PROGRESS TOWARDS THE NATURAL PRODUCTS BAUMYCIN AND THE AlPHA-2,3-SIALYL T ANTIGEN

By

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CHAPTER I

THE ALPHA-2,3_SIALYL T ANTIGEN

Infection and Pathogenesis

Infective endocarditis is a condition characterized by vegetative growths on the heart valves and surrounding tissues. The illness is associated with symptoms including fever, fatigue, and weight loss.[1,2] Ultimately the damage done by the vegetation can lead to abscess and heart failure in the infected individual. The cause of the condition can be traced to cellular adhesion of *Streptococcus gordonii* to blood platelets and subsequent colonization of vascular tissue. While rarely affecting healthy hearts, those suffering from damage are at a higher risk as platelets deposited on the affected tissue offer the bacteria an environment in which to proliferate.

Streptococcus gordonii is a Gram positive bacteria typically associated with the oral cavity and the buildup of plaque.[2] However, should a patient undergo oral surgery or should they have a wound exposed by some other means to *S. gordonii* , the bacteria can enter the blood system and cause infection. Typically this would not present an issue as the bacteria would swiftly be carried out naturally by the body's immune system, but in the case of patients who may be suffering from heart damage the bacteria becomes more virulent. Deposits of platelets left behind provide a surface for bacterial adhesion and once the cells adhere, a biofilm is excreted which prevents the colony from being washed

away into the bloodstream. With this protective barrier, the bacteria can grow more freely and becomes more invasive causing inflammation and damage to the tissue in and around the heart.

The typical course of treatment for the disease is a schedule of antibiotics, but other interests lay in the prevention of adherence. In this sense, a small molecule could potentially be developed that would block binding of the bacteria to blood cells. Little is known of the actual mode of binding of *S. gordonii* to platelets, but many groups have begun to study these events and some light has been shed on the subject in recent years.

In their studies, Bensing and Sullam discovered a mutant strain of *S. gordonii* which had its binding activity reduced to 50% of the wild type strain.[2,3] While phenotypically the same, genetic sequencing showed the deletion of a 8kb region of DNA from the genome. Encoded in this region were two secretory proteins secY and secA. Typically, such proteins are necessary for the translocation of vital proteins across cell membranes, but in the case of *S. gordonii* they were not necessary for cell growth and survival. Further examination of the surrounding genes lead to the discovery of an upfield, cell surface protein that the researchers dubbed GspB. It was assumed that this protein must be related to the ability of the bacteria to colonize heart tissue and adhere to cellular tissue.

In further publications, Bensing and Sullam have confirmed the dependence of *S. gordonii* on the protein GspB for platelet binding in addition to the fact that nine other species of Gram positive bacteria are known to contain homologues of this highly conserved protein. A key difference in the structure of GspB and its homologues resides

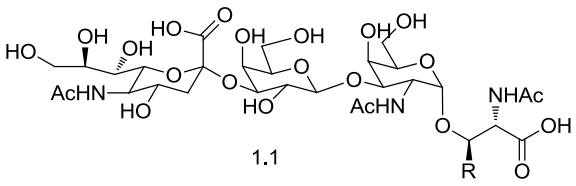
in BR (basic region) of the enzyme. It is believed that these differences are what impart the selectivity associated with the members of the family for binding to cellular surfaces. In particular, GspB has been shown to selectively bind the alpha linked 2,3-ST antigen over related carbohydrates. In the attempt to identify the target receptor protein for cell surface binding, Western Blot analysis showed the predominant candidate was the known cell surface protein GPIba, in which the sialic acid moiety is bound in the requisite configuration. It was also determined through use of fusion proteins that GspB did not bind any other associated cell surface proteins.[3] This information gives us some insight into how and where these proteins interact.

The mucin family of sialic acid associated glycopeptides was first discovered in the 1930's by two separate researchers.[4] Blix isolated sialic acid from the submaxillary mucin and Klenk found a neuraminic acid derivative in the brain glycolipids. Since that time, much research has been done on the synthesis of glycoproteins and investigation into their importance. Sialic acid itself is associated with cell surface recognition, cell communication, and immune system response. It is of little surprise that it should play a role in the mediation of cell surface interaction between *S. gordonii* and blood platelets.

Epithelial cancer cells are known to be rich in aberrantly expressed alpha-O-linked gylcosides and related studies have shown that introducing a synthetic carbohydrate can induce an immune-response in both mouse and human studies. This sparked the interest of using and developing these types of carbohydrates as synthetic vaccines against various forms of epithelial cancers. In one study by Danishefsky and co-workers, it was shown that by using a T-antigen disaccharide coupled to a carrier

protein, mice could be immunized against MCF-7 cancer cells leading to cell specific lysis.[5]

Fukuda has done significant work in the structural determination of the tumor associated oligosaccharides, many of which differ simply in the saccharide units or the linkages.[6] This work was published in two subsequent papers, of which one elucidates the structure of the trisaccharide associated with the 2,3-ST antigen. One of the predominant researchers in the field, Samuel Danishefsky, has published numerous syntheses of various blood associated antigens, including the 2,3-ST antigen which is of interest in the case of GspB and binding of *S. gordonii* to platelets. It is this work that we follow in order to synthesize our glycopeptides.[7,8,9]



1.1

Figure 1. Structure of the 2,3-ST Antigen (R = Me or R =H).

Given that the selectivity for GspB and its homologues is imparted by the BR, it is assumed that this region contains the active site responsible for mediating binding to GPIba. The short section of amino acids has been cloned, purified, and crystallized in an attempt to determine the structure of the active site by the lab of Dr. Tina Iverson (Vanderbilt University). Unfortunately, there has not been much luck in elucidating

where the protein would actually bind the respective carbohydrate. Our work revolves around the synthesis of the known 2,3-ST antigen in the hopes that co-crystallization would divulge definitively where and how the protein binds its associated carbohydrate ligand (i.e. 2,3-ST antigen). We hope to successfully visualize the native protein and carbohydrate without modification, however, given the serine rich nature of the protein we may require attachment of a tag to the carbohydrate ligand in order to aid in locating the binding site.

GspB Probe Synthesis

Starting from commercially available D-galactal, we were able to protect the C6 alcohol as a silyl ether. From here, initially we had tried to protect the 3,4-diol as an acetonide but unfortunately found that during the process, we added methanol to the glycal double bond to form the corresponding methyl acetal. In a subsequent attempt, we were able to successfully protect the diol as the carbonate, but attempts to oxidize the compound to the azido nitrate proved unfruitful. Consulting the literature, it was discovered that under the neutral conditions of DDQ and 2,2 dimethoxypropane we could successfully protect the 3,4-diol of **1.3**.[10] Using CAN and NaN_3, we were then able to form the azido-nitrate as described in Danishefsky's earlier work. Hydrolysis of the nitrate using thiophenol gave the free hydroxyl at the anomeric position, which was converted to the trichloroacetimidate to provide the glycosyl donor.

The serine donor required for the coupling to glycosyl donor **1.6** was prepared starting from commercially available O-t-butyl-N-Fmoc-serine via benzylation of the

carboxylic acid followed by acid promoted hydrolysis of the t-butyl ether to give **1.7a**.[11] The donor and acceptor were subsequently coupled using TMSOTf as the promoter to afford the alpha-linked product. Using iodine and methanol, both the silyl group and acetonide were cleaved to afford an intermediate triol which was in turn protected as the C6 TBS silyl ether (1.8??) according to literature precedence.[12]

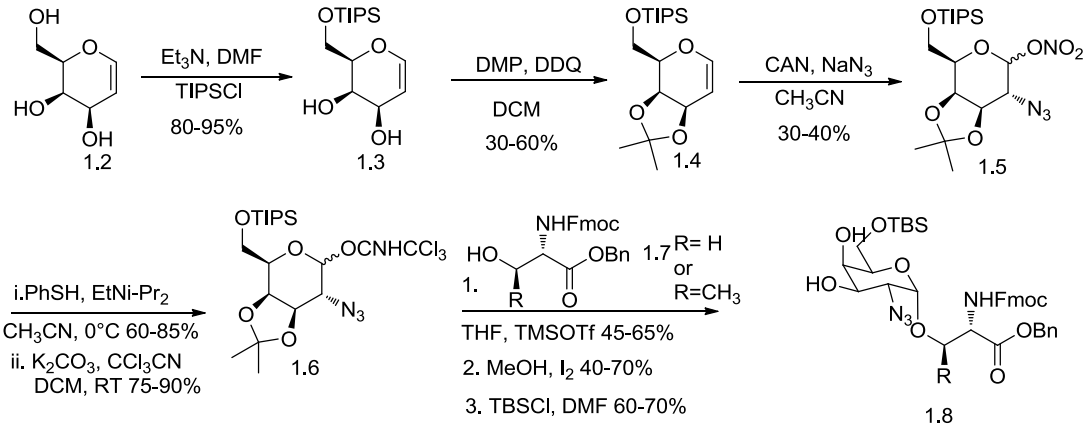


Figure 2. Synthesis of serine appended D-galactal moiety

To form the requisite disaccharide for the final coupling, commercially available tetra-O-acetyl, N-acetyl neuraminic acid was condensed with chloro pinacolphosphite to give **1.10** as a white solid. This glycosyl donor was subjected to TMSOTf promoted coupling with previously prepared 6-O-TIPS-D-galactal (**1.3**) to form disaccharide **1.11**. Epoxidation of glycal **1.10** with dimethyldioxirane followed by immediate oxirane opening with ethane thiol under acidic conditions gave thioglycoside **1.11**. The C2

hydroxyl group was protected as a benzoate and the silyl group was exchanged for an acetate to furnish our required glyclosyl donor.

