whether it exhibits polymorphism. One also needs to investigate: Does the compound exist in hydrate or solvate form? If so, how is such a form affected by temperature and moisture? How stable is a particular form in solid state and in solution? The ultimate selection of the 'optimal form' of a new drug candidate for development depends on a balance among the physicochemical properties of its various available chemical and physical forms.

Another critical element of a salt-selection process in any drug-development program is the timing. Here, the critical questions are: When does one start salt selection? Should a new drug candidate be selected after consideration of its feasibility for salt formation? Or should any such consideration be postponed, until the new candidate has been selected and forwarded to the development stage? How can the salt selection be integrated in the development process such that it does not become a rate-limiting step or does not extend development time?

The success of a salt-selection program also depends on how various disciplines within drug discovery and development interact and collaborate. The composition of a salt-selection team and the responsibilities of individual team members may have profound effects on time and resources spent on a salt-selection program.

Based on the above considerations, salt-selection strategies for new drug candidates may have the following components:

i) selection of chemical forms of salts,
ii) selection of physical forms of salts,
iii) salt-selection timing,
iv) composition of salt-selection team

In the present chapter, strategies for the selection of chemical forms of salts will be described in detail. Strategies for the selection of physical forms will be discussed in less detail, since *Chapt. 3* and *7* will also cover several aspects of these strategies. Salt-selection timing and composition of salt-selection teams will be discussed only briefly, since no clear picture of how these are practiced in various drug companies has emerged yet.

2. Selection of Chemical Forms of Salts

At the outset of any salt-selection program, it is important to determine whether a particular acid or base is amenable to salt formation. If the salt formation appears to be feasible, the question then arises is which one of the many available counter-ions would be most suitable for the purpose. Some of these issues will be addressed in this section.

2.1. Feasibility Assessment for Salt Formation

No predictive procedure to determine whether a particular acidic or basic drug would form a salt with a particular counter-ion has been reported in the literature. *Anderson* and *Flora* [4] reported that successful salt formation gen-

erally requires that the pK_a value of a conjugate acid should be smaller than the pK_a value of the conjugate base to ensure sufficient proton transfer from the acidic to the basic species. Thus, relatively stronger acids like HBr, HCl, H_2SO_4, or one of the sulfonic acids ($pK_a < 2.0$) would be suitable for the preparation of salts of weakly basic amines having $pK_a < 4$. Other investigators also provided similar but rather general guidelines for the selection of counter-ions. *Wells* [5], and also *Tong* and *Whitesell* [6] recommended that, for the preparation of salt forms of a basic drug, the pK_a of the acid used should be at least 2 pH units lower than the pK_a of the drug. Although these are valuable guidelines, a more predictive method for assessing the feasibility of salt formation would be necessary to minimize trials and errors in a salt-selection program.

As described in *Chapt. 2*, the pH–solubility interrelationship and the location of pH_{max} in the pH scale play critical roles in determining which salt, if any, can be synthesized for a particular free acid or base. *Dittert et al.* [7] reported as early as in 1964, although not for the specific purpose of salt selection, that whether a basic drug would exist as the free base or as a salt under certain pH conditions can be determined by studying its solubility *vs.* pH relationship. Later, *Kramer* and *Flynn* [8] demonstrated that the pH–solubility relationship of a basic drug could be expressed by two independent curves, and the point where the two curves intersected was the pH_{max}, the pH of maximum solubility. This is shown in *Fig. 1*, and the relevant equations are given below:

$$\text{At } pH > pH_{max}: \quad S_T = [BH^+] + [B]_s = [B]_s \cdot (1 + [H_3O^+]/K_a) \tag{1}$$

$$\text{At } pH < pH_{max}: \quad S_T = [BH^+]_s + [B] = [BH^+]_s \cdot (1 + K_a/[H_3O^+]) \tag{2}$$

In both *Eqns. 1* and *2*, S_T is the total or equilibrium solubility under a particular pH condition, [B] and $[BH^+]$ are concentrations of free and protonated species of the base, respectively, and the subscript s represents the concentration in equilibrium with the solid phase. *Fig. 1* essentially illustrates that a salt would not be formed in an aqueous medium, unless the pH of the saturated solution of a basic drug is not lowered below the pH_{max}, and any salt formed would be reconverted to its free base form, if the pH of a saturated salt solution is raised above the pH_{max}. In other words, solid phases that remain in equilibrium with solutions at pH below and above pH_{max} are a salt and the free base, respectively.

Similar pH–solubility relationship also exists for acidic drugs [9] [10]. As illustrated in *Fig. 2*, for a monoprotic acid, the free acid would be the equilibrium species at a pH below the pH_{max}, and a salt would be formed only if the pH is raised above the pH_{max} by using suitable counter-ions. The relevant

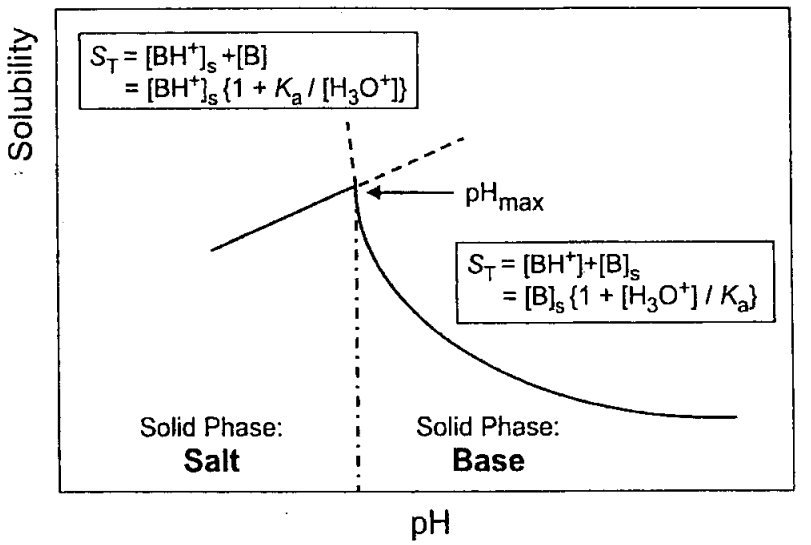


Fig. 1. *Schematic representation of the pH–solubility profile of a monobasic compound, showing that solubilities of base and salt can be expressed by two independent curves corresponding to two independent equations.* The point where the two curves intersect is the pH_{max}.

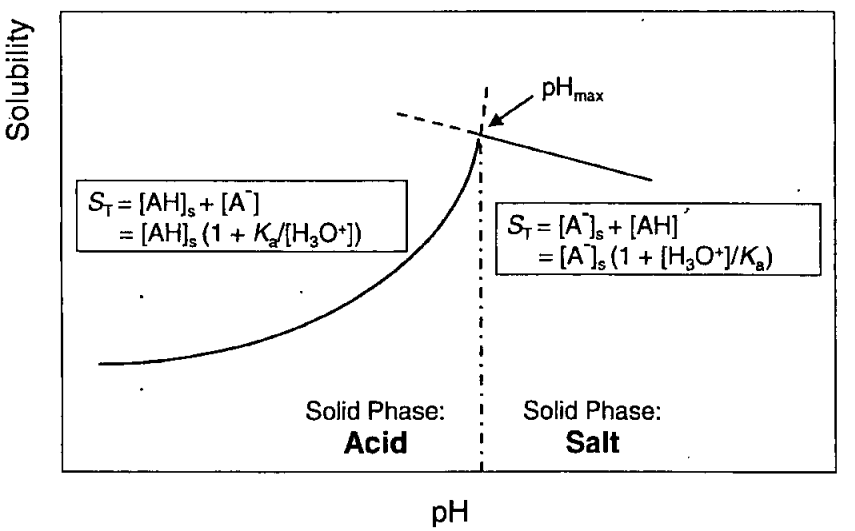


Fig. 2. *pH–Solubility profile analogous to* Fig. 1 *of a monoprotic acid*

equations are given below:

$$\text{At } pH < pH_{max}: \quad S_T = [AH]_s + [A^-] = [AH]_s \cdot (1 + K_a/[H_3O^+]) \tag{3}$$

$$\text{At } pH > pH_{max}: \quad S_T = [A^-]_s + [AH] = [A^-]_s \cdot (1 + [H_3O^+]/K_a) \tag{4}$$

In *Eqns. 3* and *4*, S_T is again the total solubility under a particular pH condition, [AH] and [A^-] are concentrations of free and ionized species of the acid, respectively, and the subscript s represents the concentration in equilibrium with the solid phase.

