SYNTHESIS OF 4-(2-AMINOETHYL)-6-DIBENZOFURAN PROPOINIC ACID:

AN UNNATURAL AMINO ACID THAT INITIALIZES B SHEET FOLDING

A Senior Thesis

By

Niki Zacharias

1996-97 University Undergraduate Research Fellow

Texas A&M University

Group: BIOCHEMISTRY/CHEMISTRY

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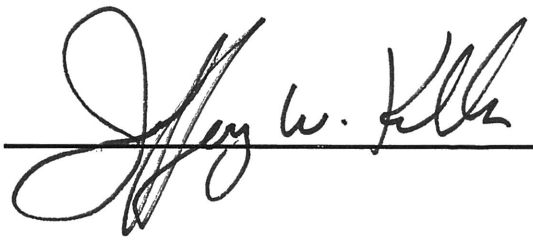
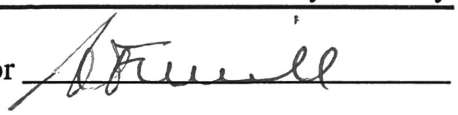
Niki Zacharias
University Undergraduate Fellow, 1996-1997
Texas A&M University
Department of Chemistry



Advisor Dr. Jeffrey W. Kelly

Honors Director 

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I want to thank everyone that has helped me this past year especially Haimi Beckele and Dr. Kelly. Both of you have helped develop me into a chemist. Haimi is the best role model I could have ever chosen. She is an intelligent and hard working graduate student. She has given me an ideal to strive for and I thank her for this. Dr. Jeff Kelly is a hard working professor. I thank him for all the time he has found for me in his hectic schedule.

I have learned a vast amount of knowledge and experience by doing research. However, everyday I find out how much I really do not know. Everyday I realize how little I know on organic techniques and analytical equipment. In addition, I realize how many mistakes I have made this past year. Nuclear Magnetic Resonance and I seem not to get along to well. But, practice makes perfect and that is why I am going to graduate school. I will hopefully not make the same mistakes twice.

Thank you for the opportunity to learn.

Abstract

Synthesis of 4-(2-aminoethyl)-6-dibenzofuran propoinic acid: An Unnatural Amino Acid that initializes B sheet Folding. Niki Zacharias (Jeff Kelly), Chemistry, Texas A&M University

Dr. Jeff Kelly's laboratory has been using 4-(2-aminoethyl)-6-dibenzofuran propoinic acid an unnatural amino acid to initiate β sheet folding. The unnatural amino acid mimics a β turn in an antiparallel β sheet structure. Peptides that incorporate the dibenzofuran based unnatural amino acid are being used to study the mechanism of folding and the synthesis of novel materials. A new synthesis of 4-(2-aminoethyl)-6-dibenzofuran propoinic acid has been created. The synthesis has a high yield and simple purification. The synthesis is based on the desymmetrization of the dibenzofuran ring using a monosilylation reaction.

Introduction

Peptides fold into two different secondary structures such as α -helical and β sheet conformations. α - helical conformation can be thought of as a spiral staircase of amino acids stacked upon each other with hydrogen bonding in the center of the staircase. β sheet conformation is obtained when amino acids form long hydrogen bonded chains called β strands which bend at particular sites called β turns (Fig 1). A β sheet is the

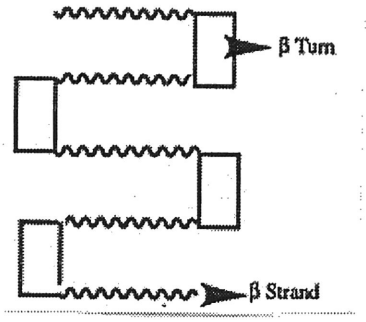


Fig 1
Schematic representation of a β sheet with β turn and strand identified

hydrogen bonded β strand network. These extended chains can hydrogen bond either parallel in nature or antiparallel. Parallel is where both strands run from C to N terminus. Antiparallel is where one strand runs C to N terminus while the other runs N to C terminus (Fig 2, Fig 3). The structure and folding mechanisms of α helical structures are better understood than β. In addition, biophysical studies have shown that folding in β sheets is slower than helix folding.[1] This observation shows that sheets and helices fold differently. A model system of β sheet conformation and folding has been elusive.[2] One

of the main difficulties with understanding β sheets is self association. Self association is when polypeptides aggregate into large and generally insoluble quartenary β sheet structures.[2] True intramolecular folding does not occur during aggregation. A relatively new technique in understanding and creating a β sheet folding model is to make synthetic β sheets using rigid unnatural amino acids.

Figure 2

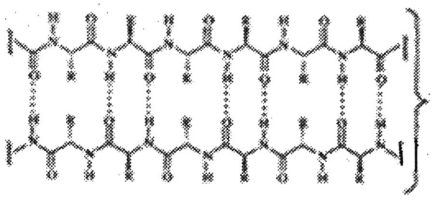
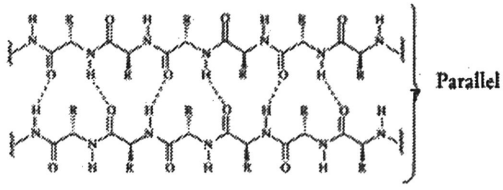


Figure 3



It is difficult to design a linear sequence that will fold into a β sheet conformation. The magnitude of electrostatic and hydrophobic interactions are all context dependent; therefore, thermodynamic stability of the peptide can not be predicted.[1] However, it is known that hydrophobic clustering in folding is important. Rose and Dill were the first to propose that protein folding progresses through the formation of hydrophobic clusters which direct folding of peptides into α helices or β sheets.[3] In addition, Karplus and Mattice's theoretical paper on β sheets predicted that folding could occur from a partial β sheet in an energetically unfavorable nucleation step.[3] It is also known that the folded state's stabilization energy is the difference in free energy between the folded and unfolded state which is derived from both the entropic and enthalpic contributions from both the solvent and the polypeptide.[1] The need for the stabilization of the folded state lead

Hirschman at Merck to introduce a rigid molecule into a peptide. [4, 5] A rigid molecule stabilizes a single conformation and lowers the entropic difference between the unfolded and folded state of the polypeptide.

Templates induce folding by creating intermolecular hydrogen bonds and hydrophobic interactions. A template is a way to achieve folding without understanding how to design a linear sequence that will fold.[1] Kemp and colleagues were the first to incorporate a template to create β sheets. They incorporated an epindolidione skeleton which mimics the central strand of a β sheet conformation (Fig 4).[4, 5] Dr. Jeff Kelly

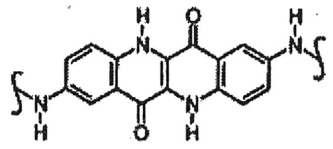
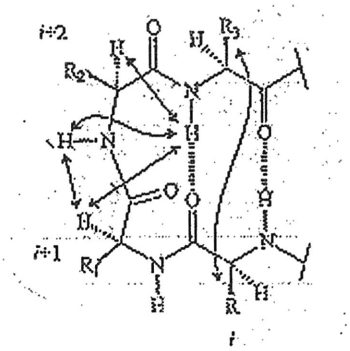


Figure 4

and his research team decided to create a β turn which did not resemble a natural turn structure but instead concentrated on creating a molecule that would reverse the peptide chain, promote intramolecular hydrogen bonding, and mediate tertiary hydrophobic interactions.[3, 6-7]