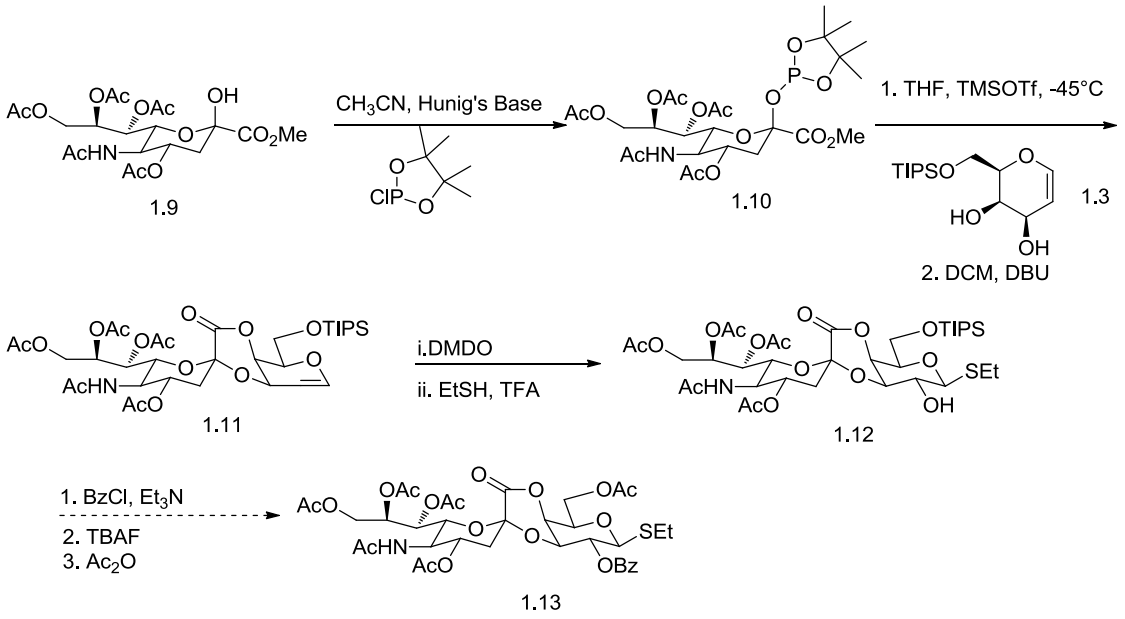


Figure 3. Synthesis of the sialic acid disaccharide donor **1.13**

For the key coupling of **1.8** and **1.13** we planned to employ the Fraiser-Reid protocol (NIS/TFA in dichloromethane). From that point, the silyl group will be removed using HCl in THF followed by acetylation of the primary alcohol. The azide will be reduced/coupled using thioacetic acid to furnish the corresponding acetamide. Morpholine will remove the Fmoc protecting group and the free amine will be acetylated. All of these protocols are based on Danishefsky's earlier synthesis of the 2,3-ST antigen. It is at this point that we could exchange the acetyl group for a linked fluorescent tag. This could aid in the visualization of our compound bound to the protein structure.

Hydrogenation removes the benzyl group and saponification opens up the lactone and the benzoate is removed to yield the desired compound.

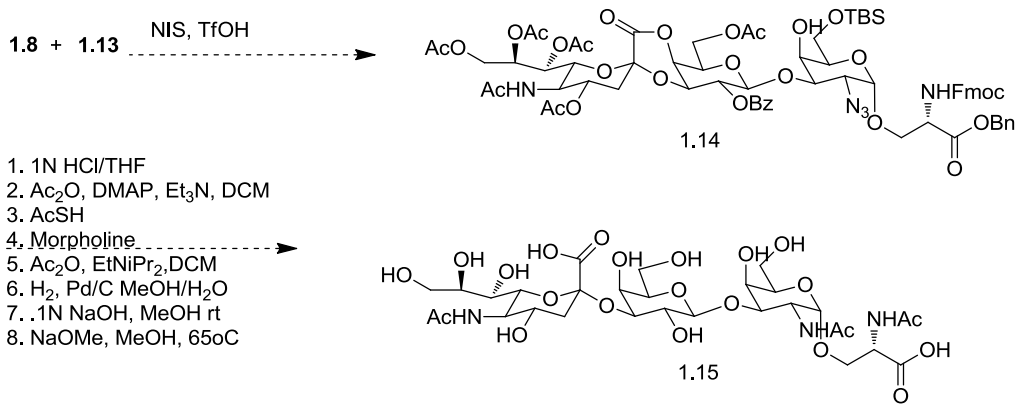


Figure 4. Completion of the 2,3-ST Antigen

Conclusions

The basic region is responsible for determining the selectivity of GspB and related proteins. Using the 2,3-ST antigen, we hope to probe the small basic region of the protein. After completion of the synthetic route, future work will entail the co-crystallization of our synthetic trisachharide with the BR of GspB followed by crystallographic experiments to visualize the protein-carbohydrate complex. We hope that this would help to determine the size, shape, and environment of the active site. Furthermore, once the active site has been identified it is foreseeable that a library of small molecule inhibitors could be developed to screen against GspB for activity. Using this information, one could envision developing a treatment for the disease which would not rely on the efficacy of antibiotics on killing the infection, but would rather prevent the infection from spreading.

CHAPTER II

BAUMYCIN

History in Brief

Anthracyclines are an interesting class of natural products of which the first reported isolation of rhodomycin dates back to 1950 by Brockman and Bauer.[13] This class of natural products has shown high efficacy as antitumor agents in various lines of cancer cells as well as antibiotic activity. Doxorubicin (adriamycin) isolated from *S. peucettius* was the first of the class to be approved for clinical usage in 1974.[13]

The mechanism of action of the anthracycline class is in part what makes them interesting to researchers. Anthracyclines are known to intercalate DNA and cause an unwinding of the helical structure. Additionally certain members such as doxorubicin and daunorubicin can covalently crosslink segments of DNA. The amine moiety of the daunosamine sugar can act as a crosslinking agent in combination with formaldehyde and irreversibly link with DNA through a guanine amine. Furthermore, it has been shown that such drugs can stabilize a transient form of the topoisomerase II enzyme in its cleavable state. This inhibits DNA and RNA synthesis halting the process of cellular division.[13]

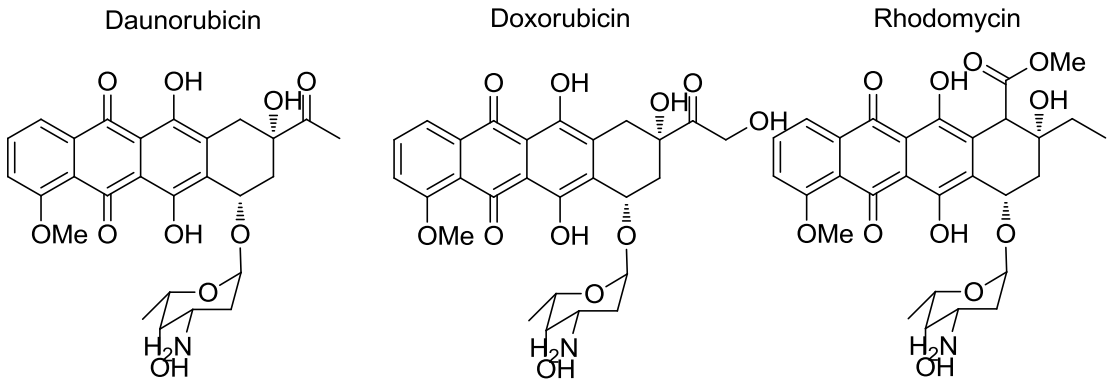


Figure 5. Some common anthracyclines.

Baumycins were first described by Umezawa et al in 1977 as 3 epimeric pairs of anthracycline compounds (A_1/A_2, B_1/B_2, C_1/C_2), isolated from *S. coeruleorubidus* ME130-A4.[14] It has been shown that baumycins A_1/A_2 are the major product of fermentations for several species of *Streptomyces,* leading to the assumption they are the final biosynthetic product rather than daunomycin and that the hydrolysis of the acetal is an artifact of isolation.[13]

Baumycin A_1 exhibited high efficacy against leukemia cells (L1210) but the members of the baumycin family were not pursued as clinical candidates because of their instability and difficulty in recovering them from fermentation broths.[13] The acetal moiety of baumycin is conjectured to derive from the condensation of the 4'-hydroxyl of daunosamine with a carbohydrate via a glycosyltransferase, however, conversion to the acetal is not fully understood and is under investigation. Of particular note as well is that the stereochemistry of the acetal has never been elucidated.