Solubilities of salts, as described by *Eqns. 2* and *4*, can be influenced by excess counter-ions present in solution. However, counter-ions influence solubilities through solubility products only after salts are formed and, therefore, might not adversely affect the feasibility of salt formation. The issue of solubility product on the salt-selection strategy will be discussed in a later section of this chapter.

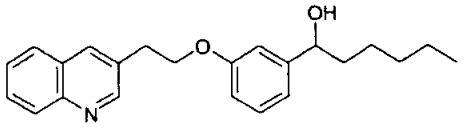
Serajuddin and co-workers [10–14], and numerous other authors [8] [9] [15–18] confirmed the application of the above-mentioned pH–solubility relationships in determining under which pH conditions salts of particular acidic and basic drugs can be formed.

2.2. Application of pH–Solubility Relationship: Case Histories

The application of pH–solubility relationships in determining the feasibility of salt formation can be explained by a few case histories.

2.2.1. *Case History 1: REV5901*

To determine the feasibility of salt formation for REV-5901 (*Fig. 3*), a base with the pK_a value 3.7, *Serajuddin et al.* [14] determined its pH–solubility profile as shown in *Fig. 4*. An identical profile was obtained, when either the free base or the hydrochloride salt was used as the starting solid phase. The pH_{max} of the compound was 1, indicating, as mentioned above, a salt form would exist only at pH below 1.0. Indeed, only two salts, a hydrochloride salt and a sulfate salt, could be prepared for REV-5901, since only strong acids like HCl and H_2SO_4 could lower the pH of a saturated solution below the pH_{max} of 1. A salt formation with relatively weaker acids like phosphoric acid, acetic acid, lactic acid, tartaric acid, and so forth, would not be feasible, since such acids would be unable to lower the pH below 1.0. Thus, just from the pH–solubility relationship, one can narrow down the type and the number of salts that can be prepared, saving much efforts and resources that could otherwise be wasted in attempting to synthesize many different salt forms. Based on *Fig. 4*, one would even question the suitability of hydrochloride and sulfate salts for development, because such salts would be converted to the free base form, when the microenvironmental pH in presence of moisture rises above 1.0. It was, indeed, observed that both of these salts



$pK_a = 3.6$
$S_0 = 0.002$ mg/ml

Fig. 3. *Chemical structure of REV5901, the compound used in* Case History 1

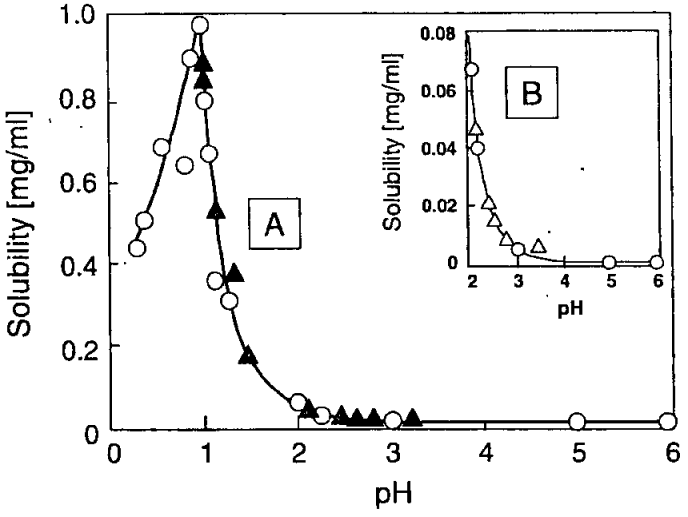


Fig. 4. *pH–Solubility profile of REV5901* (A), *where triangles represent the solubility obtained with the free base and circles the data obtained with the hydrochloride salt. In the insert* (B), *the solubility is shown on an expanded scale.* Either HCl or NaOH was used to adjust pH.

did not have acceptable properties for development, and the free base form of REV-5901 was ultimately selected.

2.2.2. *Case History 2: GW1818*

Tong and *Whitesell* [6] studied the feasibility of salt formation of a basic drug GW1818, which had the pK_a value of 8.0 and the intrinsic free base solubility of 0.0044 mg/ml. For this compound, the pH_{max} was *ca.* 5, and, as a result, the formation of stable salts with both strong and weak counter-ions, such as hydrochloride, methanesulfonate, phosphate, and succinate, was feasible. This is because all of these counter-ions could lower the pH below 5.

2.2.3. *Case History 3: Phenytoin*

The feasibility of salt formation for an acidic drug can be illustrated by the pH–solubility profile of phenytoin (*Fig. 5*), a compound with a pK_a value of 8.4 and the intrinsic free acid solubility of 0.02 mg/ml at 37 °C [19]. The sodium salt is the commercially available salt form for phenytoin, and there are numerous reports in the literature demonstrating that the free acid form of phenytoin precipitate out of salt solutions depending on pH. There is also the propensity for the conversion of salt to free acid in solid dosage form. It is apparent from *Fig. 5* that the salt formation for phenytoin is feasible only with strong alkalis like NaOH because it can raise the pH above the pH_{max} value of 11. Since relatively weaker bases like $Mg(OH)_2$, $Ca(OH)_2$, *etc.*, and the commonly used amine bases like arginine, lysine, *etc.*, would not raise the pH of an aqueous solution above 11, they will not form salts with phenytoin. *Fig. 5* also indicates that any salt formed would be converted to the free acid if the microenvironmental pH were below 11. If, unlike phenytoin, the pH_{max} of an acid were, for example, around 8, there would be a much better option for salt formation, because the pH could be raised above 8 by using a larger selection of alkalis and bases.

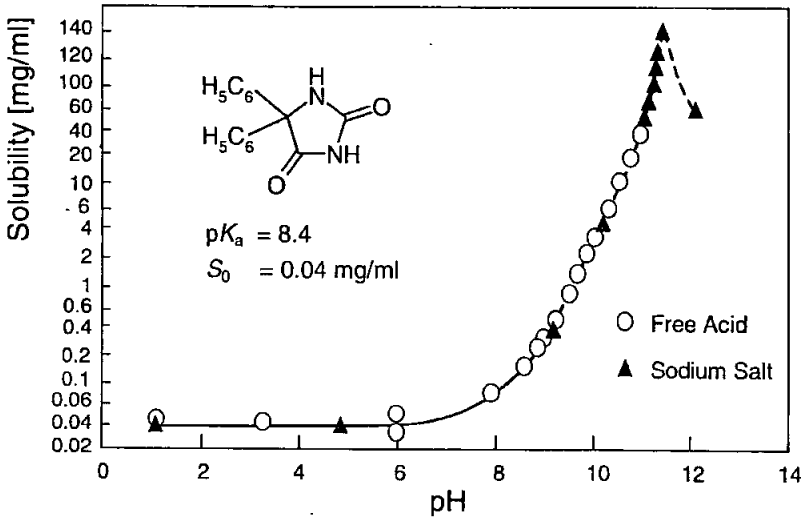


Fig. 5. *pH–Solubility profile of phenytoin at 37 °C indicating pH_{max} at 11.* Identical profiles were obtained when either free acid or the sodium salt of the drug substance was used. pH was adjusted using either NaOH or HCl.