Jeff Kelly's research is centered on β sheet folding in relatively small peptides. Folding is initialized by a β turn mimic that replaces the i+1 and i+2 residues of a β turn (Fig 5). While other β turn mimics have been designed in our laboratory, most of the

Figure 5



effort has been centered on dibenzofuran based mimics. 4-(2-aminoethyl) -6-dibenzofuran propoinic acid was synthesized and designed to initialize intramolecular hydrogen bonding between amides and create a hydrophobic cluster between the dibenzofuran ring and hydrophobic side chains flanking the turn. [6](Fig 6) Dibenzofuran

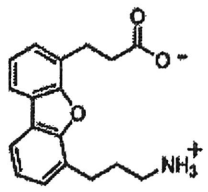


Figure 6

was chosen as the backbone for the unnatural amino acid β turn structure because the distance between C_4 and C_6 is 4.9 A which is close to the distance between two strands of an antiparallel β sheet (4.85 A).[8, 9] In addition, it has been shown by physical studies that the low energy conformation for phenylethylamine is with the diphatic CH_2-CH_2 perpendicular to the plane of the aromatic ring.[6] Aminoethyl and carboxy ethyl were picked to be the functionalities on C_4 and C_6 of dibenzofuran to create the same

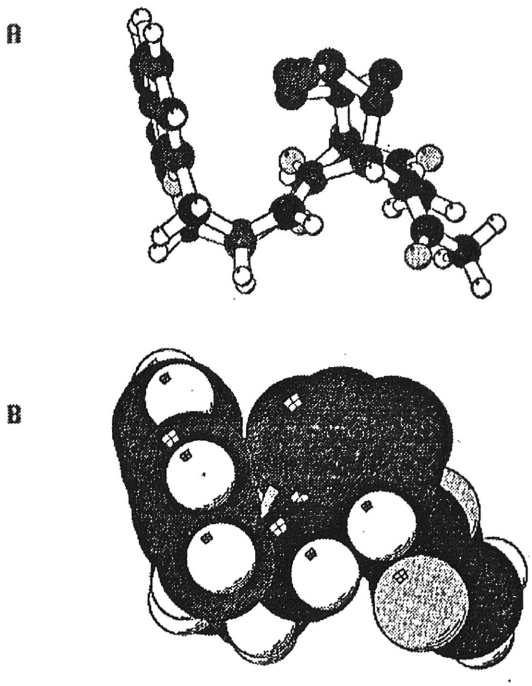


Figure 7 a) ball and stick representation of the hydrophobic cluster b) CPK representation of the hydrophobic cluster

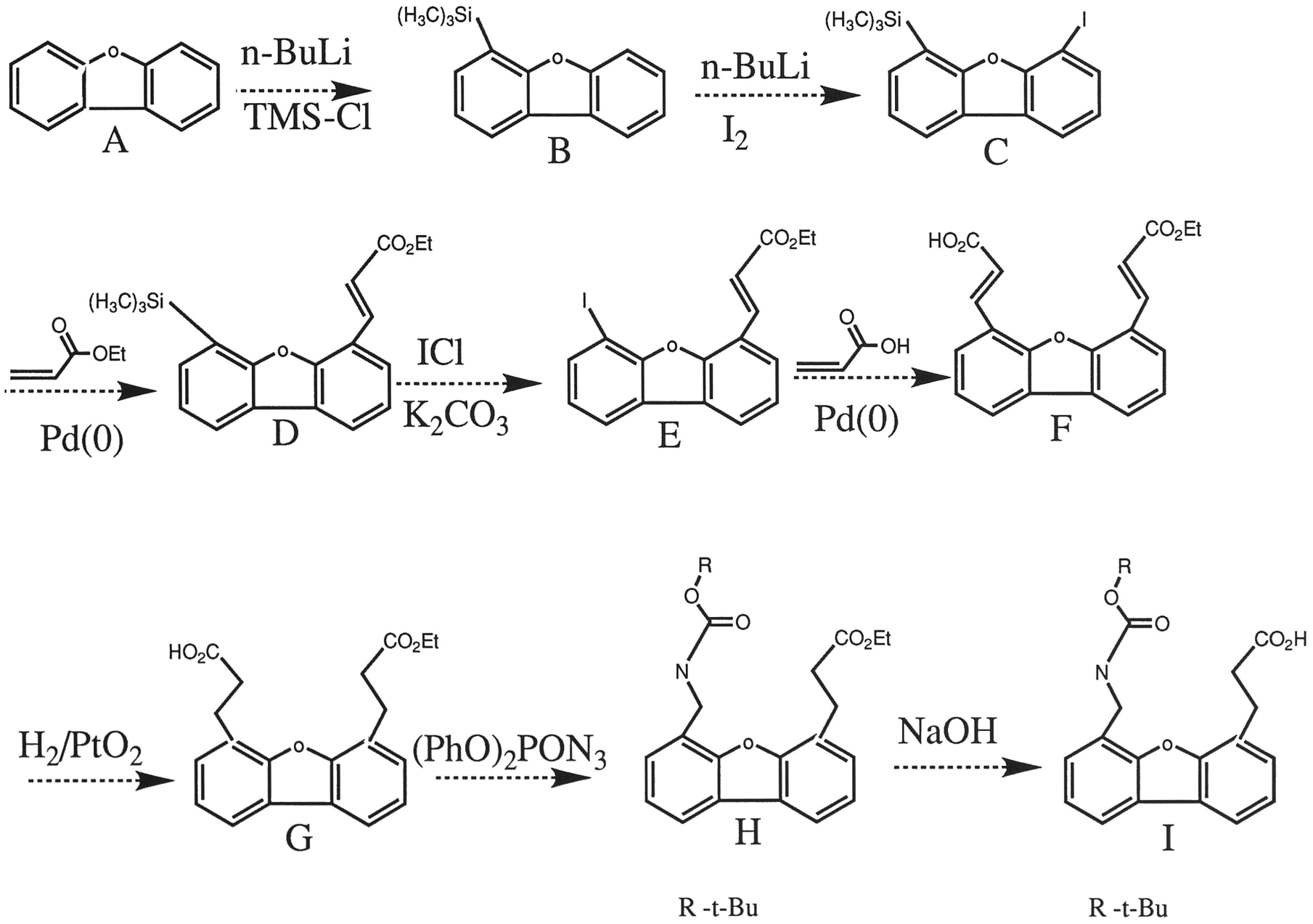
conformations as phenylethylamine.[6](Fig 7). The formation of the hydrophobic cluster nucleates a wide variety of sequences to fold into β sheet hairpin conformation in aqueous solution. However, it has been shown that 4-(2-aminoethyl)-6-dibenzofuran propoinic acid is capable of β sheet folding only when flanked by hydrophobic amino acid residues like Val, Leu, Phe and etc.[3, 6-7] In addition, self association of peptides was succumbed by replacing two exterior amide protons in tridecapeptides with methyl groups to prevent intermolecular hydrogen bonding. These methylated amino acids are usually the fourth or third amino acid in the sequence.[6] N-methylated tridecapeptides which incorporate a dibenzofuran β turn have been shown to create a monomeric β hairpin like structures.[10]

The dibenzofuran based β sheets are used for understanding amyloidotic disease and the design of biomaterials. Kelly's lab studies familial amyloidotic polyneuropathy or FAP which is a lethal disease caused by deposits in the lung, stomach, heart, and other organs. Transthyretin protein in blood plasma begins to aggregate and form amyloid plaques. These amyloid plaques have been shown to have β sheet conformation.[11] The unnatural dibenzofuran β sheet folding model is used to try to understand the folding mechanism of the transthyretin. In addition, the unnatural dibenzofuran β sheets are being used to create biomolecules and biominerals like silk and calcite crystals. Silk lamellar conformations have been shown to be in β sheet conformation.[12] Silk like materials have been made in the Kelly research group using dibenzofuran based unnatural amino acid as the β turn. [12] Biominerals use structured biomolecules to organize inorganic ions into minerals like calcite and magnite crystals. Our β sheets are in the process of being used to orient inorganic ions during the formation of minerals.