Baumycin has been isolated from a cave dwelling actinomycete bacteria by Dr. Brian Bachmann's group (Vanderbilt University). This suggests that the secluded ecosystems found in subterranean environments may yield species which produce interesting, active secondary metabolites. Starting from the advanced intermediate daunomycin, a semisynthetic route to baumycin has been proposed. Through synthetic methods, the different stereoisomers can be generated in order to assign the relative configuration of the acetal substituent of the natural product.

Three stereocenters are unassigned for the dissacharide, namely the configuration of the anomeric center, the relative stereochemistry of the secondary alcohol at C3 as well as the stereochemistry at C5. It is hypothesized that the diastereomers could arise from a mixed reduction product at the C3 center, but we cannot rule out the possibility of a mixture of D and L sugars as evidenced by work done on rubeomycin or potentially an anomeric mixture[15]. One stereocenter is set using L-rhamnose in our synthesis while the anomeric position and the geometry about the secondary alcohol are to be determined via experiment. However, as nature tends to favor the alpha-anomer, this will be our main consideration for the stereochemistry at C1.

Synthetic Strategy

It has been hypothesized that baumycin could come from the oxidative cleavage of a 3,4 hydroxy sugar followed by reduction of the corresponding keto-aldehyde[13]. This 2-deoxy-glycoside could arise from the oxidative coupling of daunomycin with an appropriately protected glycal followed by reduction and deprotection. The 2-deoxy glycal can be obtained from the sugar rhamnose (Figure 6).

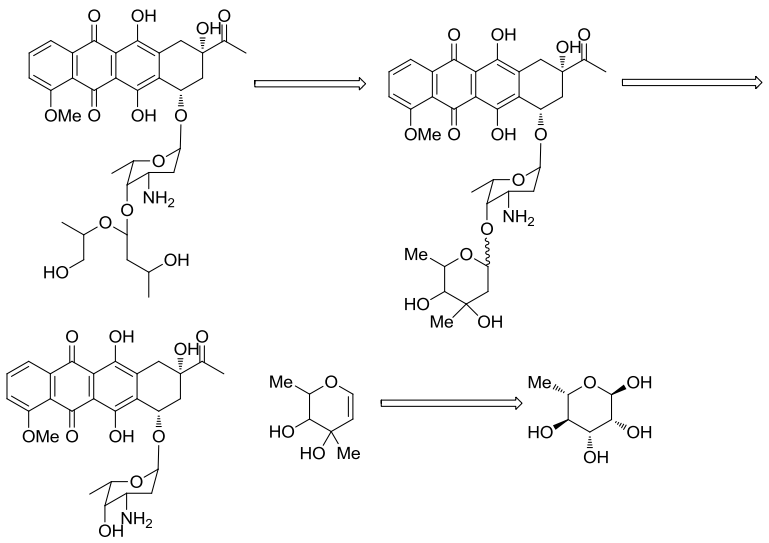


Figure 6. Baumycin retrosynthesis

Conversion of L-rhamnose to L-rhamnal serves as a starting point for the synthesis and defines the C5' stereocenter. Following a modified Fischer-Zach protocol, L-rhamose is peracetylated followed by conversion to the crude bromide (Scheme 2). Using a buffered solution of zinc and copper sulfate, the bromide is converted to the 3,4-di-O-acetyl rhamnal.[16] Deprotection of the alcohols allows the selective oxidation of the allylic alcohol by PDC and treatment with methyllithium yields the known C_3 branched glycals in a 4:1 ratio.[17] Protection of the secondary alcohol as the acetate provides the necessary substrate for the key oxidative coupling of **2.6** to daunomycin.

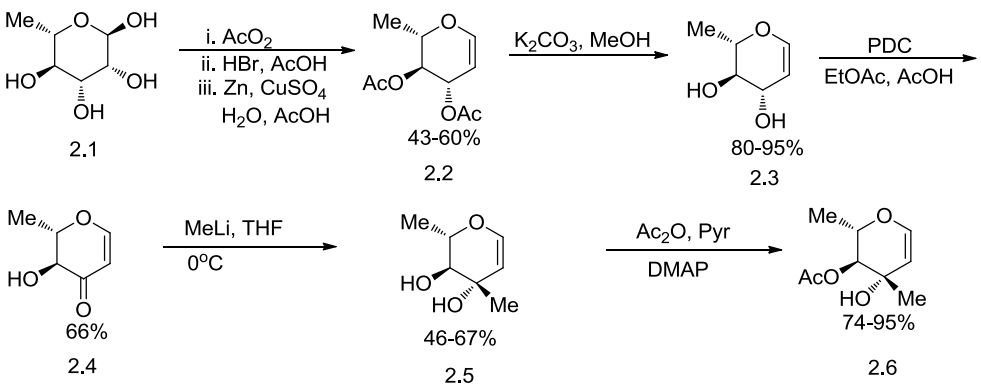


Figure 7. Glycosyl donor synthesis.

Using cyclohexanol for a model system in place of expensive daunomycin, the glycosylation was performed using N-iodosuccinimide as the promoter affording a mixture of alphaand beta isomers of the iodo-pyranose product which were separated by flash chromatography.[17] Using triethylborane as the radical promoter in the presence of atmospheric oxygen and triphenyltinhydride, the iodo group was reduced to afford in good yield the 2-deoxy glycoside.[18] We chose this method as typical reflux conditions (AIBN initiator) led to decomposition. Removal of the acetate followed by oxidative cleavage of the diol using lead (IV) acetate produced the crude keto-aldehyde which was immediately subjected to $NaBH_4$ reduction to furnish the acetal **2.10**.[19] as a 1:1 mixture isomers. The mixture of isomeric alcohols was treated with benzoic anhydride resulting in selective protection of the primary alcohol to afford **2.11**.

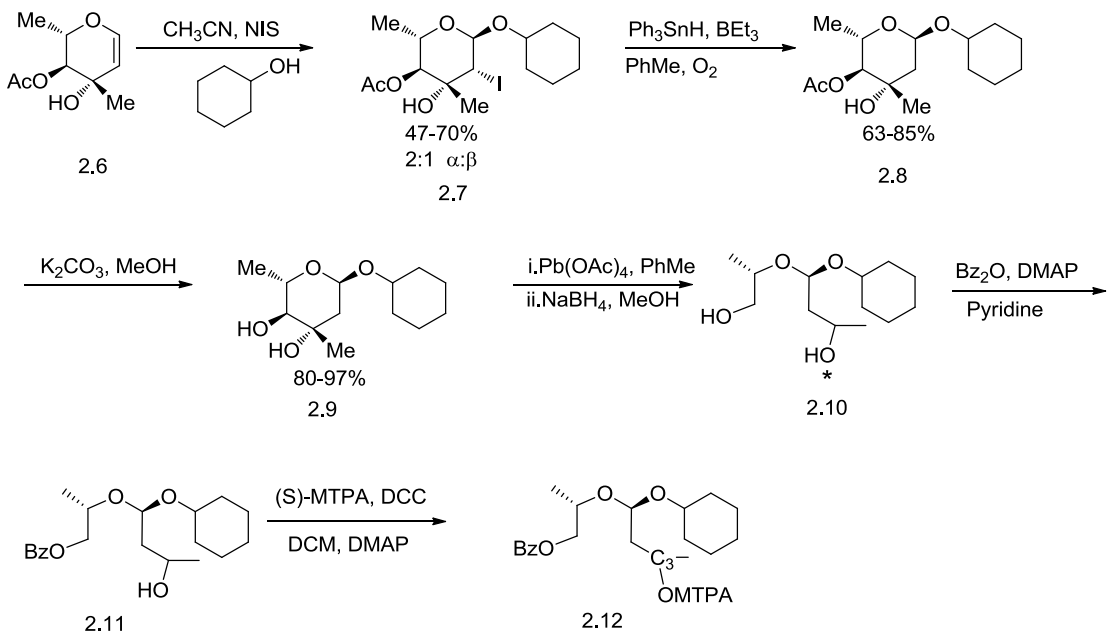


Figure 8. Cyclohexyl model system.

Selective protection of the primary alcohol allowed for esterification of the remaining secondary alcohol using (S)-Mosher's acid in an attempt to assign stereochemisty. Comparing the chemical shifts of the diastereomers by ^{1}H NMR, we assigned the relative stereochemistry at C3. According to a paper by Riguera, the ap (antiperiplanar) conformer is the major contributor leading to an overall deshielding of the C3' proton of one isomer relative the other, so we correlated the shifts expected of the corresponding Mosher esters to what we observed in our experimental evidence.[20] This differs from the classical method Mosher analysis where both the (R)- and (S)- acids are used. Traditionally it is the difference of the chemical shifts for substituents which indicates stereochemistry.