2.3. Theoretical Modeling of pH–Solubility Relationship

In the case histories mentioned above, pH–solubility profiles, which were determined experimentally, have been used to identify the pH_{max} and to determine the feasibility of salt formation. However, at drug discovery and early development stages, when the supply of drug substances is limited, it might not be practical to determine pH–solubility profiles experimentally. In such a situation, the pH–solubility relationships corresponding to *Eqns. 1* and *3* for basic and acidic drugs, respectively, can be generated theoretically, if pK_a and S_0 values are available. Then, an estimate of pH_{max} values can be made by assuming certain values for salt solubilities.

Fig. 6 shows pH–solubility profiles of a basic compound generated theoretically according to *Eqn. 1* by using a fixed pK_a value of 8.0 and various S_0 values ranging from 0.0001 to 10 mg/ml. In this case, solubilities of salt forms corresponding to various theoretical curves are unknown. Any particular value for the salt solubility can be assumed for the purpose of estimating pH_{max} values. If, for example, a salt solubility of 20 mg/ml is assumed corresponding to each curve in *Fig. 5*, the estimated pH_{max} values corresponding to S_0 values of 0.0001, 0.001, 0.01, 0.1, 1, and 10 mg/ml would be 3, 4, 5, 6, 7, and 8, respectively. The pH_{max} values would not differ much even if the solubility of salt form somewhat differs, because, as mentioned in *Chapt. 2*, for a ten-fold difference in salt solubility, the pH_{max} differs by one unit only. Thus, from the theoretical analysis of pH–solubility relationships in *Fig. 6*, it may be concluded that the salt formation of a base with the pK_a value of 8.0 might be feasible with most commonly used acids when S_0 val-

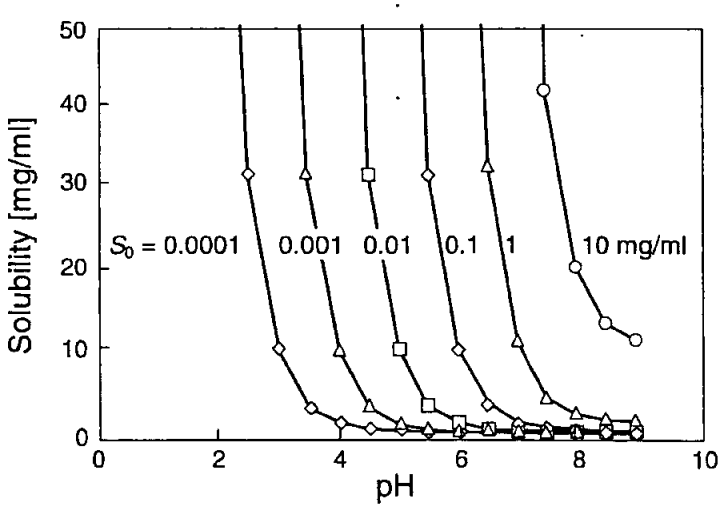
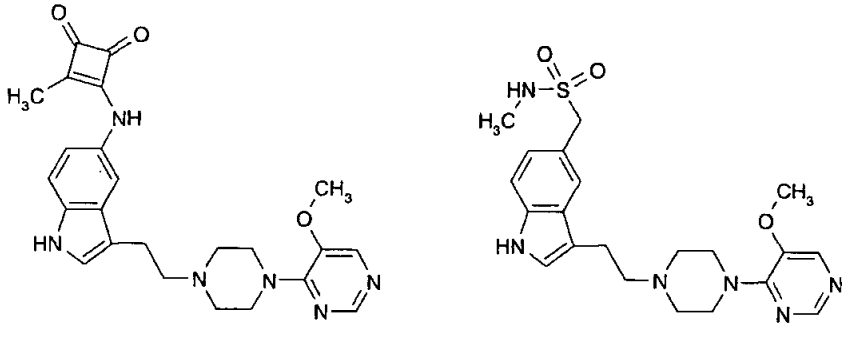


Fig. 6. *Theoretical pH–solubility profiles demonstrating the effect of intrinsic solubility (S_0) ranging from 0.0001 to 10 mg/ml of a basic drug with the $pK_a = 8.0$*

ues are *ca.* 0.01 mg/ml and higher. This is because pH_{max} values in these cases would be around 5 and higher. For S_0 values of 0.001 and 0.0001 mg/ml, however, relatively stronger acids would be required to form salts because pH_{max} values would be *ca.* 4 and *ca.* 3, respectively.

The theoretical analysis in *Fig. 6* will change if the pK_a value of a basic drug is lower. As shown in *Chapt. 2*, there is a direct relationship between pK_a and pH_{max}; the pH_{max} decreases by 1 for each unit decrease in the pK_a value. Thus, if the pK_a value in *Fig. 6* would be 4.0 instead of 8.0, the pH_{max} would be 3 and lower for S_0 values of 1 mg/ml and lower. In such a situation, the possibility of salt formation becomes limited, because only relatively stronger acids like HCl, methanesulfonic acid, ethanesulfonic acid, *etc.*, can lower the pH of saturated solutions below 3. The salt formation may not at all be feasible if the S_0 is below 0.01 mg/ml because the pH_{max} in this case would be less than 1.

A confirmation of the validity of above theoretical analysis may be obtained from the work of *Lakkaraju et al.* [20], where the authors studied pH–solubility relationships of two structurally similar compounds, avitriptan and BMS-181885 (*Fig. 7*). The compounds were dibasic in nature, each of them with pK_a values of 8.0 and 3.6. However, the S_0 values of the compounds differed; they were 0.006 and 0.0007 mg/ml for avitriptan and BMS-181885, respectively. Because of this difference in S_0 values, the pH_{max} values, due to the effect of the higher pK_a (8.0), were *ca.* 5 for avitriptan and *ca.* 4 for BMS-181885. As a consequence, salts with many different counterions, including acetate, lactate, succinate, and tartrate, could be synthesized for avitriptan. But, with BMS-181885, it was not possible to lower the pH of a saturated solution below 4 by using acetic acid, lactic acid, succinic acid,



Avitriptan
$pK_{a,1} = 8.0$; $pK_{a,2} = 3.6$
$S_0 = 0.006$ mg/ml

BMS-181885
$pK_{a,1} = 8.0$; $pK_{a,2} = 3.6$
$S_0 = 0.0007$ mg/ml

Fig. 7. *Chemical structures of avitriptan and BMS-181885*

or tartaric acid, and, therefore, the salt formation with any of these counter-ions was not feasible for this compound. However, BMS-181885 formed salts with stronger acids like H_3PO_4 and HCl.

It should be noted here that self-association of drug molecules in solutions may sometimes lead to deviations in pH–solubility profiles predicted from pK_a and solubility. Nevertheless, the theoretical modeling can still serve as a useful method of predicting the feasibility of salt formation because a pH_{max} value may be estimated within a reasonable range. Also, the self-association often shifts pH_{max} in favor of salt formation.