4-(2-aminoethyl)-6-dibenzofuran propoinic acid is used extensively in Kelly's lab. Due to the high demand of the compound, it was necessary to improve its synthesis. Initial design was composed of synthesis of the dibenzofuran to a diethyl ester on both the C_4 and C_6 to unsymmetrical monoester monacid . [3, 6-7] The old synthesis of the monoester monoacid gives an overall yield of 25%.[3, 6-7] The new synthesis gives a yield of 39%. The problem with the first synthesis is that C_4 and C_6 had the same side groups throughout the synthesis. Therefore, the dibenzofuran was kept symmetrical throughout the synthesis. The new synthesis is designed to desymmetrize the dibenzofuran early in the synthesis, therefore, allowing regioselective reactions to be utilized.

The new synthesis is centered around the desymmetrization of dibenzofuran early. The desymmetrization is done using a deprotonation-silylation reaction with n-BuLi and trimethylsilyl chloride. The silyation gives a quantitative yield and desymmetrizes the dibenzofuran ring at the first step of the synthesis. The new products are easier to purify and gives a yield of 39% of the monoester monoacid (Fig 5 reaction scheme).

Outline of the Synthesis



Experimental Section

General Methods. Unless otherwise noted, materials were purchased from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium/benzophenone ketyl under nitrogen (N_2). Trimethylsilyl chloride (TMSCl) was distilled from calcium hydride under N_2. Triethylamine (TEA) and N,N,N',N'- tetramethylethylenediamine (TMEDA) were refluxed over ninhydrin, distilled and then redistilled from calcium hydride or potassium hydroxide. Routine ^1H NMR spectra were recorded on Varian Sun System 200 spectrometer and are reported in parts per million (δ) relative to acetone (2.20 ppm). Column chromatography was performed as described by Still[13] using forced flow (flash) chromatography with the indicated solvents on Baxter SIP silica gel 60A. Mass spectra were obtained on a Hewlett Packard 5971 mass spectrometer. Analytical HPLC was done on a Waters 600 preparative HPLC using a Waters RCM Delta Pak C_{18} column and Knauer 86 variable wavelength detector. Unless otherwise noted, all reactions were run under Argon or Nitrogen.

4-trimethylsilydibenzofuran (B)

An oven dried 300 ml three necked round bottom with stir bar was cooled under Ar and charged with dibenzofuran (10.14 g, 59.6 mmol). The round bottom was then fitted with an oven dried condenser and addition funnel. THF (120 ml) was added to the system via syringe through the condenser. The solution was cooled down to 0°C and n-BuLi (45 ml of 1.6 M hexane solution, 72 mmol) was added via syringe over a 15 minute period. The orange solution was allowed to stir at room temperature for 5 hours. The solution was

then cooled again to 0°C. TMSCl (15 ml, 118.2 mmol) and THF (15 ml) was added to the round bottom via syringe through the addition funnel dropwise over a 10 minute period. After half of TMSCl solution had been added, the reaction solution turned yellow. After complete addition, the solution was allowed to stir at room temperature for 20 minutes. The solution was then refluxed for two hours after which it was allowed to stir overnight at room temperature. The reaction mixture was then poured over crushed ice (115 g) in (50 ml) of ether. After the ice melted, solution was transferred into a separatory funnel. Aqueous layer was extracted with 2 X 50 ml of ether. The combined organic layers were washed with saturated sodium bicarbonate solution (70 ml). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. A pale yellow oil (13.13 g) was achieved. Yield of 97%. Mass spectra gives a molecular ion peak at 240 m/z. ^1H NMR (d_6 acetone) δ 7.9-7.6 (broad peak, 2H, Ar H's), 7.4-7.0 (m, 5H, Ar H's), 1.7 (impurity), 0.2 (m, 8H, $-Si(CH_3)_3$)

4-iodo-6-trimethylsilyldibenzofuran (C)

A 250 ml oven dried round bottom was charged with monosilyl dibenzofuran (6.80 g, 28.3 mmol). The flask was then dried under high vacuum over night. The round bottom was fitted with a stir bar and sealed with a septum. Diethyl ether (54 ml) and TMEDA (6.03 ml, 39.9 mmol) were added via syringe. The solution was then cooled to 0°C and n-BuLi (25ml in 1.6 M hexane solution, 40 mmol) was added via syringe. A dark orange brown solution occurred. The solution was stirred for 5 hours at room temperature. During that time, a dry 500 ml round bottom with stir bar was charged with I_2 (14.6 g, 57.5 mmol) and

sealed. EtO_2 (51 ml) was added via syringe to the iodine. The iodine round bottom was cooled to -78°C. The copper colored dibenzofuran solution was cannulated to the iodine containing round bottom slowly. The mixture was stirred overnight at room temperature. A 450 ml solution of 20% $NaHSO_3$ was made in 600 ml Erlenmeyer and cooled in an ice bath. The iodine reaction mixture was added directly to the sodium thiosulfate and 120 ml of methylene chloride was added. The mixture was then stirred at room temperature overnight with periodical additions of $NaHSO_3$ until it became a pale yellow. The solution was then poured into a separatory funnel. Aqueous layer extracted with 2 X 100 ml CH_2Cl_2 and, the combined organic layers were washed with 3 X100 ml of 20 % $NaHSO_3$. The organic layer was then dried with $MgSO_4$ and the solvent removed under low pressure. The crude was then purified by flash chromatography (95:5 hexane : ethyl acetate) to afford pure compound 7.19 g (83.4 %). Mass spectra gave peaks at 240 and 366 m/z corresponding to the starting material and product. No NMR data.

4-trimethylsilyl-6-dibenzofuran ethyl propenoate (D)

An oven dried 250 ml round bottom was charged with 4-iodo-6-trimethylsilyldibenzofuran (11.56 g, 91.5 % purity by HPLC, 28.9 mmol), $Pd(OAc)_2$ (136 mg, 5.8 mol), and $P(otol)_3$ (450.2 mg, 14.17 mol). A stir bar was added and the round bottom was put under high vacuum for 2 hours to dry. Then an oven dried condenser was added and the system sealed. CH_3CN (46 ml), Et_3N (13ml, 92.7 mmol), and ethylacrylate (9.4 ml, 86.8 mmol) were added to the round bottom via syringe. The solution was heated in an oil bath at 85°C for 3 ½ hours. The solution was then cooled to room temperature and the solvent

removed under low pressure. A dark brown yellow sludge remained. The solid was then partitioned between 350 ml CH_2Cl_2 and 450 ml water. The aqueous layer was extracted with 3 X 200 ml CH_2Cl_2 and, the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The yellow crude material was purified by flash chromatography (95:5 hexane : ethyl acetate) to afford pure yellow oil compound 8.28 g (85% yield). Mass spectra gave a molecular ion peak at 338 m/z. ^1H NMR (d_6 acetone) δ several peaks between 7.82 - 6.84 (Ar H's), 3.83 (2H, $-CH_2$), 1.7 (2H), 0.9 (3H,$-CH_3$), 0.54 (m, 8H, $-Si(CH_3)_3$)