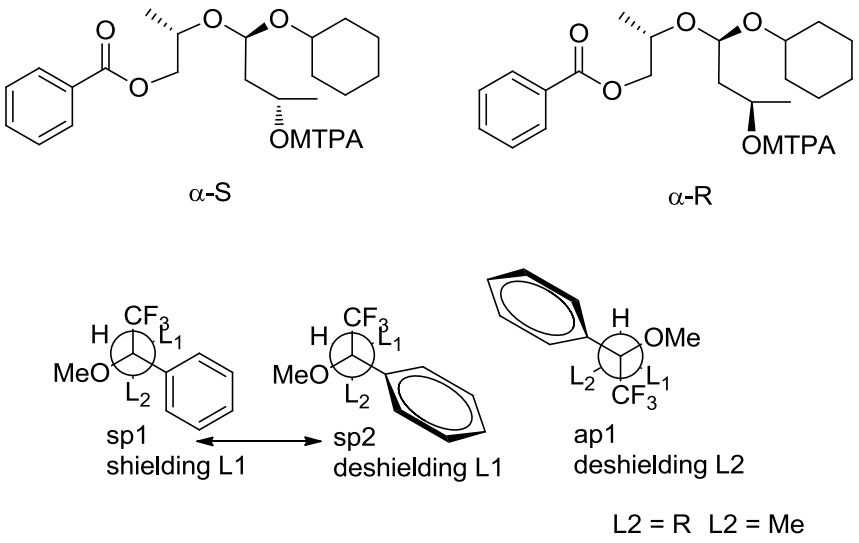


Figure 9. Mosher model comparison

Table 1. Chemical shifts of diastereomers

Proton	α-R	α-S
1	4.63	4.88
3	5.27	5.35
4	1.45	1.37
5,5'	4.26	4.27,4.39
6	4.01	4.1
7	1.17	1.21
1'	3.55	3.6

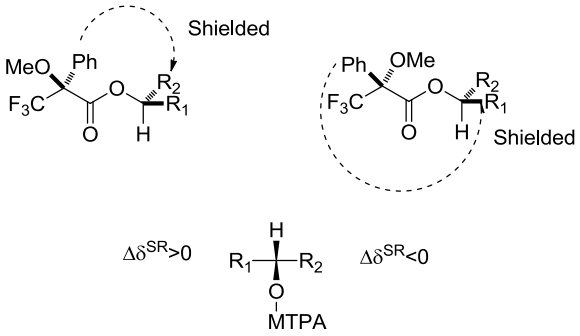


Figure 10. Mosher's Classical Model

Next we attempted to couple glycal **2.6** under identical conditions to N-acetyl daunomycin, unfortunately this reaction did not proceed. Apparently, the axial hydroxyl of the daunosamine sugar proves too unreactive towards NIS mediated glycosylation. Consulting work by Wang and co-workers we discovered that the identical daunomycin glycosyltion was reported using the a thioglycoside as the donor, the coupling was reportedly achieved using a silver salt as the promoter[21]. Starting from rhamnal (**2.1**), we affected coupling with isopropyl alcohol in the anomeric position yielding a mixture of known monosaccharide after reduction. Lewis acid catalyzed the exchange of the isopropyl acetal with thiophenol under the promotion of $BF_3 \cdot OEt_2$ furnished our desired thioglycoside donor. Starting from daunomycin hydrochloride salt, we were able to synthesize the trifluoroamide to provide glycosyl acceptor **2.14** in good yield. Unfortunately, all attempts to couple thioglycoside **2.17** with the N-trifluoroacetamide daunomycin failed to yield desired disaccharide leading instead to slow decomposition.

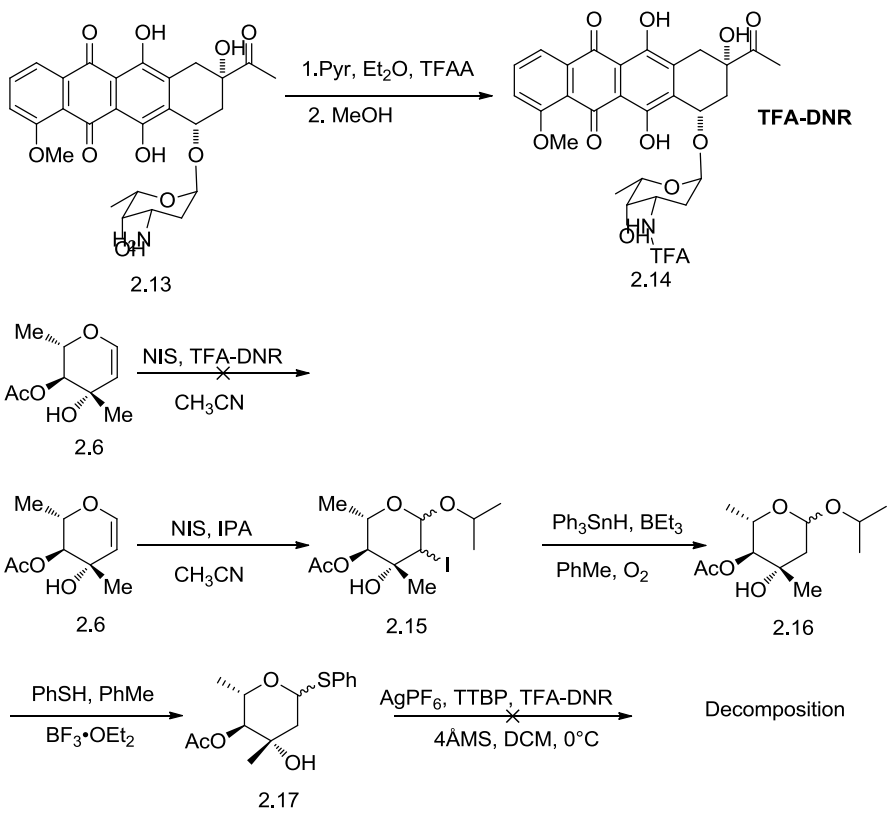


Figure 11. Attempted coupling of DNR and glyosidic donor

Conclusions

Future directions of this project would obviously entail formation of the disaccharide followed by the chemical transformation of the product to the requisite diol. It is hoped from that point we could derivatize the compound to form the Mosher ester to determine the stereochemistry about C3. Once we know this, it is foreseeable that derivatization of the natural product in the same manor would tell us if the compound were a mixture of R and S isomers about C3 and determine which configuration we have. However, if the spectra of the synthetic product and the natural do not match, our

assumption of C3 being the source of the diastereomers is incorrect and either it is a mixture of anomeric products or D and L sugars. To determine this, it would be possible to start from the D-rhamnose substrate and compare the isomeric series. Despite the prevalence of baumycin, it is surprising that the stereochemistry still remains unknown. Given the fragile nature of the acetal, this natural product is extremely difficult to work with.

As an interesting side project Brian Bachmann's lab, which isolated the product in question, has discovered an interesting oxidative enzyme. It is proposed that this enzyme may be responsible for oxidizing the disaccharide to effect a Criegee type fragmentation to the keto-aldehyde. With the disaccharide in hand, it would be interesting to see if this enzyme could in fact produce the product of the oxidative cleavage. With some positive results, potential justification for exploring more isolated cave ecosystems in attempt to discover more novel natural products could be found. The discovery of this new enzyme could also be a significant find.

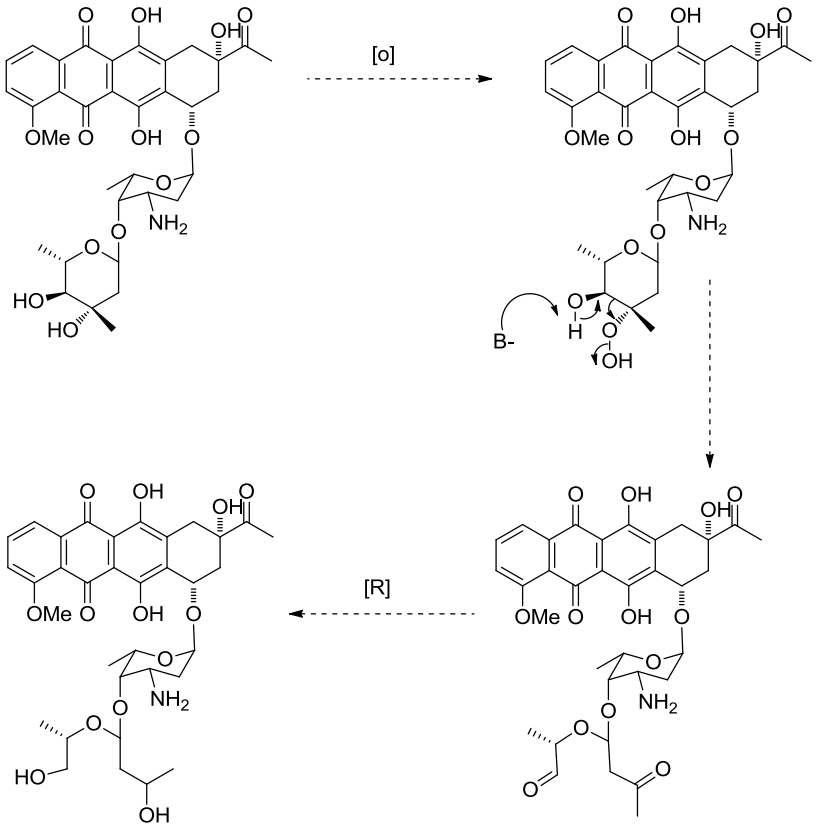
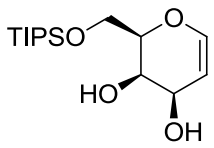