2.4. Feasibility of Disalt Formation

Eqns. 1–4 are applicable to compounds with only one pK_a value, and, therefore, the discussion in this chapter has so far focused primarily around the feasibility of salt formation for bases with one protonatable moiety and acids with one ionizable species. Such compounds can form only mono-salts (*e.g.*, mono-hydrochloride, mono-sodium, *etc.*). In addition, a compound may have both basic and acidic moieties. Such a compound can also be classified as one forming a mono-salt, because only one of these groups can be used at one time for salt formation. In contrast, drugs can also be polybasic or polyprotic, which might be able to form poly-salts. Examples of disalts, such as dihydrochloride, disodium, *etc.*, are common in the literature. Some of the questions that arise for compounds with multiple basic moieties or multiple acidic moieties are: Should mono- or poly-salt be synthesized for such compounds? Is the formation of poly-salt feasible? If the synthesis of both forms of salts is feasible, which one is preferred for a particular drug candidate? Some of these issues are addressed below.

2.4.1. *Feasibility of Salt Formation for Dibasic Compounds*

Serajuddin and co-workers [20] [21] have demonstrated that the feasibility of salt formation for a dibasic compound can also be predicted from its pH–solubility relationship. As illustrated schematically in *Fig. 8*, the solubility of a free base increases with a decrease in pH, and, after the first pH_{max} (or $pH_{max,\,1}$) is reached, a mono-salt might be formed. The solubility of the mono-salt formed then increases because of the protonation of the second basic moiety, thus reaching $pH_{max,\,2}$. Below $pH_{max,\,2}$, a disalt could be formed. Depending on pH and counter-ions used to prepare salts, there could be three distinct solid phases (free base, mono-salt, and disalt) in equilibrium with aqueous solutions. The equations corresponding to solubilities of these three

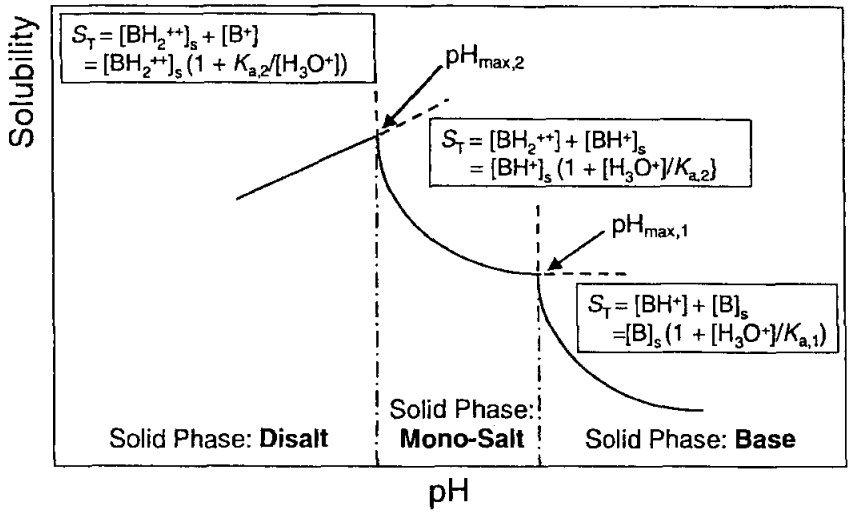


Fig. 8. *A schematic representation of the pH–solubility profile of a dibasic compound, showing that solubilities of the base and its mono- and disalt forms can be expressed by three independent curves corresponding to three independent equations.* The profile indicates that two pH_{max} values, $pH_{max,1}$ and $pH_{max,2}$, may exist for such a compound.

phases are given below:

At $pH > pH_{max,1}$: $$S_T = [BH^+] + [B]_s = [B]_s \cdot (1 + [H_3O^+]/K_{a,1}) \quad (5)$$

At $pH < pH_{max,1}$ and $> pH_{max,2}$: $$S_T = [BH_2^{++}] + [BH^+]_s = [BH^+]_s \cdot (1 + [H_3O^+]/K_{a,2}) \quad (6)$$

At $pH < pH_{max,2}$: $$S_T = [BH_2^{++}]_s + [B^+] = [BH_2^{++}]_s \cdot (1 + K_{a,2}/[H_3O^+]) \quad (7)$$

For the sake of simplicity, no consideration of the common-ion effect and the solubility product was made in deriving the above equations. It should also be mentioned here that distinct regions in the pH–solubility profile corresponding to mono- and disalt forms may not be obtained if pK_a and/or pH_{max} values of the compound are not far apart (*ca.* 2 units). If two pH_{max} values are indistinguishable, only the disalt may be isolated in pure form.

Avitriptan (*Fig. 7*), a dibasic compound, was used as the test compound for salt formation. As shown in *Fig. 9*, protonation of the piperazine N-atom and the pyrimidine N-atom was responsible for pK_a values of 8.0 and 3.6, respectively, for the compound. By using HCl to adjust pH, it was established that the compound could have two pH_{max} values, one at pH 5 ($pH_{max,1}$) and the other at pH *ca.* 2 ($pH_{max,2}$). This is shown in *Fig. 10*. It is evident from

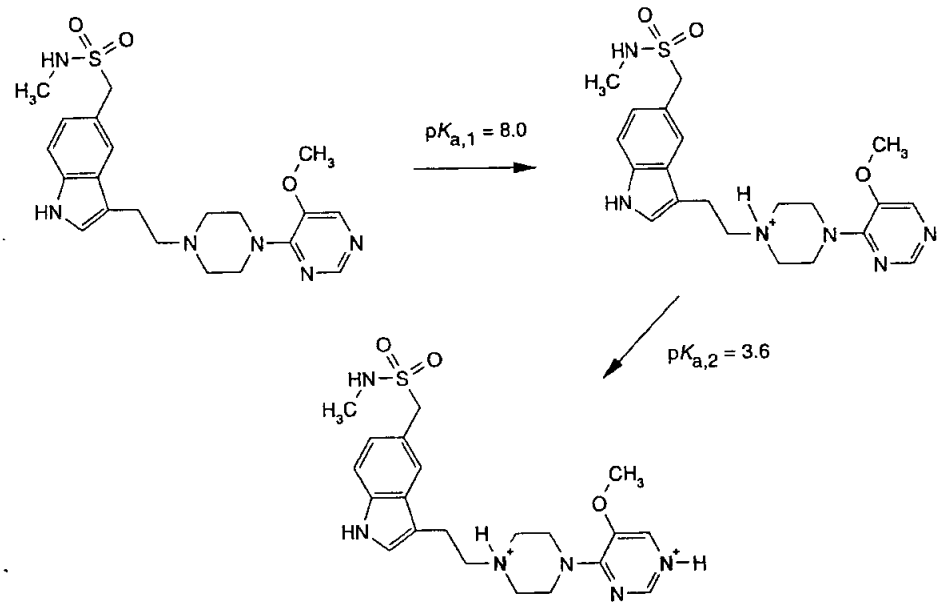


Fig. 9. *Protonation of avitriptan corresponding to its two* pK_a *values*

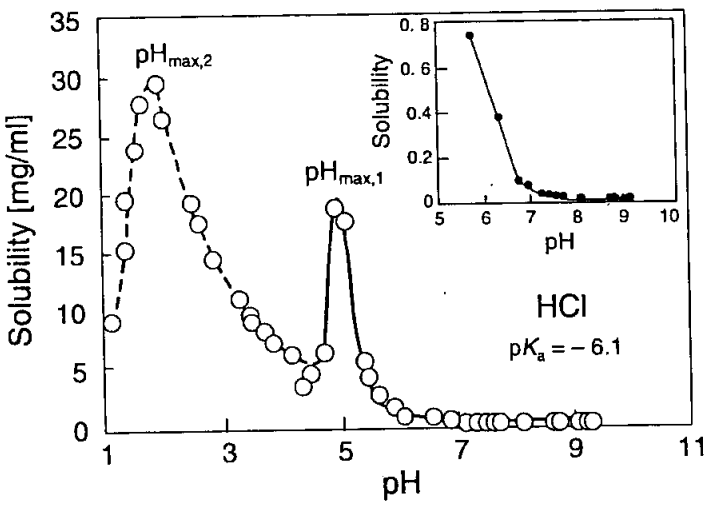


Fig. 10. *pH–Solubility profile of avitriptan at 25 °C where HCl was used to adjust pH, indicating two* pH_{max} *values. Solubility profile at pH above 5 is shown in the inset.*