4-iodo-6-dibenzofuran ethylpropenoate (E)

A dried 50 ml round bottom was charged with 4-trimethylsilyl-6-dibenzofuran ethyl propenoate (500mg, 1.48 mmol). The round bottom was then dried under high vacuum over night. K_2CO_3 (0.626 g, 4.54 mmol) was added to the round bottom. The flask was again dried under vacuum for another 45 minutes. Carbon tetrachloride (10.12 ml) was added via syringe and the mixture was cooled to 0°C. Then ICl (0.22 ml, 4.19 mmol) was transferred via teflon cannula to a septum capped graduated cylinder which had been oven dried over night and charged with CCl_4 (1.5 ml). The ICl solution was then canulated via teflon tubing into the cold reaction flask. The red wine colored solution was allowed to warm to room temperature and was stirred in the dark overnight. The reaction solution was then transferred into a 600 ml Erlenmeyer with 20% $Na_2S_2O_3$ solution (50 ml). The solution was stirred overnight with periodical additions of extra sodium thiosulfate until the dark wine solution turned a pale yellow. Solution was transferred to a separatory

funnel. The aqueous layer was extracted with 3 X 10 ml CH_2Cl_2 . The combined organic layers were washed with (3 X 22 ml) 20% $Na_2S_2O_3$ solution, (3 X 22ml) 10% HCl, (3 X22ml) 1 N NaOH, and (1 X 22 ml) water. The organic layer was then dried over $MgSO_4$ and the solvent was then removed under low pressure. A pink solid formed. After further drying under high vacuum, the pink color was removed by washing with a 95:5 hexane: ethyl acetate solution (3 X 5ml). A white solid was finally achieved. Product was dried under high vacuum for 45 minutes. Yield of 90 % achieved. Mass spectra gives a molecular ion peak at 392 m/z. ^{1}H NMR (d_6 acetone) δ hard to distinguish peaks 8.15-7.15 (7Hs, Ar H's), 4.29 (q, 2H, -C*H*$_2$CH$_3$), 1.34 (t, 3H, -CH$_2$C*H*$_3$)

4-propenoic acid 6- dibenzofuran ethyl propenoate (F)

A dried 20 ml round bottom was charged with dried 4-iodo-6-dibenzofuran ethylpropenoate (1.18 g, purity 70 % by HPLC, 2.11 mmol), palladium acetate (14.89 mg, 0.999 mol), and tri-*ortho*-tolyphosphine (46.23 mg, 2.268 mol). Round bottom was then dried under high vacuum for 45 minutes. An oven dried condenser was attached to the round bottom. Acetonitrile (20ml), triethylamine (1.51 ml, 10.78 mmol), and acrylic acid (0.7 ml, 9.29 mmol) were added via syringe to the round bottom. The solution was then refluxed at 85°C for ≈5 ½ hours and stirred at room temperature overnight. The solvent was removed under reduced pressure. The crude material was then dissolved in 50 ml CH_2Cl_2 and washed with 40 ml of water. The water was then extracted with 4 X 25 ml CH_2Cl_2. Combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to give a yellow solid. Purification by flash chromatography (70:29:1

hexane: ethyl acetate: acetic acid to 60:40 hexane: ethyl acetate to 100 % ethyl acetate) afforded 1.18 g (68%) of a yellowish white solid. Mass spectra gave a molecular ion peak at 334 m/z. ^{1}H NMR (d_6 acetone) δ several small peaks 8.22 - 7.00 (6H, Ar H's), 4.18 (2H, -C*H*$_2$CH$_3$), 2.6 (impurity), 1.15 (3H, -CH$_2$C*H*$_3$)

4-propionic acid-6-dibenzofuran ethyl propionate (G)

An oven dried 100 ml round bottom was charged with 4-propenoic acid 6- dibenzofuran ethyl propenoate (2 g, 5.99 mmol), 40 ml of methanol, and Pd/C (0.067 g, 0.57 mmol, 5 mol %). The mixture was hydrogenated using a hydrogen balloon giving a pressure ≈ 12 psi overnight. The catalyst was removed by filtration through a nylon membrane and the solvent was removed under reduced pressure. A pale yellow solid resulted 1.73 g (86%). Mass spectra has a peak at 336 m/z . ^{1}H NMR (d_6 acetone) δ 7.8 (d, 2H), 7.4-7.2 (4H, Ar H's), 3.96 (q, 2H, -C*H*$_2$CH$_3$), 3.2 (3H, -CH$_2$COR), 2.75 (3H, -C*H*$_2$-CH$_2$), 1.09 (m, 6H, -CH$_3$)

4-(2-t-butoxycarbamylethyl)-6-dibenzofuran ethyl propoinate (H)

A oven dried 20 ml round bottom was charged with 4-propionic acid-6-dibenzofuran ethyl propionate (680 mg, 2.023 mmol) and dried under high vacuum for two days. A stir bar and oven dried condenser were added. The system was then sealed and dried under nitrogen. t-Butanol (6.1 ml, 9.76 mmol), diphenylphosphonic azide (5.52 ml, 2.40mmol), Et$_2$N (0.28 ml, 1.998 mmol) were added to round bottom via syringe through the condenser. The solution was refluxed overnight. Solution was allowed to cool and

concentrated under reduced pressure. The yellow oil was dissolved in ether (9 ml) and transferred to a separatory funnel. The solution was washed with (3 X 6ml) 2M citric acid, (3 X 6ml) 20% sodium bicarbonate, and (2 X 8ml) water. Combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography purification performed (70:30 hexane: ethyl acetate). Solvent was removed under reduced pressure and 0.44g (55.8%) of white solid formed. Mass spectra gave a molecular ion peak at 390 m/z which corresponds to the molecular weight of the product. No NMR datat.

4-(2-t-butyloxycarbamylethyl)-6-dibenzofuram propanoic acid (I)

A dried 50 ml round bottom was charged with 4-(2-t-butoxycarbamylethyl)-6-dibenzofuran ethyl propionate (0.44 g, 1.128 mmol), 4 ml ethanol, and NaOH (64 mg, 1.6 mmol). A stir bar and condenser were added. Solution was refluxed for five hours. After the solution was cooled, the solvent was removed under reduced pressure to yield a yellow oil 0.49 g (100%). Mass spectra gave a molecular ion peak at 362 m/z. No NMR data.

Results and Discussion

The old synthesis of 4-(2-aminoethyl)-6-dibenzofuran propionic acid was composed of reactions that created a diacid, a diester, and after a 50 hour ethanol reflux gave the monoacid monoester. The ethanol reflux step only gave a percent yield of 53% when desymmetrization is accomplished. The new synthesis is centered on eliminating this late desymmetrization step. We believed that a better yield would be accomplished if desymmetrization is done early in the reaction pathway.

The new synthesis is begun by creating a monoanion and reacting with trimethylsilyl chloride to give monosilyl dibenzofuran (B). The dibenzofuran ring is now unsymmetrical. The product can then be deprotonated again and reacted with iodine to form 4-iodo-6-trimethylsilyldibenzofuran (C).

The monometallation reactions are easily accomplished by using 1.2 equivalents of n-BuLi in THF. Both deprotonation-metalation reactions achieve nice yields of 97 and 83 % yield, respectively.