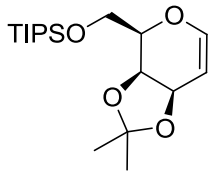
Figure 12. Criegee Rearrangement

Experimental



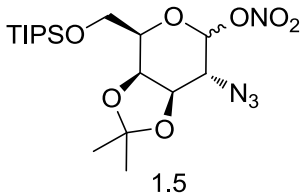
1.3

To D-galactal (3.125 g, 21.4 mmol) in DMF (8.5 mL) was added Et_3N (8.9 mL) with stirring at room temperature. After 30 min, TIPSCl (5.15 mL, 23.5 mmol) was added and the reaction was stirred for 24 hours. The reaction was washed with 50mL of H_2O and extracted 3x30mL with EtOAc, dried (MgSO4), concentrated and purified by silica gel chromatography with Hexane/EtOAc (3:1). Product was obtained as a pale, yellow oil (5.048g, 78%) . The 1H NMR was identical to published data. [8]



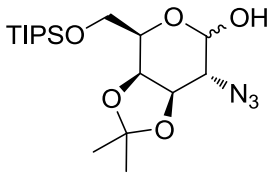
1.4

To 6-O-TIPS-d-galactal (5.048g,16.7mmol) in dichloromethane (104mL) at room temperature was added 2,2-dimethoxypropane (6.14Ml, 50.1mmol) followed by a catalytic amount of DDQ (379mg, 1.67mmol). The reaction was allowed to stir at room temperature for 3 days at which time the solvent was evaporated and the residue was purified by flash chromatography with Hexane/EtOAc (30:1) to give 2.877g (50%) of viscous oil. The 1H NMR was identical to published data. [8]



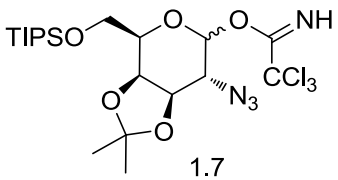
1.5

To the acetonide (698.7mg, 2.09mmol) in CH_3CN was added CAN (3.355g, 6.12mmol) followed by NaN_3 (265mg, 4.08mmol) at -20˚C with vigorous stirring. After 20 hours the reaction appears to be complete. It was diluted with EtOAc , washed with cold H_2O and saturated brine solution, then dried (MgSO4), concentrated and the residue was purified by flash chromatography Hexane/EtOAc (40:1) to give 298mg (32%) of a clear oil. The 1H NMR was identical to published data.[8]



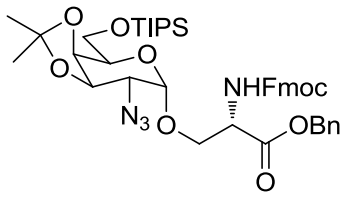
1.6

To the azido-nitrate (289mg, .648mmol) in acetonitrile (2.2mL) at 0˚C was added Hunig's base (113uL, .648mmol) followed by PhSH (197uL, 1.944mmol) slowly dropwise. The reaction was allowed to stir for 2 hours and the solvent was evaporated. The product was purified by flash chromatography Hexane/EtOAc (25:1) to give 160mg (61%) of a clear oil. The 1H NMR was identical to published data.[8]

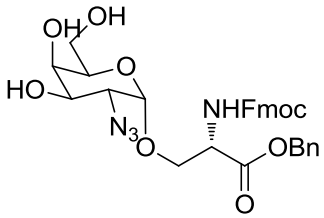


1.7

To the azido sugar (100mg, .249mmol) in dichloromethane (2.5mL) was added K_2CO_3 at 0˚C followed by trichloroacetonitrile (250uL, 2.49 mmol) with stirring. After five hours, the reaction was filtered through Celite and concentrated. The residue was purified by flash chromatograpy Hexane/EtOAc (25:1) to give 92.4mg (70%) of a white solid and 21.4mg of starting material. The 1H NMR was identical to published data.[8]

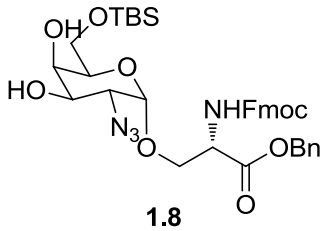


To a mixture of peptide(230mg,.54mmol) and trichloroacetimidate (200mg,) in THF (3.6mL) was added freshly activated, powdered 4A molecular sieves (800mg). After 30 minutes of stirring, the reaction was cooled to -78˚C and TMSOTf (33uL in .76mL of THF) was added dropwise. After stirring for two hours, the reaction was quenched with Et_3N and filtered through Celite with EtOAc. The reaction was washed with H_20 and saturated brine, dried (MgSO4), concentrated and purified by flash chromatography Hexane/EtOAc (10:1) with 2% Et_3N. Product was obtained as a clear oil (161mg, 56%)



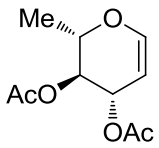
To the glycopeptide (400.4mg, .50mmol) in MeOH (8.3mL) was added I_2 (381mg, 1.50mmol) with stirring at room temperature. The reaction was allowed to stir overnight

(16hours) and was diluted with EtOAc. It was quenched by washing with saturated $Na_2S_2O_3$ then washed with brine and dried ($MgSO_4$) and concentrated. Purification via column with 1:1 Hex:EtOAc gave 208mg of white foam (69%). The 1H NMR was identical to published data.[12]



1.8

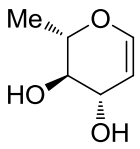
To the triol (66mg, .11mmol) in DMF (.55mL) was added imidazole (30mg, .44mmol) with stirring. The reaction was cooled to 0˚C and TBSCl (33mg, .22mmol) was added. The reaction was allowed to slowly warm to rt and was stirred overnight (16hrs) then diluted with EtOAc and washed with H_2O then brine solution. The organics were dried ($MgSO_4$), concentrated, and purified by flash chromatography with 2:1 Hex:EtOAc to give a white foam (55mg, 70%),



2.2

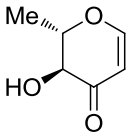
To a solution of Ac_2O(30.6mL, .324mol) was added l-rhamnose monohydrate (10g, .055mol) slowly. A water bath was used to maintain the reaction at room temperature. After stirring for 5hr, 33%HBr in AcOH (18.8mL, .104mol) was added dropwise and the reaction was stirred overnight (17hr). A buffered solution was prepared by mixing $NaOAc{\cdot}3H_2O$ (7.076g, 52mmol) in water (60mL) with AcOH (61mL) at 0^oC with

mechanical stirring. The crude bromide was added slowly, dropwise at 0°C. The reaction was stirred 4hr at room temperature then filtered through celite and extracted 4x100mL of EtOAc. The organics were washed with cold water cold saturated sodium bicarbonate, saturated brine and water . The organics were dried ($MgSO_4$) and concentrated. The residue was purified by gradient elution of 6:1 Hex:EtOAc to 4:1 Hex:EtOAc until elution. A white crystalline solid (6.99g, 60%) was obtained. The 1H NMR was identical to published data.[3]



2.3

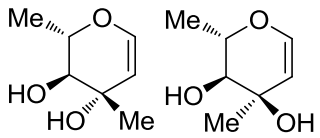
To MeOH(120mL) was added l-rhamnal (2.52g, 11.8mmol) with stirring followed by K_2CO_3 (4.88g, 35.34mmol) at ambient temperature. After 24hr the solvent was evaporated and the product was dissolved in Et_2O/EtOAc and washed with water to remove residual salt. The product was dried ($MgSO_4$) and concentrated to yield l-rhamnal (1.33g, 87%). The 1H NMR was identical to published data.[16]



2.4

To a solution of EtOAc (220mL) and AcOH (4.8mL) was added l-rhamnal (2.871g, 22.1mmol) with stirring at ambient temperature. After 17hr the reaction was filtered through a mixture of celite/silica gel to remove Cr. The organics were washed with saturated $NaHCO_3$ and dried ($MgSO_4$). Evaporation of the solvents yields white crystals

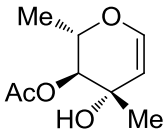
(1.86g, 65%). ^{1}H NMR ($CDCl_3$, 300MHz) 7.38 (d, J = 5.7Hz, 1H), 5.45 (d J = 5.8Hz, 1H), 4.19 (dq, 1H), 3.97 (d, J = 13Hz, 1H), 1.56 (d, J = 6.2Hz, 3H). The 1H NMR was identical to published data.[16]



2.5a 2.5b

To THF (72mL) at-78 ^{o}C was added the glycal (1.86g,14.4mmol) with stirring. To this was added MeLi (18mL, 1.6M in Hexanes). After 30 min an additional aliquot of MeLi was added to push the reaction to completion. The reaction was poured onto ice and extracted with EtOAc. The organics were dried ($MgSO_4$) and then concentrated. Flash chromatography (3:1 Hex:EtOAc) gave 943mg (46%) of product (**1:4**). ^{1}H NMR ($CDCl_3$,300MHz) 4b: 6.18 (d J = 6Hz, 1H), 4.69 (d, J = 6Hz, 1H), 3.86 (m, 1H) 3.62 (d, J = 9.9Hz, 1H) 3.02 (bs 1H), 2.32(bs, 1H) 1.37 (d, J = 6.2Hz, 3H), 1.32 (s, 3H)

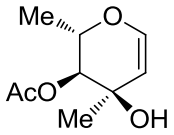
4a: 6.34(d, J = 5.9Hz, 1H), 4.81 (d, J = 5.9Hz, 1H), 3.71 (m, 1H), 3.23 (t, J = 10.1Hz, 1H), 2.49 (d, J = 9.9Hz, 1H), 1.80 (s, 1H), 1.40 (d, J = 6.2Hz, 3H), 1.34 (s, 3H).