Fig. 10 that both mono- and dihydrochloride salts can possibly be prepared for avitriptan; the monohydrochloride salt would be the equilibrium species at pH between 2 and 5, and the dihydrochloride salt would be the equilibrium species at pH below 2. Among various acids used by *Lakkaraju et al.* [20] to form salts with avitriptan, only HCl could lower the pH of a saturated av-

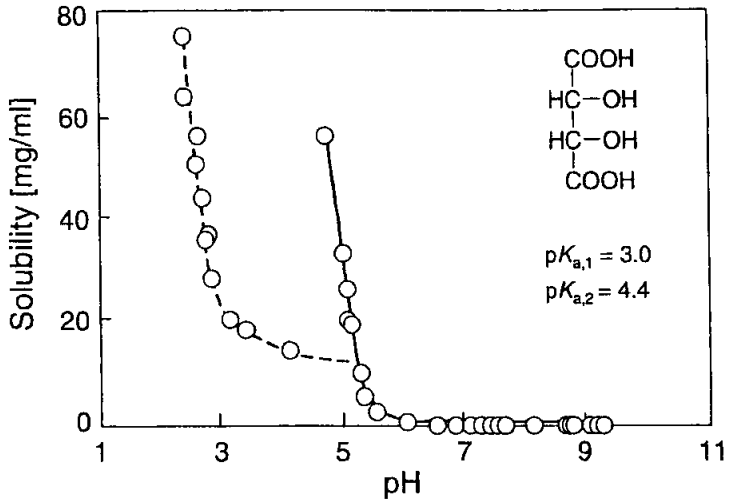


Fig. 11. *pH–Solubility profile of avitriptan at 25 °C where tartaric acid was used to adjust pH, indicating the presence of only one pH_{max} value*

itriptan solution below 2 and thus could form a disalt. The pH of an avitriptan solution could be lowered below 5 ($pH_{max,1}$) but not below 2 ($pH_{max,2}$), when methanesulfonic acid, acetic acid, lactic acid, tartaric acid, and succinic acid were used, indicating that these acids would form only mono-salts with avitriptan. As a typical example, the pH–solubility profile of avitriptan in presence of tartaric acid is shown in *Fig. 11*, where the pH could not be lowered below 2.5 by adding excess amount of tartaric acid. In agreement with these pH–solubility considerations, the salt-selection program of avitriptan yielded mono-salts for all counter-ions used except for hydrochloride, although the existence of two basic moieties in the molecule intuitively suggested that attempts for the synthesis of disalts using various counter-ions should be made. Thus, conducting a feasibility analysis based on pH–solubility relationships can save considerable time and efforts in a salt-synthesis program.

2.4.2. *Feasibility of Salt Formation for Diprotic Acids*

Equations analogous to *Eqns. 5–7* above can also be derived for acids with two ionizable groups in order to study the feasibility of mono- or disalt formation. For such a compound, the solubility of the acid initially increases with an increase in pH due to the ionization of the first ionizable group (*i.e.*, the stronger ionizable group with lower pK_a value). At a certain pH, the first pH_{max} ($pH_{max,1}$) will be reached, and above that a mono-salt will form.

With a further increase in pH, the solubility of the mono-salt will increase due to the ionization of the second ionizable group in the molecule, and the second pH_{max} ($pH_{max,2}$) will be reached. A disalt will be formed above $pH_{max,2}$. The relevant equations are given below:

$$\text{At } pH < pH_{max,1}: \quad S_T = [AH]_s + [A^-] = [AH]_s \cdot (1 + K_{a,1}/[H_3O^+]) \tag{8}$$

$$\text{At } pH > pH_{max,1} \text{ but } < pH_{max,2}: \quad S_T = [A^-]_s + [A^{--}] = [A^-]_s \cdot (1 + [H_3O^+]/K_{a,1}) \tag{9}$$

$$\text{At } pH > pH_{max,2}: \quad S_T = [A^{--}]_s + [A^-] = [A^{--}]_s \cdot (1 + [H_3O^+]/K_{a,2}) \tag{10}$$

In any salt-screening program for diprotic acids, the pH–solubility relationships can be studied either experimentally or by theoretical considerations using *Eqns. 8* and *9*. Then, whether a compound will at all form a salt, and, if it forms a salt, whether it will form a mono- or disalt can be ascertained by studying the effect of counter-ion on the pH of a saturated solution. If the pH of a saturated solution cannot be raised above $pH_{max,1}$ by adding a particular counter-ion, a salt would not be formed. If the pH remains between $pH_{max,1}$ and $pH_{max,2}$, a mono-salt would be formed. A disalt would be formed only if the pH rises above $pH_{max,2}$. In other instances, when pK_a values of two acidic moieties are closer, and, as a consequence, $pH_{max,1}$ and $pH_{max,2}$ are also closer or indistinguishable, it might be difficult to isolate mono-salts in pure forms; either a disalt or a mixture of mono- and disalts might be formed. Under such a situation, the preparation of only disalts may be considered, and, if acceptable disalts are not available, due consideration to the free form of the drug should be given.

2.5. Effect of Counter-Ions on Salt Solubility

It has been reported extensively in the literature that aqueous solubilities of different salt forms of a compound may vary depending on counter-ions used [9] [16] [17] [22–25]. *Streng et al.* [16] attributed the difference in aqueous solubilities of lactic acid, methanesulfonic acid, HCl, and H_3PO_4 salts of terfenadine on the difference in their K_{sp} values with different counter-ions. *Anderson* and *Flora* [4] reviewed the literature for this aspect of salt formation; however, no predictive relationship emerged. It is often difficult to predict *a priori* how solubilities of different salt forms of a particular drug will differ from each other. The reported differences in salt solubilities could sometimes be due to artifacts; some possible difficulties in the determination

of accurate salt solubility have been discussed in *Chapt. 2* of this volume and also by *Anderson* and *Flora* [4]. It is possible that, for a particular salt, the excess solid present in equilibrium with a saturated solution during the solubility determination may not be a salt, because the salt may dissociate into its free form, and thus the 'apparent solubility' may not reflect the true solubility of the salt form. This can lead to an inaccurate and misleading solubility value. Other factors such as crystal lattice energy, solvation energy, common-ion effect, hydrated state of crystals, and so forth, could also be responsible for differences in solubilities of different salt forms of a particular compound. *Anderson* and *Flora* [4] noted that contributions of salt-forming counter-ions on salt solubility must be considered in terms of their separate contributions to crystal-lattice and solvation energies. Since crystal-lattice and hydration energies increase with an increase in cation or anion charges and decrease with an increase in ionic radius, the overall effect of a change in salt form on water solubility will depend on which term, the ionic charge or the ionic radius, is most sensitive to the change in structure. *Lakkaraju et al.* [20] reported that aqueous solubilities of mono-salt forms of avitriptan with five counter-ions, namely, acetate, lactate, methanesulfonate, succinate, and tartrate, were similar and ranged from 14.7 to 16.5 mg/ml, while the solubility of the monohydrochloride salt was 3.4 mg/ml. However, no analysis of contributing factors leading to the similarity in solubilities of certain salts and the difference with another was made.