After formation of the 4-iodo-6-trimethylsilyldibenzofuran, a Heck reaction can be utilized to create 4-trimethyl-6-dibenzofuran ethyl propenoate (D). The Heck reaction is catalyzed by Pd(0) and is the arlylation of ethyl acrylate.[14] The reaction occurs by oxidative addition of RX onto the palladium complex which then inserts the olefin. Steric factors always favors the migration of the R group to the less substituted carbon on the olefin when formation to the linear product occurs.[14] The Heck reaction is selective and achieves good yield of 4-trimethylsilyl-6-dibenzofuran ethyl propenoate (D).

An iododesilylation step is then performed on the 4-trimethylsilyl-6-dibenzofuran ethyl propenoate. The iododesilylation is achieved by ICl in carbon tetrachloride in the presence of potassium carbonate which gives an isolated yield 90 %. Then a second Heck reaction is performed using acrylic acid. The Heck reaction yields the unsaturated monoester monoacid. The saturation of the compound can then be achieved by either Pd/C or PtO_2 in methanol under approximately 12 psi of hydrogen. The unsymmetrical monoester monoacid is achieved in six steps with an overall isolated yield of 39%.

The monoester monoacid can be converted to amino ester (4-(2-aminoethyl)-6-dibenzofuran propoinic acid) by a Curtis rearrangement. The Curtis rearrangement is accomplished by using 1.2 equivalence of diphenylphosphoric azide and 1 equivalence of triethylamine in the presence of t-butanol. The Curtis rearrangement reaction is a mechanistically interesting reaction. The triethylamine deprotonates the carboxylic acid which then allows the oxygen to attack the phosphoryl azide. The phosphoryl azide attaches to the oxygen through an O-P bond and removes the azide group after forming a tetrahedral intermediate. The azide group then attacks the carbonyl which removes the diphenyl phosphoryl group. The rearrangement then occurs forming an isocyanate group. The reaction in our synthesis is done in t-butyl alcohol which allows for 4-(2-t-butoxycarbamylethyl)-6-dibenzofuran ethyl propoinate or the BOC derivative of the β turn to be created.

The final NaOH reflux is just the removal of the ester group to create a carboxylic acid. This step is done to prepare the turn for peptide synthesis.

Conclusion

The trimethylsilylation of dibenzofuran as the first reaction in the creation allows for differentiation of the C_4 and C_6 positions of the dibenzofuran at the very beginning of the synthesis. The desymmetrization allows for selective reactions to be used. The desymmetrization and sequential Heck reactions allows for a new practical synthesis of the BOC- 4-(2-aminoethyl)-6-dibenzofuran propionic acid. In addition, the purification of the intermediates is easier in the new synthesis than the initial synthesis because the polarities of the products and starting materials are quite different. The most important difference between the new and old synthesis is that a 39% yield of monoester monoacid can be achieved with the new synthesis while only a 25 % yield is achieved with the old synthesis.

References

1 Dill, K.A. *Biochemistry*. 1990, *29*, 7133.

2 Freidinger, R.M.; Veber, D.F. ; Perlow, D.S.; Brooks, J.R.; Saperstein, R. *Science* 1980, *210*, 656

3 Schneider, J.P.; Kelly, J.W. *Chem Rev*. 1995, *95*, 2169-2187.
And references within.

4 Kemp, D.S.; Bowen, B.R. *Tetrahedron Lett*. 1988, *29*, 5081.

5 Kemp, D.S. *Trends Biotechnol*. 1990, *6*, 246.

6 Tsang, K.Y.; Diaz, H.; Graciani, N; Kelly, J.W. *J. Am. Chem. Soc*. 1994, *116*, 3988-4005.

7 Nesloney, C.L.; Kelly, J.W. *J. Org. Chem*. 1996, *61*, 3127-3137.

8 Pauling, Li; Corey, R.B. Proc. *Natl. Acad. Sci*. USA 1953, *39*, 253-256.

9 Reppart, W. J.; Galluci, J.C.; Lundstedt, A.P.; Gerkin R.E. *Acta Crystallogr*. 1984, *C40,* 1572-1576.

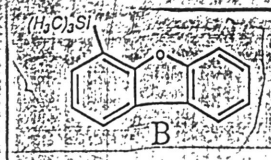
10 Frori, W.R.; Choo D.W.; Kelly, J.W. *Unpublished results*.

11 Taubes, C. *Science*., 1996, *271*, 1493-1495.

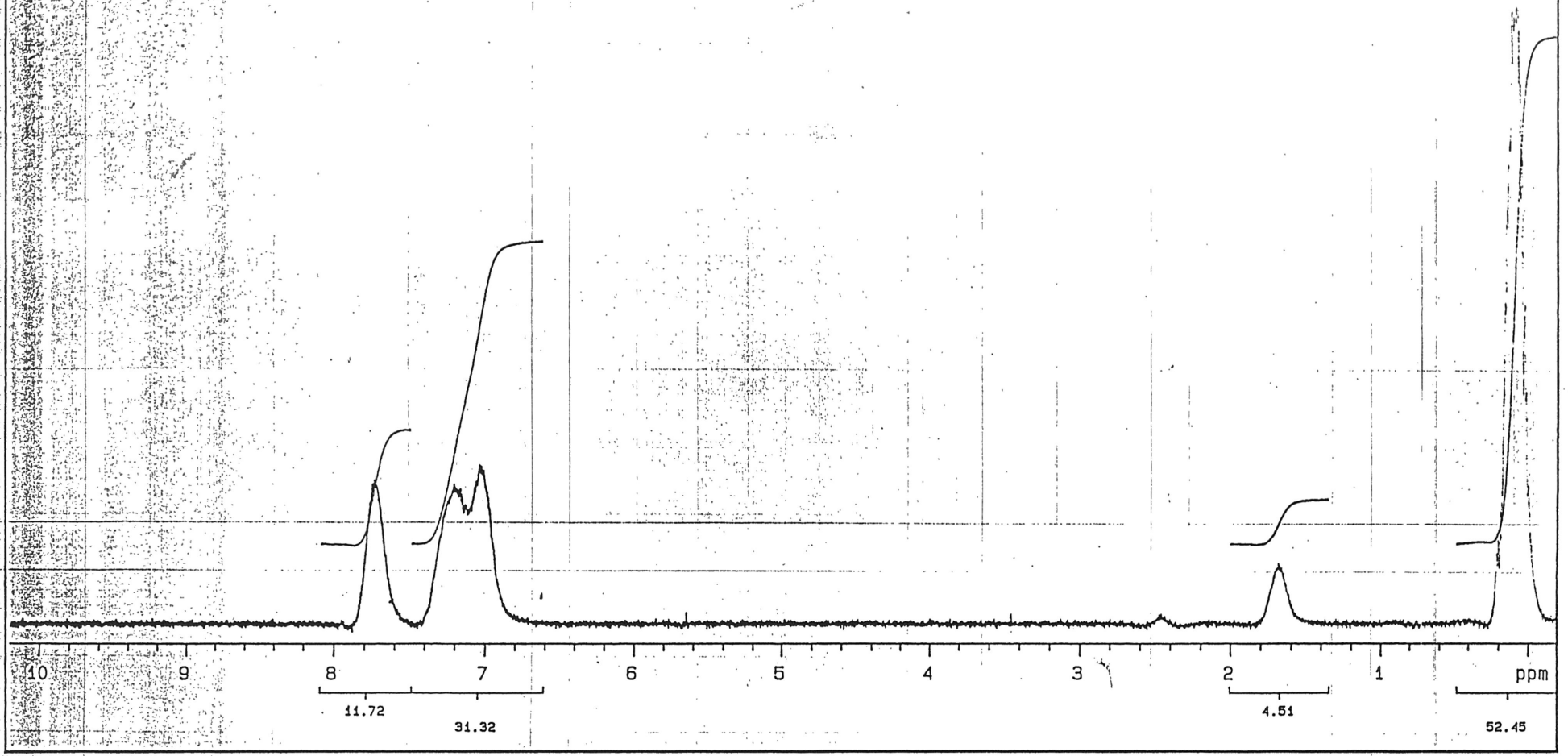
12 Kurt Wayne McWilliams, Ph.D Dissertation Thesis, University of Texas A&M, 1996.