2.6a

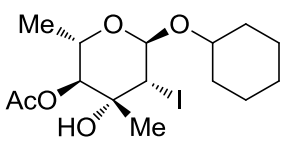
To the glycal (100mg, .694mmol) in pyridine (6mL) was added Ac_2O (131µL, 1.39mmol) and a catalytic amount of DMAP. The reaction was stirred for 24hr then

azeotroped with toluene. Flash chromatography with 2:1 Hex:EtOAc yield product as a clear oil (115mg, 89%): $[\alpha]^{20}_{D}$ = -92.29° (*c*. 0.0858, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400MHz) 6.19 (d, J = 6Hz, 1H), 4.90 (d, J = 10Hz, 1H), 4.73 (d, J = 6Hz, 1H), 3.93 (dq, J = 10Hz, J = 6Hz, 1H), 2.11(s, 3H), 1.26 (s, 3H), 1.24 (d, J = 6Hz, 3H) ^{13}C: 171.2, 142.3, 108.0, 78.8, 71.9, 69.6, 24.6, 20.8, 17.3. IR: 3449, 2984, 1735, 1649, 1453, 1376, 1237, 1078, 1055.

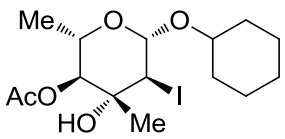


2.6b

To the glycal (100mg, 0.694mmol) in pyridine (6mL) was added Ac_2O (131µL, 1.39mmol) and a catalytic amount of DMAP. The reaction was stirred for 24hr at which time an additional aliquot of Ac_2O was added. The reaction was stirred another 24hr then azeotroped with toluene. Flash chromatography with 2:1 Hex:EtOAc yield the product as a clear oil (96mg, 74%): $[\alpha]^{20}_{D}$ = -134.49° (*c*. 0.024, $CHCl_3$.) ^{1}H NMR ($CDCl_3$, 400MHz) 6.30 (d, J = 6Hz, 1H), 4.79 (d, J = 10.4Hz, 1H) 4.74 (d, J = 6Hz, 1H) 4.12 (dq, J = 6.4Hz, J = 12.8Hz) 2.13 (s, 3H), 1.20 (d, J = 6.4 Hz) 1.19 (s, 3H). ^{13}C: 170.2, 145.1, 105.6, 76.6, 69.3, 66.5, 26.1, 20.7, 16.8. IR: 3457, 2980, 2937, 1739, 1644, 1375, 1244, 1137, 1053.



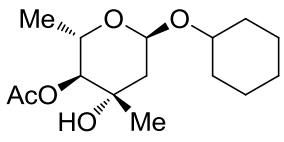
2.7a



2.7b

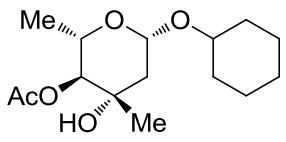
To a solution of acetylated glycal (115mg, .617mmol) in dry CH_3CN was added cyclohexanol (77µL,.741mmol) followed by NIS (208mg,.926mmol). The reaction was stirred for 3 days. The reaction was washed with saturated sodium thiosulfate and saturated sodium bicarbonate. The aqueous layer was extracted with EtOAc (2x10mL) and dried ($MgSO_4$). The organics were concentrated and the residue was purified to provide by flash chromatography alpha and beta glycosides (2:1 alpha: beta, 105mg,41%) where some residual cyclohexanol remained in the fraction with the beta product. Alpha: $[\alpha]^{20}_D$ = -34.04° (*c*. 0.0228, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400MHz) 5.35 (d, J = 2.8Hz, 1H), 4.94 (d, J = 8.4Hz, 1H), 4.33 (d, J = 2.8Hz, 1H), 3.94 (m, 1H), 3.59 (m, 1H), 2.32 (s, 1H), 2.12 (s, 3H), 1.80 (m, 2H), 1.70 (m, 2H), 1.50 (s, 3H), 1.22 (d, J = 9.2Hz, 3H) ^{13}C: 170.8, 99.8, 76.8, 76.3, 71.7, 67.6, 47.1, 33.2, 31.5, 25.6, 23.9, 23.6, 22.3, 20.9, 17.7 IR: 3482, 2932, 2857, 1745, 1451, 1376, 1233, 1036. LCMS ($M+H^+$) = 412.26, found ($M+Na^+$) = 435.1

Beta: $[\alpha]^{20}_D$ = -42.93° (*c*. 0.027, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400MHz) 4.76 (d, J = 9.6Hz, 1H), 4.57 (d, J = 9.2Hz, 1H), 4.08 (d, J = 8.8Hz, 1H), 3.52-3.63 (m, 2H), 2.45 (s, 1H), 2.12 (s, 3H), 1.36 (s, 3H), 1.20 (d, J = 6Hz, 3H). ^{13}C: 170.7, 99.5, 78.0, 76.9, 73.53, 69.0, 46.5, 33.2, 31.4, 25.5, 23.9, 23.8, 21.6, 20.9, 17.7. IR: 3435, 2934, 1732, 1644, 1231, 1016. LCMS ($M+H^+$) = 412.26, found ($2M+ Na^+$) = 847.1.



2.8a

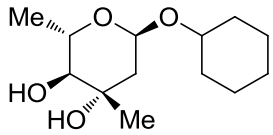
To the iodopyranoside (72mg, .175mmol) in PhMe (1.8mL) at 0°C was added Ph_3SnH (74mg, .210mmol) and BEt_3(1.0M in Hexanes, 52µL, .052mmol) with stirring. The reaction was allowed to come to room temperature and stirred for 3hr open to atmosphere. The solution was washed with saturated potassium fluoride for 1hr and filtered through celite. The organic phase was dried ($MgSO_4$) and concentrated. The residue was chromatographed 5:1 Hex:EtOAc to give 42mg (84%) of white solid. $[\alpha]^{20}_D$ = -132.81° (*c*. 0.0205, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400MHz) 4.98 (d, J = 3.2Hz, 1H) 4.58 (d, J = 9.6Hz, 1H), 3.82 (dq, J = 6Hz, J = 3.6Hz), 3.52 (m, 1H), 2.40 (s, 1H), 2.12 (s, 3H), 1.88-2.00 (m, 2H), 1.69-1.78 (m, 4H), 1.59 (s, 3H), 1.49 (m, 1H), 1.24-1.35 (m, 5H), 1.14 (d, J = 6.4Hz, 3H). ^{13}C: 171.6, 94.8, 80.8, 74.5, 70.9, 64.9, 44.3, 33.3, 31.4, 25.7, 24.0, 23.6, 22.7, 21.0, 17.8. IR: 3472, 2933, 2857, 1742, 1727, 1452, 1374, 1237, 1126, 1048, 1014. LCMS ($M+H^+$) = 286.2, found ($M+ Na^+$) 309.2.



2.8b

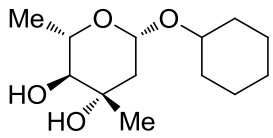
To the glycoside (54mg, .131mmol) in PhMe (1.3mL) at 0°C was added Ph_3SnH (55mg, .157mmol) and BEt_3 (1.0M in Hexanes, 39µL, .039mmol) with stirring. The reaction was allowed to come to room temperature and stirred for 3hr open to atmosphere. The solution was washed with saturated potassium fluoride for 1hr and filtered through celite. The organic phase was dried ($MgSO_4$) and concentrated. The residue was chromatographed 5:1 Hex:EtOAc to give 29mg (77%) of white solid. $[\alpha]^{20}_D$ = 12.65° (*c*. 0.0109, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400MHz) 4.65 (dd, J = 9.2Hz, J = 2Hz, 1H) 4.56 (d, J = 7.6Hz, 1H) 3.62 (m, 1H), 3.48 (dq, J = 8.8Hz, J = 6Hz, 1H), 2.67 (s, 1H), 2.11 (s, 3H)

1.7-2.0 (m, 7H), 1.20-1.53 (m, 6H) 1.24 (s, 3H), 1.21 (d, J = 6Hz, 3H). ^{13}C: 171.5, 97.0, 80.2, 76.5, 71.2, 69.5, 45.9, 33.6, 31.9, 25.6, 24.2, 24.1, 21.4, 21.0, 18.4. IR: 3436, 2982, 2932, 2856, 1733, 1370, 1229, 1099, 1059, 1050. LCMS ($M+H^+$) = 286.2, found ($M+Na^+$) 309.2.