2.5.1. *Common-Ion Effect on Salt Solubility and Dissolution*

When a basic drug forms a salt with a relatively strong acid, namely, HCl, the aqueous solubility of the salt is strongly influenced by the common-ion effect [11–16]. In such a case, *Eqn. 2*, which depicts the solubility of such a salt, becomes

$$S_T = (1 + K_a/[H_3O^+])\sqrt{K_{sp}} \tag{11}$$

where, for a hydrochloride salt, the solubility product, K_{sp}, is defined by

$$K_{sp} = [BH^+]_s \cdot [Cl^-].$$

It is evident from *Eqn. 11* that as the chloride ion concentration increases with a decrease in pH, the solubility of the salt would decrease due to the common-ion effect and in accordance with the K_{sp} value. This fact should carefully be considered in selecting a hydrochloride salt for development, because, under the acidic pH condition of stomach in the gastro-intestinal tract, the solubility and the dissolution rate of the salt will decrease. The common-ion effect due to a decrease in pH is relatively less pronounced in case of salts

of basic drugs with relatively weak acids, such as acetic acid, lactic acid, *etc.*, because the ionization of such acids decreases with the lowering of pH.

Solubilities of alkali salts of acidic drugs are also governed by the solubility product (K_{sp}) and decrease with an increase in common-ion. *Serajuddin et al.* [26] reported that the solubility of the sodium salt of an experimental drug REV3164 decreased from 7.8 mg/ml in distilled water to 1.1 mg/ml in a 0.1 M NaCl solution. This common-ion effect on solubility adversely influenced the development of REV3164 as a solution dosage form. Such an effect of K_{sp} on the solubility of the salt form of an acidic drug can be studied using the following equation:

$$S_T = (1 + [H_3O^+]/K_a)\sqrt{K_{sp}} \tag{12}$$

where, for a sodium salt, $K_{sp} = [Na^+][A^-]$. The possible effect of K_{sp} on salt solubility and its influence on dosage form design should, therefore, be carefully analyzed during the salt-form selection for acidic drugs. Although no systematic study has been reported in the literature, it is usually assumed that organic counter-ions (*e.g.*, amines) exert relatively less effects on solubility as compared to the inorganic ones.

The use of counter-ions other than hydrochloride may also provide higher dissolution rates for salts of a basic drug as compared to its hydrochloride salt form in the gastric fluid where the presence of chloride ions is prevalent. Indeed, *Bogardus* and *Blackwood* [27] reported that the dissolution rate of doxycycline hydrochloride in 0.1M HCl was adversely influenced by the chloride-ion concentration, whereas a hyclate salt was not similarly affected. Unless the non-hydrochloride salt forms of a particular drug are converted to the crystalline hydrochloride salt during dissolution, no common-ion effect on the dissolution rate is expected. Since the dissolution is a dynamic process, the hydrochloride salt may not readily form on surfaces of dissolving non-hydrochloride salts. Also, any dissolved drug may not crystallize out in the gastric fluid as the hydrochloride salt, unless the solubility of the hydrochloride salt is extremely low. When solubilities of different salt forms for a particular drug are relatively low, such a lack of common-ion effect on the dissolution rate may result in higher bioavailability for a non-hydrochloride salt. Recently, *Engel et al.* [28] reported that the methanesulfonate salts of two basic drugs had 2.6 and 5 times higher bioavailability in dogs than their corresponding hydrochloride salts.

2.5.2. in-situ *Screening of Counter-Ion Effects on Salt Solubility*

Since salts with different aqueous solubilities can be produced for a particular compound by using different counter-ions, *Shanker et al.* [29] report-

ed a method whereby salt solubilities can be screened *in situ* using small amounts of drug substances. In this method, small volumes of concentrated drug solutions using different counter-ions are prepared, and the solutions are then set aside for the crystallization of salts. In such solutions, the counter-ion concentrations are usually in stoichiometric ratios (or slightly in excess of stoichiometric ratios) with drugs. Once the crystals are formed and equilibria are established, drug concentrations in the solutions are measured. *Tong* and *Whitesell* [6] later used this method for the *in-situ* screening of salt solubilities for a basic drug having a pK_a value of 8.02 and the free base solubility of 0.0044 mg/ml. However, care must be taken in any routine use of this method for determining solubility and assessing the feasibility of salt formation. Supersaturated solutions with pH around the pH_{max} are often formed, when a free acid or base and its counter-ions are mixed together [10] [12] [13] [18]. Unless crystal forms of salts are produced and equilibria are reached, any measurement of drug concentration may lead to erroneous conclusions regarding salt solubility. It is also difficult during the initial set-up of such experiments to ascertain whether adequate amounts of drugs and counter-ions have been added. A salt would not crystallize, unless the drug concentration is adequate and the pH of solution is favorable for salt formation (for example, its position with respect to pH_{max} in the pH–solubility profile).

One should also keep in mind that a negative result with respect to crystallization during *in-situ* screening in aqueous media does not necessarily mean that a salt would not be formed. When salts are not produced in aqueous media, it might still be possible to crystallize them from organic solvents or water/organic cosolvent systems. For this reason, there is a recent trend where multiple counter-ions and multiple solvent systems are used in an attempt to prepare salts for a particular compound. In addition to a greater effort in salt synthesis, this makes the number of samples for subsequent physicochemical characterization very large. For example, if 10 counter-ions are tested for a compound, and for each counter-ion 10 solvent systems are used, the total number of samples generated would be 100. Salt-selection strategies based on pH–solubility principles, as reported in the present chapter, may greatly reduce the number of such samples and thus accelerate the salt-selection process.

2.6. Effect of Organic Solvents on Salt Formation

The pH–solubility relationships in aqueous media and their influences on the salt formation of acidic and basic drugs have been the primary focus of this chapter. However, organic solvents or water/organic cosolvent systems

are frequently used for the preparation of salts. Although not much has been reported in the literature, it has been the experience of present authors that pH–solubility theories may also be applied in assessing the feasibility of salt formation from organic systems.

An organic solvent may influence the solubility of a compound in several ways: *i*) increase solubility of unionized species, *ii*) decrease protonation or ionization of the molecule, and *iii*) decrease solubility of salt formed. *Kramer* and *Flynn* [8] postulated that the pH_{max} of a basic drug can increase due to an increase in S_0 value in a cosolvent system. Similarly, the pH_{max} of an acidic drug may decrease because of an increase in S_0 value in the medium. As indicated in *Figs. 1* and *2*, such an effect may favor salt formation. However, this will be true only if the ionization behavior, that is, the pK_a value, of the compound remains unchanged. It has been extensively reported in the literature that an organic solvent may adversely influence the ionization of drug due to a decrease in dielectric constant as compared to H_2O [30–32]. For example, alcohols weaken both acids and bases. *Albert* and *Serjeant* [30] noted that the pK_a value of an acid was raised by *ca.* 1 and that of a base is lowered by *ca.* 0.5 in 60% MeOH in H_2O. A greater depression in ionization or protonation can be observed in mixtures of acetone, dioxane, *etc.*, with H_2O. Thus, any positive effect of an organic solvent on pH_{max} due to an increase in S_0 of a drug molecule may be negated by the depression of its ionization. The net effect might be such that the pH_{max} of the molecule becomes even less favorable to salt formation. The most positive effect of organic solvents on salt formation is the decrease in salt solubility, when suitable organic solvents with relatively low dielectric constants are used [33]. This favors crystallization and isolation of salts.