13 Still, W.C.; Kahn, M.; Maitra, A. *J. Org. Chem*. 1978, *43*, 2923-2925.

14 Cabri, W.; Candiani, I. *Acc. Chem. Res*. 1995, *28*, 2-7.



4-trimethylsilyldibenzofuran



OBSERVE
Nucleus H1 Freq. 199.9772 MHz
Spec. Width 2999.6 Hz Offset 100.0 Hz
Acq. Time 1.995 sec Delay 1.000 sec
Pulse Width 10.0 µsec Transients 16

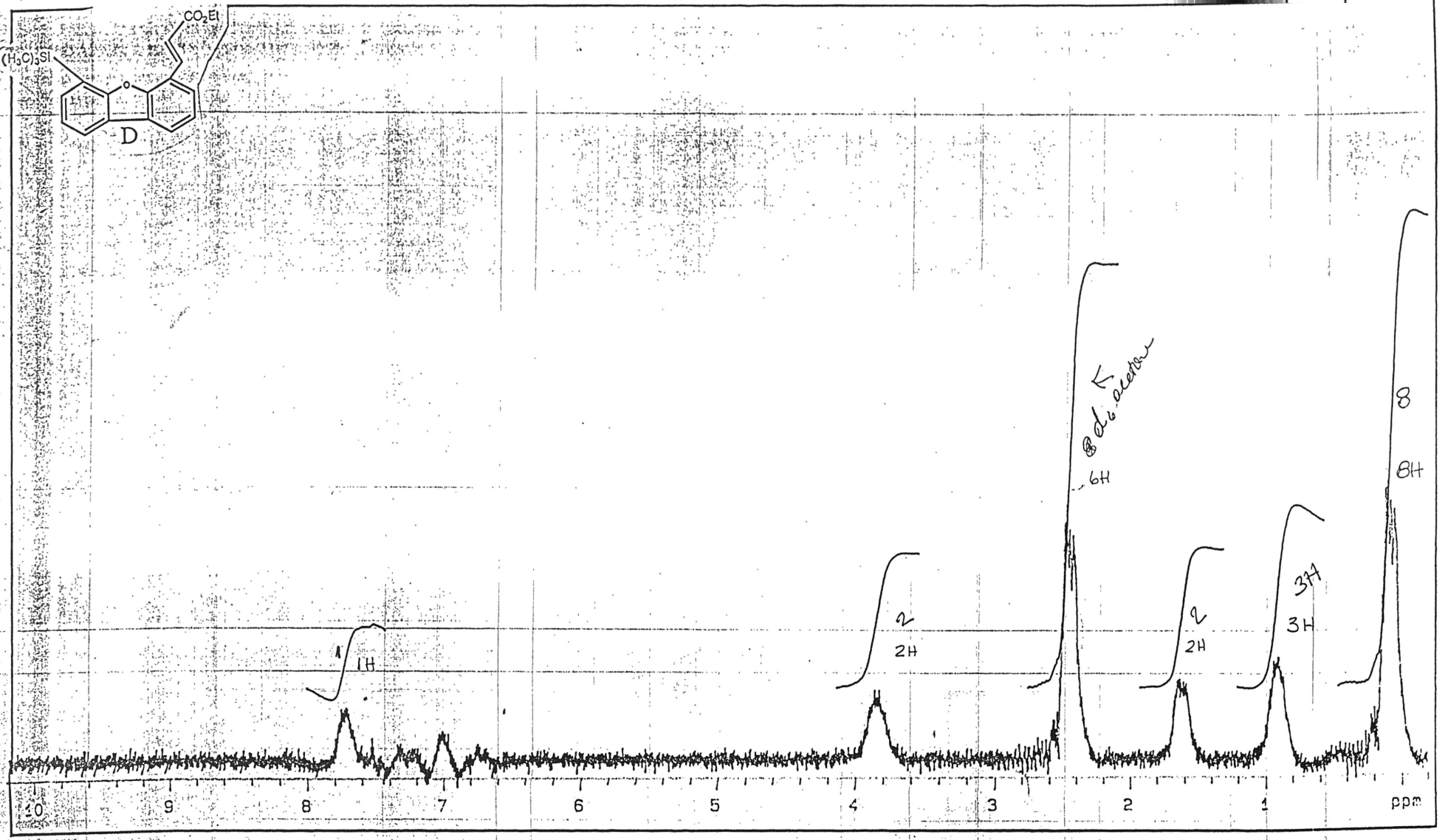
DECOUPLE
Nucleus H1 Offset 0 Hz
Mode nnn Power 1023 db
Modulation: Mode c Freq. 200 Hz
Pulse Width µsec Power Mode not used

PLOT/PROCESSING
FN not used K RE sec CD sec
LB not used Hz not used sec CCD
Width 2079.8 Hz/ppm Start -40.0 Hz/ppm
Reference

EXPERIMENT
Pulse Sequence s2pul
Probe
Temp. 25.0 °C
Solvent acetone

SAMPLE
STANDARD 1H OBSERVE
trimethylsilyl in dacetone

User
File exp
Date Feb 20 98
200



OBSERVE: Nucleus H1 | Freq. 199.9772 MHz | Spec. Width 2999.6 Hz | Offset 400.0 Hz | Acq. Time 1.995 sec | Delay 1.000 sec | Pulse Width 10.0 µsec | Transients 16

DECOUPLE: Nucleus H1 | Offset 0 Hz | Mode nnn | Power 1023 db | Modulation Mode C | Freq. 200 Hz | Pulse Width µsec | Power Mode not used

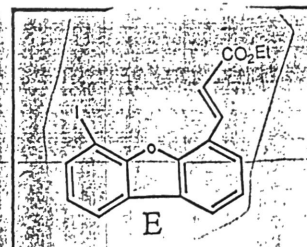
PLOT/PROCESSING: FN not used K RE sec CD sec | LB not used Hz not used sec CCD | Width 2079.7 Hz/ppm Start -40.3 Hz/ppm | Reference

EXPERIMENT: Pulse Sequence 2pul | Probe | Temp. 25.0 °C | Solvent acetone

SAMPLE

STANDARD 1H OBSERVE
ethylpropionate trimethylsilydib
e
nzofuran

User | File exp | Date May 8 97 | 200



Aromatics

2

Acetone

3

10 9 8 7 6 5 4 3 2 1 ppm

OBSERVE: Nucleus H1 Freq. 199.9772 MHz; Spec. Width 2999.6 Hz Offset 100.0 Hz; Acq. Time 1.995 sec Delay 1.000 sec; Pulse Width 10.0 µsec Transients 16