2.9a

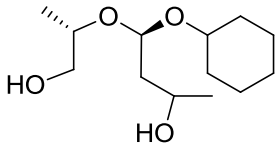
To MeOH (1.5mL) was added the protected glycoside (42mg, .147mmol) with stirring followed by K_2CO_3 (2mg, .015mmol) at ambient temperature. After 24hr the solvent was evaporated and the product dissolved in EtOAc then filtered through celite to give the deacetylated product (35mg, 97%). $[\alpha]^{20}_D$ = -88.57° (*c*. 0.0205, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400MHz) 4.97 (d, J = 4Hz, 1H) 3.70 (dq, J = 6.4Hz, J = 9.6Hz, 1H), 3.54 (m, 1H), 3.25 (d, J = 9.6Hz, 1H), 2.29 (bs, 1H) 1.83-1.95 (m, 3H), 1.77 (m, 2H), 1.70 (m, 2H) 1.50 (m, 1H), 1.43 (s, 3H), 1.27 (d, J = 6.4Hz, 3H), 1.25(m, 3H). ^{13}C: 94.9, 79.9, 74.2, 71.7, 66.6, 43.7, 33.4, 31.4, 25.8, 24.0, 23.7, 22.1, 18.0. IR: 3400, 2931, 2856, 1451, 1327, 1125, 1054, 1011. LCMS ($M+H^+$) = 244.2, found ($M+ Na^+$) 267.2.



2.9b

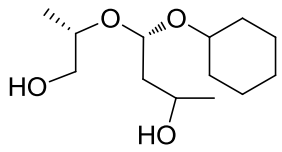
To the protected glycoside (22mg, .077mmol) in MeOH (1mL) with stirring was added K_2CO_3 (ca 1mg,) at ambient temperature. After 24hr the solvent was evaporated and the product dissolved in EtOAc then filtered through Celite to give the deacetylated product

(16mg, 85%). $[\alpha]^{20}_{D}$ = 19.39° (*c*. 0.0109, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400MHz) 4.63 (dd, J = 9.6Hz, J = 2 Hz, 1H), 3.60 (m, 1H), 3.34 (dq, J = 9.6Hz, J = 6Hz, 1H) 3.23 (d, 9.2Hz, 1H), 2.71 (s, 1H), 2.41 (s, 1H), 1.30 (d, J = 6Hz, 3H), 1.25 (s, 3H), 1.2-1.4 (m, 6H). ^{13}C: 97.0, 79.5, 72.0, 70.7, 45.7, 33.6, 31.9, 25.5, 24.2, 24.1, 20.4, 18.3. IR: 3400, 2931, 2856, 1450, 1363, 1073, 1050, 1023. LCMS ($M+H^+$) = 244.2, found ($M+ Na^+$) 267.2.



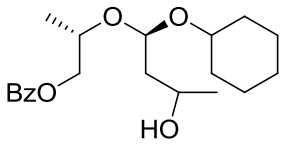
2.10a

To the free diol (29mg, .120mmol) in PhMe (1.2mL) was added lead (IV) acetate (57mg, .132mmol) with stirring at rt. After two hours, the reaction was filtered through a short silica plug. The crude keto-aldehyde was immediately concentrated to give a crude, yellow oil (29.0 mg, 99%) and redissolved in MeOH (1.2mL) at 0˚C and $NaBH_4$ (.479mmol) was added with stirring. After 1.5hr, the reaction was evaporated, dissolved in ether, and filtered through a short silica plug to give 22.8mg (77%) of product. The diol proved unstable and necessitated use immediately. Crude Aldehyde: ^{1}H NMR ($CDCl_3$, 400MHz) 9.58 (d, 2.4Hz, 1H), 5.03 (dd, 4.4Hz, 6.4Hz, 1H), 3.95 (m, 1H), 3.42 (m, 1H), 2.82 (dd, 6.4Hz, 15.2Hz, 1H), 2.67 (dd, 4.4Hz, 7.2Hz, 1H), 2.18 (s, 3H), 1.6-1.9 (m, 5H) 1.5 (m, 1H), 1.18-1.28 (m, 7H) Crude diol mixture 0.9:1.0 : 4.84(t, 4.8Hz, 1H) 4.78(t, 5.6Hz, .9H), 4.03 (m, 1.9H), 3.82 (m, 1.9H), 3.48 (m, 5.7H), 1.9 (m, 4.2H), 1.76 (m, 7.4H), 1.53 (m, 1.9H) 1.21-1.41 (m, 10H), 1.18 (m, 6H), 1.14 (m, 5.9H)



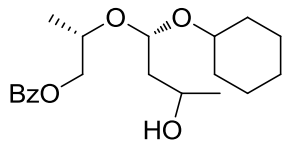
2.10b

To the free diol (37mg, .12051mmol) in PhMe (1.5mL) was added lead (IV) acetate (74mg, .167mmol) with stirring at rt. After one hour, the reaction was filtered through a short silica plug. The crude keto-aldehyde was immediately concentrated to give a crude, yellow oil (35.8 mg, 98%) and redissolved in MeOH (1.5mL) at 0˚C and $NaBH_4$(22mg, .591mmol) was added with stirring. After 1.5hr, the reaction was evaporated, dissolved in ether, and filtered through a short silica plug to give 21.5mg (59%) of product. The diol proved unstable and necessitated use immediately. Crude Aldehyde: ^{1}H NMR ($CDCl_3$, 400MHz) 9.59 (d, 1.6Hz, 1H), 5.12 (t, 5.2Hz, 1H), 4.11 (m, 1H), 3.50 (m, 1H), 2.79 (dd, 5.6Hz, 3.2Hz, 2H), 2.19 (s, 3H) 1.8 (m, 3H), 1.7 (m, 3H), 1.5 (m, 1H), 1.2 (m, 5H). Diol mixture 1:1.2 diastereomers: 5.02 (t, 4.8Hz, 1H), 4.88 (t, 4.4Hz, 1.6H), 4.17 (m, 1H), 4.0 (m, 2.4H), 3.72 (m, 3.3H), 3.44(m, 9.9H) 1.6-1.8 (m, 18H), 1.04-1.20 (m,11H)



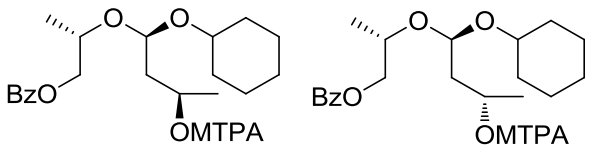
2.11a

To the reduced diol (22.8mg, .093mmol) in pyridine (1mL) was added benzoic anhydride (26mg, .102mmol) at room temperature with stirring. A catalytic amount of DMAP was added to the reaction and stirred for three hours. The reaction was diluted with ethyl acetate and washed with saturated sodium bicarbonate solution, dried ($MgSO_4$) and concentrated. The product was purified by flash chromatography with Hexanes:EtOAc (3:1) to give 9.9mg of product (30%).^{1}H NMR ($CDCl_3$, 400MHz) 8.08 (d, 8.8Hz, 2H), 7.65 (m, 1H), 7.54 (t, 5.6Hz, 2H), 5.00 (m, 1H), 4.35 (m, 1H), 4.27 (m, 1H), 4.17 (m, 1H), 3.95 (m, 1H), 3.64 (m, 1H), 1.86 (m, 2H), 1.73 (m, 2H), 1.67(m, 2H), 1.49 (m, 1H), 1.2-1.30 (10H) 1.15 (m, 3H)



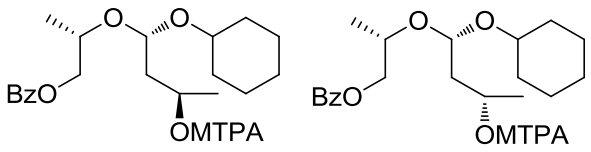
2.11b

To the reduced diol (21.5mg, .087mmol) in pyridine (.9mL) was added benzoic anhydride (24mg, .096mmol) at room temperature with stirring. A catalytic amount of DMAP was added to the reaction and stirred for three hours. The reaction was diluted with ethyl acetate and washed with saturated sodium bicarbonate solution, dried ($MgSO_4$) and concentrated. The product was purified by flash chromatography with Hexanes:EtOAc (3:1) to give 14mg of product (46%).^{1}H NMR ($CDCl_3$, 400MHz) 8.1 (m, 2H), 7.69 (m, 1H), 7.56 (t, 8Hz, 2H), 5.06 (t, 1H), 4.3 (m, 1H), 4.16 (m, 1H), 3.80 (m, 1H), 3.66 (m, 1H), 1.93 (m, 2H), 1.70 (m, 4H), 1.53(m, 1H), 1.33 (d, 6Hz, 1H), 1.2-1.40 (6H) 1.15 (d, 6Hz, 3H)