While assessing the influence of organic solvents on the salt formation of drugs, one should also consider their effects on the ionization of counter-ion species used. For example, in forming salt of a basic drug with a carboxylic acid, the organic solvent may not only decrease the pK_a of the base, it may also increase the pK_a of the conjugate acid as compared to its value in H_2O. This will have a negative impact on salt formation.

Thus, due to conflicting effects of organic solvents on S_0, pK_a, pH_{max}, salt solubility, *etc.*, the study of pH–solubility relationships of drugs in aqueous media remains the most useful tool in assessing the feasibility of salt formation. Organic solvents may, however, be conveniently be used to isolate salts. If, under certain circumstances, a salt is obtained from an organic solvent despite an unfavorable pH–solubility relationship in an aqueous medium, one should keep in mind that such a salt would be prone to disproportionation in presence of H_2O or moisture to produce its free acid or base form. Therefore the salt may not be optimal for dosage form development.

3. Selection of Physical Form

In the above section, we discussed how a rational strategy for the selection of chemical forms of salts can be developed based on the application of pH–solubility principles. Whether certain counter-ions have potentials for salt formation with a particular drug can be determined from a pH–solubility profile of the drug and the location of pH_{max} values in the solubility profile. If it becomes obvious from such an analysis that a stable salt would not form, no attempt should be made to synthesize such a salt, thus saving time and efforts in the salt-selection program. However, many drugs can still form multiple salts, and the number of potential salts depends on pK_a and S_0 values. When the synthesis of multiple salts is feasible, it is important to narrow down the number of salts and ultimately select the optimal salt form based on physicochemical characterization of solids.

3.1. A Multi-Tier Approach

Morris et al. [34] reported a multi-tier approach whereby salts can be screened for their optimal physical form. An updated version of this approach is shown schematically in *Fig. 12*. In this approach, certain physicochemical properties of salts are studied at each tier, and critical 'Go/No Go' decisions are made based on the results of those studies. The number of tiers usually

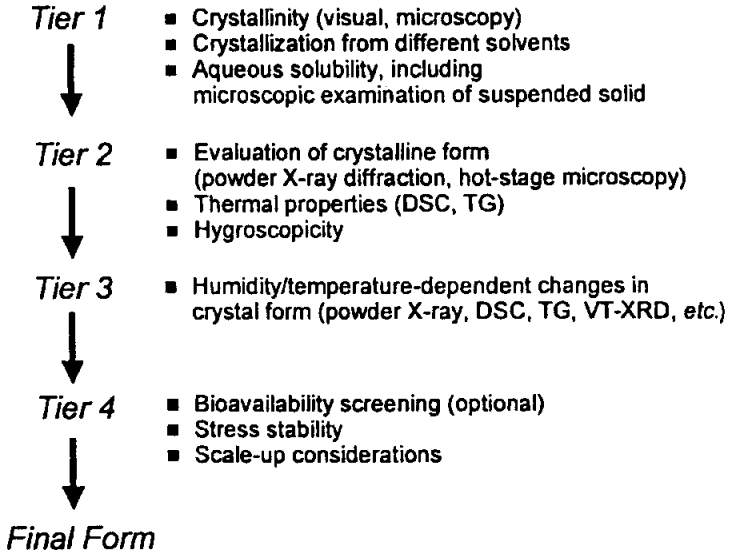


Fig. 12. *Schematic representation of a multi-tier approach for the selection of optimal salt form for a drug*

depends on the number of salt forms available for a compound. Various methods for the characterization of physicochemical properties of salts have been described in *Chapt. 3* which can be applied in a systematic manner in the salt-selection process as discussed here.

In *Tier 1*, the crystallinity of salts is examined by simple visual or microscopic method. If the results of visual and microscopic examinations are inconclusive, powder X-ray diffraction may be used. Equipment is now available for the use of relatively small sample in powder X-ray diffraction studies. If a particular salt is found to be noncrystalline, attempts are made to crystallize it from alternate solvents. In many cases, more than one solvent is tried for the crystallization of drugs. Aqueous solubilities are then determined for those salts that are found to be crystalline. During the determination of aqueous solubility, excess solids in equilibrium with solutions are examined to determine any change in crystal form. Based on these studies, salts that are deemed to have acceptable crystallinity and aqueous solubility are elevated to *Tier 2*. Which aqueous solubility is acceptable for a particular drug often depends on the scope of the drug-development project. If a salt needs to be administered as a solution, a certain minimum solubility might be necessary depending on the dose. For a salt designed specifically for oral administration as a solid dosage form, it is not necessary that a salt with the highest aqueous solubility must be chosen; a salt with a relatively low solubility can also provide adequate dissolution rate for the product. Since many salts and their solid forms may possibly be produced at this stage, any attempt to conduct full physicochemical characterization of all those forms should be restricted at this time, because the results would be useless if the salts are not elevated to the next higher tier. For this reason, the microscopic examination of salts is recommended in *Tier 1*, and powder X-ray diffraction should be used only if microscopic studies are inconclusive. It should also be mentioned that an amorphous form of drug should be elevated to *Tier 2* only in exceptional situations.

In *Tier 2*, an in-depth characterization of crystal properties are conducted by using such techniques as powder X-ray diffraction, hot-stage microscopy, differential scanning calorimetry, thermal gravimetric study, and so forth. Hygroscopicity of salts as a function of relative humidity is also studied in this tier. Based on crystal properties and hygroscopicity, certain salts are then elevated to the next higher tier. If any salt selected in *Tier 2* is found to exist as a hydrate or solvate, or if it is found to be hygroscopic, further studies in *Tier 3* are conducted to determine the effect of temperature and humidity on the crystal form. In this way, the number of salts elevated to *Tier 4* can be minimized.

Salts selected in *Tier 3* are subjected to accelerated stability testing in *Tier 4*. Effects of temperature, humidity, and light are usually studied. If necessary, any potential incompatibility of salts with selected excipients and the effect of processing conditions on salt properties may also be studied at this

tier. Since the stress stability testing of salts is labor-intensive and require much time, conducting this study in *Tier 4* with a limited number of salts avoids the generation of unnecessary data with other salt forms. Screening for the polymorphism of salts may also be conducted in *Tier 4* by crystallization from different solvent systems. Again, time and effort are saved by conducting polymorphism screening in this tier rather than with a larger number of chemical forms at an earlier tier.

Salts are usually prepared in test tubes and beakers during salt screening. However, prior to selecting a salt for development, appropriate consideration must be given in *Tier 4* whether the manufacturing process can be scaled up, and what would be the relative ease or difficulty in the scale-up of different salts studied in this tier. In many cases, bioavailability testing of different salt and acid/base forms of drugs in animal models is also conducted at this stage. A dog model is commonly used. However, to save developmental time and resources, one must be judicious in determining whether the bioavailability test should be conducted or not. The salt formation is an additional step in the manufacture of a drug substance. If a free acid or base has acceptable physicochemical properties and has comparable bioavailability to its salt forms, the free form of the compound might be preferred for development. Prior to reaching such a decision, a comparative bioavailability testing between a salt and the free form might be necessary. However, if the physicochemical properties clearly indicate that a salt form would have superior bioavailability (for example, the free form is extremely water-insoluble), a comparative bioavailability testing will not be necessary for the selection of a salt form. Similarly, a comparative bioavailability testing of different salt forms would not be necessary if the salts have acceptable dissolution rates, even though their aqueous solubilities might differ to a considerable extent.