DECOUPLE: Nucleus H1 Offset 0 Hz; Mode nnn Power 1023 db; Modulation: Mode c Freq. 200 Hz; Pulse Width — µsec Power Mode not used

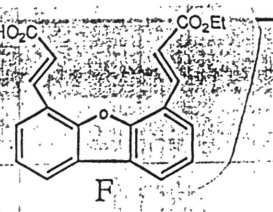
PLOT/PROCESSING: FN not used K RE — sec CD — sec; LB not used Hz not used sec CCD —; Width 2250.7 Hz/ppm Start -78.4 Hz/ppm; Reference —

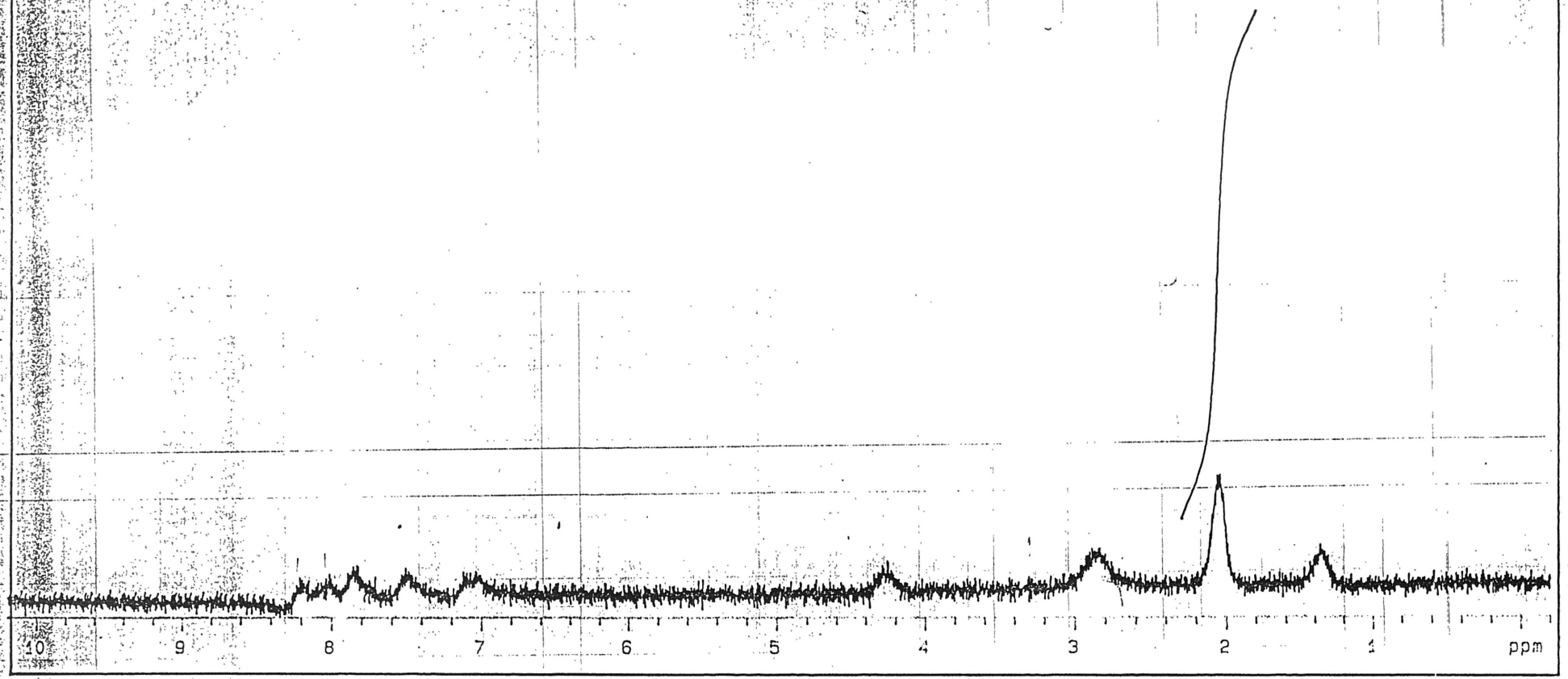
EXPERIMENT: Pulse Sequence 2pul; Probe —; Temp. 25.0 °C; Solvent acetone

SAMPLE

STANDARD 1H OBSERVE
iodo ethyl propoinate

User —
File exp
Date May 13 97
200





OBSERVE
Nucleus H1 Freq. 199.9772 MHz
Spec. Width 2999.6 Hz Offset 100.0 Hz
Acq. Time 1.995 sec Delay 1.000 sec
Pulse Width 10.0 µsec Transients 16

DECOUPLE
Nucleus H1 Offset 0 Hz
Mode nnn Power 1023 db
Modulation: Mode c Freq. 200 Hz
Pulse Width not used µsec Power Mode not used

PLOT/PROCESSING
FN not used K RE sec CD sec
LB not used Hz not used sec CCD
Width 2079.7 Hz/ppm Start -40.4 Hz/ppm
Reference

EXPERIMENT
Pulse Sequence s2pul
Probe
Temp. 25.0 °C
Solvent acetone

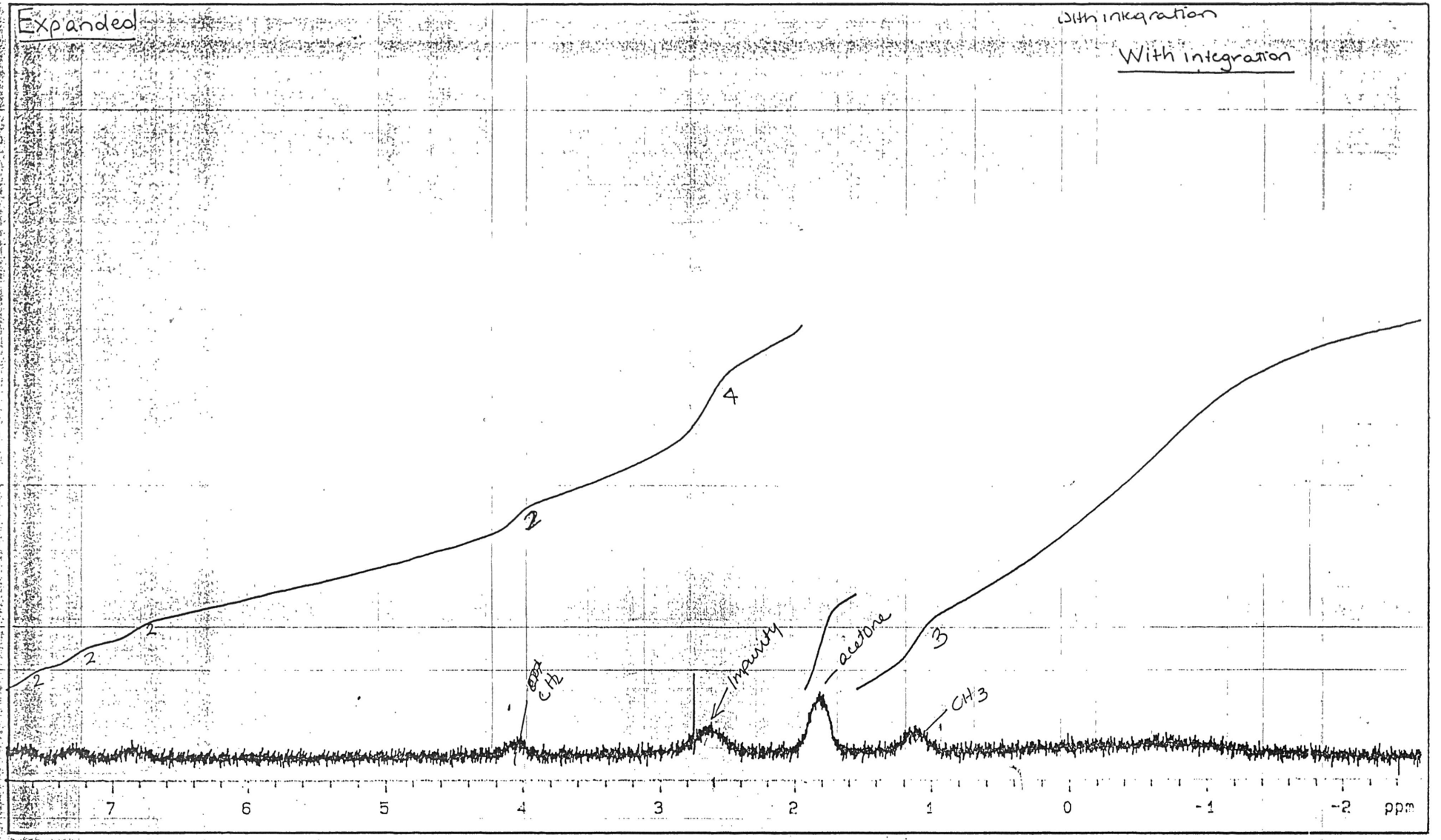
SAMPLE

STANDARD 1H OBSERVE
ethyl propenate dibenzofuran pro
p
oinic acid before hydragenation

User
File exp
Date May 13 97
200

Expanded

With integration



OBSERVE

Nucleus H1 Freq. 199.9762 MHz
Spec. Width 2999.6 Hz Offset -937.8 Hz
Acq. Time 1.995 sec Delay 1.000 sec
Pulse Width 10.0 µsec Transients 16

DECOUPLE

Nucleus H1 Offset 0 Hz
Mode nnn Power 1023 db
Modulation: Mode c Freq. 200 Hz
Pulse Width — µsec Power Mode not used

PLOT/PROCESSING

FN not used K RE — sec CD — sec
LB not used Hz not used sec CCD —
Width 2079.7 Hz/ppm Start -517.8 Hz/ppm
Reference —

EXPERIMENT

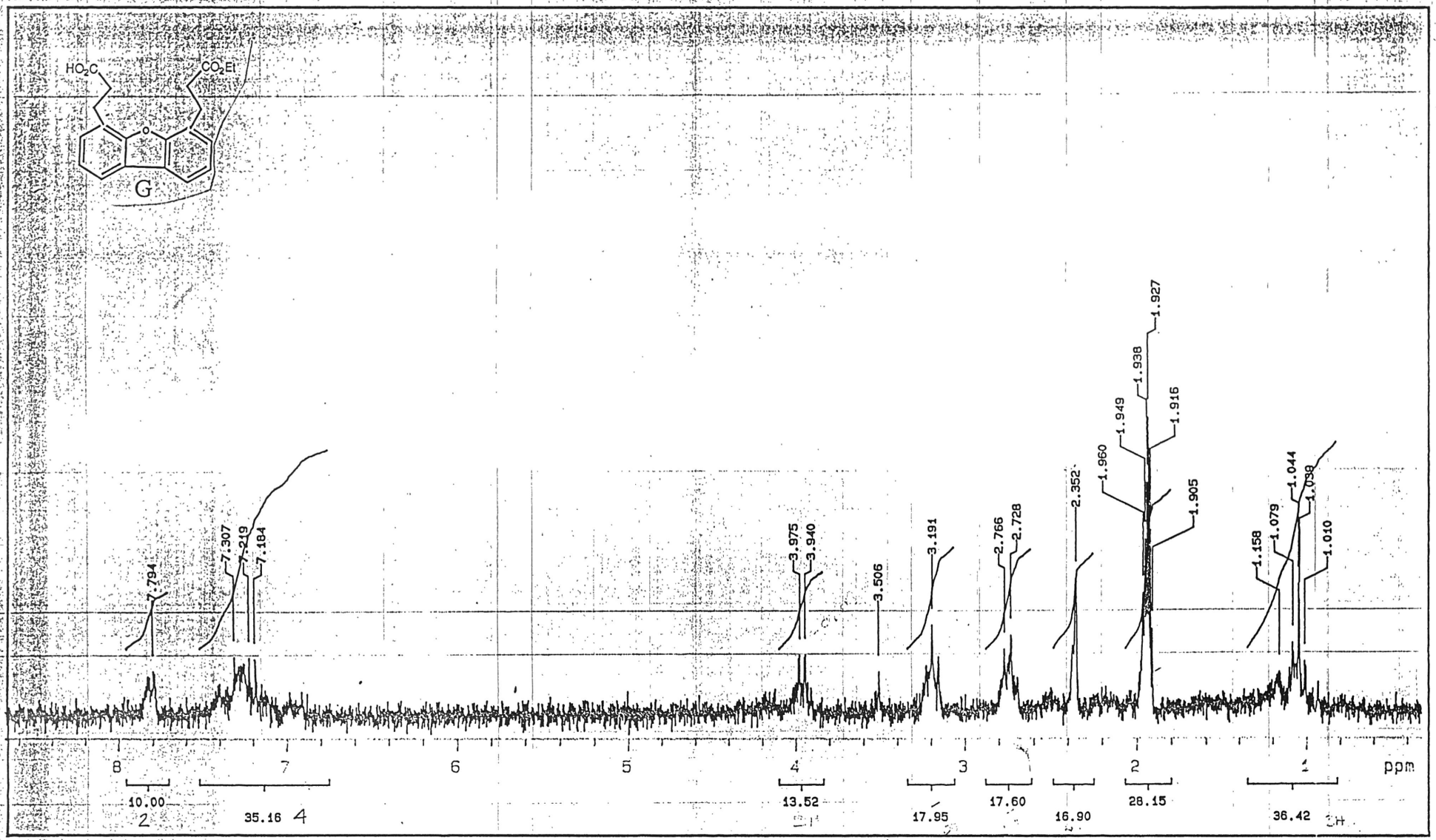
Pulse Sequence s2pul
Probe —
Temp. 25.0 °C
Solvent acetone

SAMPLE

STANDARD 1H OBSERVE
monoester monoacid no hydrogenat
1
on

User —
File exp
Date May 13 97
200

A



OBSERVE: Nucleus H1 — Freq. 199.9771 MHz; Spec. Width 3000.3 Hz — Offset 0 Hz; Acq. Time 2.000 sec — Delay 1.000 sec; Pulse Width 7.0 µsec — Transients 8

DECOUPLE: Nucleus H1 — Offset 0 Hz; Mode nnn — Power 1023 db; Modulation: Mode c — Freq. 200 Hz; Pulse Width — µsec — Power Mo not used

PLOT/PROCESSING: FN not used K RE — sec CD — sec; LB not used H not used sec CCD —; Width 1673.2 Hz/ppm Start 62.4 Hz/ppm; Reference —

EXPERIMENT: Pulse Sequence 2pul — SAMPLE; Probe —; Temp. 25.0 °C; Solvent Acetone

STANDARD 1H OBSERVE

User —; File exp; Date May 14 97; 200