Morris et al. [34] applied the above multi-tier approach for selecting the optimal salt form for an HMG-CoA reductase inhibitor containing a carboxylic group as the acidic functionality. Seven crystalline salt forms, namely, sodium, potassium, calcium, zinc, magnesium, arginine, and lysine, were synthesized and the arginine salt form was ultimately selected for development. The authors suggested that with such a systematic approach the entire salt-selection process can be completed in 4–6 weeks. *Engel et al.* [28] has recently adopted this multi-tier approach in selecting the methanesulfonate salt form for a basic drug.

4. Salt-Selection Timing

Morris et al. [34] pointed out that the selection of suitable chemical forms of new drug candidates, which includes salt selection, must be done at the

early stage of drug development. This is because any later change in salt form may require repeating many of the developmental studies conducted prior to the change, with the consequent negative impact on the development time and cost. To prepare salts with good biopharmaceutical properties, certain attributes must be built into drug molecules. Therefore, the most appropriate time to start thinking about salt selection of any potential development candidate is in the drug discovery phase. Due to the pressure of bringing new drugs from discovery laboratories to the marketplace in the shortest possible time, the traditional discovery–development interface is getting blurred and more and more development scientists are participating in drug discovery working groups [35]. As a part of the overall developability assessment of new drug candidates, it is during this time that the developmental scientists should make assessment for the feasibility of salt formation. When a selection is made from among many potential candidates, some of the molecules may be more suitable for salt formation than the others. Also, when the medicinal chemists are still in the discovery mode, they might be able to make chemical modifications in molecules to facilitate salt formation.

Much of the assessment at the drug-discovery stage for the feasibility of salt formation can be done *in silico* based on physicochemical principles outlined in the present chapter, and any experimental work needed might be minimal. The actual synthesis and characterization of salts for the purpose of selecting an optimal form for development should preferably start as soon as a developmental candidate is identified. In many cases, the selection begins through prospective research before drug molecules are officially handed over to development groups. *Morris et al.* [34] noted that the salt selection can be removed from critical development path by completing the selection when the chemists are still involved with the scaling up of the synthetic process. According to them, a systematic salt selection may be completed in as little as 4–6 weeks.

5. Salt-Selection Team

The selection of optimal salt forms of new drug candidates involves a multi-disciplinary team approach. The team may even decide that a salt is not warranted for a particular drug, and a free acid or base form should be used instead. Since the ultimate use of a salt is in a dosage form, the formulation needs must carefully be addressed in the selection process. At the same time, a salt, as a drug substance, must be easily synthesized and manufactured. In addition, various analytical tools and techniques are required to characterize different salts prepared during the salt selection. For these reasons, in most pharmaceutical companies, representatives from pharmaceutics, chemical

process development, and analytical research form core salt-selection team. Medicinal chemists from drug discovery make the original synthesis of drug molecules and can provide valuable input towards the synthesis of salts. Representatives from drug metabolism/pharmacokinetics address various bioavailability issues and, if necessary, conduct experimental work in animal models to compare absorption and bioavailability of different chemical and physical forms. Therefore, representatives from these two disciplines participate in the expanded salt-selection team. Inputs from drug safety and marketing are also necessary for the selection of certain salt forms.

6. Summary and Conclusions

In this chapter, a systematic strategy for the selection of optimal salt forms for acidic and basic drugs has been described. The selection of an optimal salt form for a drug involves the selection of both chemical and physical forms. At the end of a study, it might also be concluded that a salt form is not suitable for a particular drug, and the free acid or base form is preferred. Based on physicochemical principles described in this chapter, it is hoped that some of the 'trials and errors' usually associated with salt selection can be avoided, thus saving valuable time and resources in a drug-development program.

Several topics of experimental, physicochemical, and procedural nature as described in this chapter are summarized below. They should be regarded as essential building blocks of any effective salt-selection strategy.

1. The first consideration in any salt-selection program is to determine whether a compound is amenable to salt formation. The presence of an ionizable moiety for an acidic drug and a protonatable moiety for a basic drug does not necessarily mean that a salt would be formed. To form a salt, the pH of the aqueous solution (or suspension) of an acidic drug must be adjusted above its pH_{max} value, and, for a basic drug, the pH of the solution must be below its pH_{max}. Counter-ions used to form salts must be suitable to achieve such pH conditions. Otherwise, salts would not be formed. From this consideration, it can be determined whether the salt formation might be feasible for a particular compound, and, if so, which counter-ions should be tested.
2. The pH_{max} principles have been discussed in detail in *Chapt. 2* of this volume. The pH_{max} value for a particular drug can be determined experimentally from the pH–solubility study. It can also be estimated theoretically form a knowledge of pK_a and solubilities of free and salt forms of the compound. It is not necessary that pH–solubility profiles for a partic-

ular compound should be determined with all counter-ions considered for salt formation. A profile of the compound with only one suitable counter-ion can give a fairly good idea of its pH_{max} value. A reasonably good estimate of the pH_{max} value can also be obtained from a theoretical pH–solubility profile generated from S_0 and pK_a values, even without the solubility of a salt form.

3. Multiple pH_{max} values might exist for compounds with more than one ionizable or protonatable groups. pH–Solubility and pH_{max} principles are also applicable in determining whether such a compound would form a mono-salt or multi-salt (*e.g.*, disalt) with a particular counter-ion. Mono- or multi-salts may selectively be synthesized by using appropriate counter-ions.
4. It is granted that aqueous solutions alone are not always used to prepare salts, and mixtures of aqueous and organic solvents are often used. There are, however, conflicting effects of organic solvents on S_0, pK_a, and salt solubility, and, as a consequence, the pH_{max} may be positively or negatively impacted. The major advantage of using an organic solvent is to lower dielectric constant of the solvent system used, which generally decreases solubility of salts and thus facilitates their crystallization and isolation from solvents. In certain situations, a salt that would not normally exist in an aqueous medium might be formed in a cosolvent system due to a more favorable pH_{max} value. However, such a salt might disproportionate into its free unionized or nonprotonated form, when it contacts an aqueous medium. For these reasons, pH–solubility relationships of drugs in aqueous media remains the most useful tool in assessing the feasibility of salt formation.
5. In selecting counter-ions for salts, the fact should be considered that certain counter-ions can exert significantly more pronounced common-ion effects in aqueous solubilities than others. Salts formed with relatively stronger counter-ions (*e.g.*, hydrochloride salt, sodium salt, *etc.*) are relatively more affected by counter-ion than the salts formed with relatively weaker counter-ions (*e.g.*, salts with carboxylic acid, amine, *etc.*).
6. When several salts for a particular compound are synthesized, physicochemical tests to characterize solids can be conducted at different tiers with a 'Go/No Go' decision at the end of each tier of testing the salts. By a systematic multi-tier approach, many different salt forms can be screened for their physicochemical properties with the minimum of experimental effort.
7. Finally, the salt selection is a team approach with representatives from pharmaceutics, chemical process development, and analytical chemistry forming the core salt-selection team. Representatives from drug discovery, drug metabolism/pharmacokinetics, drug safety, and other disciplines

participate, as needed, in an expanded team. Through such teamwork and with proper planning, the salt selection can be removed from the critical development path, thus accelerating drug development.

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