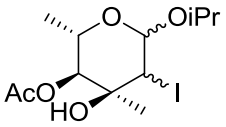
2.12a 2.12b

To 9.9mg of the benzoate (.028mmol) in DCM (.3mL) was added S-Mosher acid (8.0mg, .034mmol), DCC (8.0mg, .037mmol) and a small crystal of DMAP to catalyze the reaction. The reaction was stirred for 24 hours at room temperature at which time an additional equivalent of Mosher acid and DCC were added to drive the reaction to completion. After another 24 hours, the solvent was evaporated and the product purified by flash chromatography Hexanes:EtOAc to give a mixture of diastereomers (7.8mg, 49%). These were separable on a silica column by HPLC using Hexane:EtOAc (5:1) to give the R- and S- esters. ^{1}H NMR ($CDCl_3$, 400MHz) R-Ester: 8.08 (m, 2H), 7.7 (m, 1H), 7.67 (m, 2H), 7.5-7.63 (m, 4H), 5.27 (m, 1H), 4.62 (dd, 3.6Hz, 7.6Hz, 1H), 4.25 (m, 2H), 4.0 (m, 1H), 3.62 (d, 1.2Hz, 3H), 3.54 (m, 1H), 1.75-2.00 (m, 6H), 1.67 (m, 2H), 1.47 (m, 1H), 1.44 (d, 5.6Hz, 3H). ^{13}C 166.5, 166.2, 133.8, 133.3, 131.0, 130.5, 129.4, 129.3, 129.3, 129.1, 128.0, 127.9, 125.4, 98.2, 74.7, 72.2, 70.7, 68.6, 55.7, 42.7, 34.1, 32.7, 26.2, 24.5, 24.4, 20.3, 17.8. S-Ester: 8.08 (m, 2H), 7.5-7.7 (m, 9H), 5.35 (m, 1H), 4.80 (dd, 4.4Hz, 5.6Hz, 1H), 4.38 (dd, 5.6Hz, 11.6Hz, 1H), 4.27 (dd, 4.4Hz, 11.2Hz, 1H), 4.17 (m, 1H), 3.60 (d, 0.8Hz, 3H), 1.84-1.98 (m, 5H), 1.68 (m, 3H), 1.2-1.4 (m, 7H), 1.33 (d, 5.6Hz, 3H), 1.28 (d, 5.6Hz, 3H) ^{13}C: 166.5, 166.2, 133.8, 133.6, 133.0, 131.1, 130.4, 130.1, 130.0, 129.3, 129.23, 129.18, 129.1, 128.4, 128.2, 98.2, 75.0, 72.0, 70.5, 68.7, 55.7, 42.4, 33.8, 33.2, 26.2, 24.5, 24.4, 20, 17.6.



2.12c 2.12d

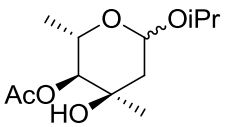
To 14mg of the benzoate (.040mmol) in DCM (.4mL) was added S-Mosher acid (11.2mg, .048mmol), DCC (11.0mg, .052mmol) and a small crystal of DMAP to catalyze the reaction. The reaction was stirred for 24 hours at room temperature at which time an additional equivalent of Mosher acid and DCC were added to drive the reaction to completion. After another 24 hours, the solvent was evaporated and the product purified by flash chromatography Hexanes:EtOAc (5:1) to give a mixture of diastereomers (12mg, 53%). These diastereomers proved inseparable thus far by HPLC. ^{1}H NMR ($CDCl_3$, 400MHz) 8.08 (m, 2H), 7.67 (m, 1H), 7.53 (m, 6H), 5.32 (m, 1H), 4.92 (dd, 4.8Hz, 6.8Hz, .6H), 4.65 (dd, 4.4Hz, 6.8Hz, .4H), 4.32 (m, 2H), 4.05-4.18 (m, 1H), 3.70 (m, .6H), 3.66 (s, 1.2H), 3.58 (s, 1.65H), 3.48 (m, .4H), 2.84(bs, 3.59H), 1.92 (m, 3H), 1.7 (m, 2.4H), 1.53 (m, 1H), 1.40 (d, 6Hz, 1.6H), 1.28-1.34 (m, 7H).



2.15

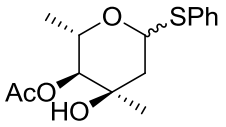
To the acetonide (1.5345g, 8.24mmol) in acetonitrile (82mL) was added NIS (2.781g, 12.36mmol) and IPA (756μL, 9.89mmol) at ambient temperature with stirring. After 3 days, the reaction was washed with $Na_2S_2O_3$ solution and saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc and the organics were dried ($MgSO_4$) and

concentrated. Flash chromatography provided a clear oil mixture of the alpha and beta anomers.



2.16

To the isopropyl iodo-glycoside (816mg, 2.19mmol) in toluene (22mL) at 0˚C was added Ph_3SnH (924mg, 2.64mmol) and 1.0M BEt_3 in hexanes (.66mL, .66mmol) open to atmosphere and allowed to warm to room temperature for 3 hours. The solvent was evaporated and the residue was purified by flash chromatography to give 543g (100%) of white solid. The 1H NMR was identical to published data.[21].



2.17

To the isopropyl glycoside (359mg, 1.46mmol) and PhSH (149µL,1.46mmol) in toluene (14.6mL) at 0˚C was added $BF_3{\cdot}EtO_2$ (166µL, 1.31mmol) and the reaction was allowed to slowly come to room temperature and stirred for 3 hours. The starting material was replaced by a UV active spot and the reaction was washed with 5% NaOH and separated. The organics were dried ($MgSO_4$), concentrated, and purified by 10:1 Hex:EtOAc to give 378mg of compound (87%). The 1H NMR was identical to published data.[21]

APPENDIX A: SPECTRA

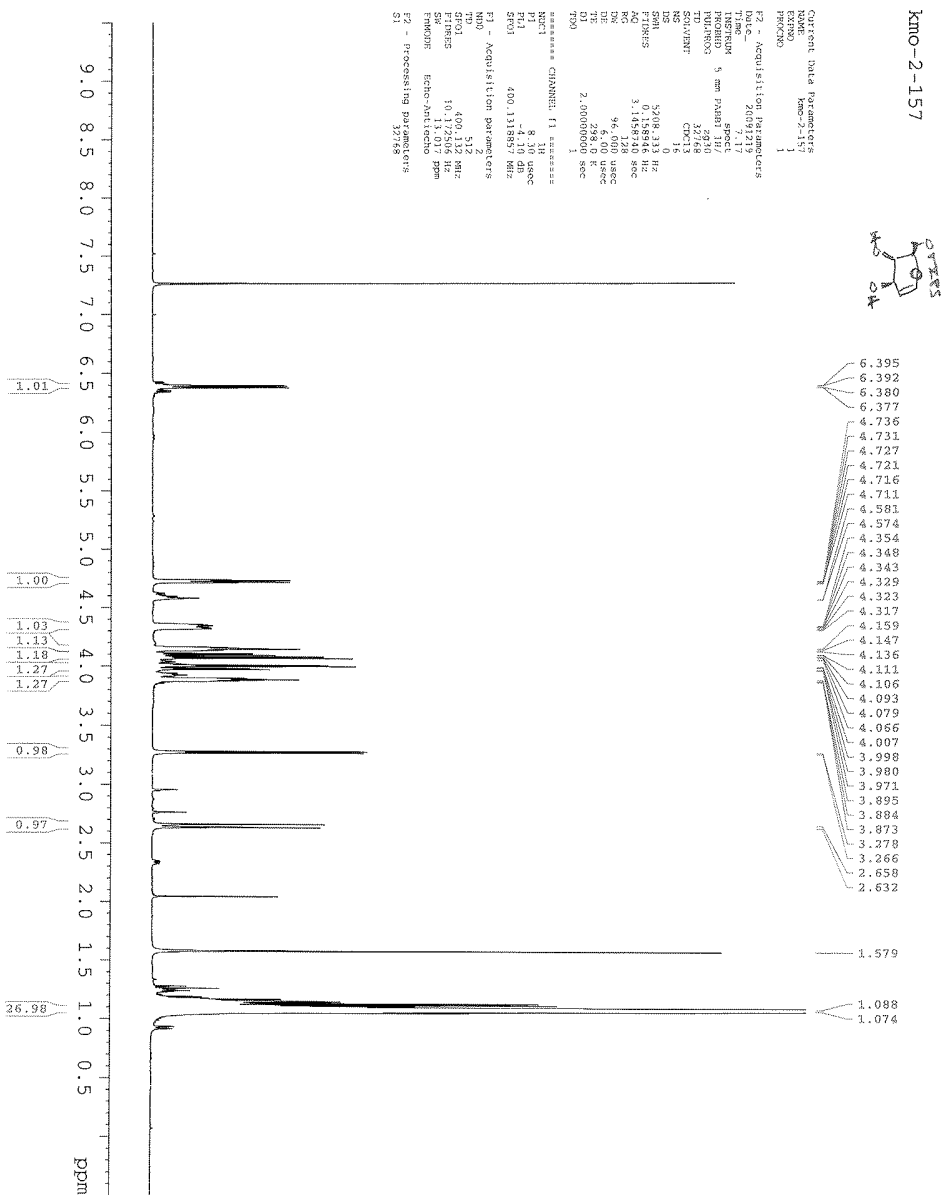


Figure A1 The 400MHz ^{1}H NMR of 1.3 in $CDCl_3$

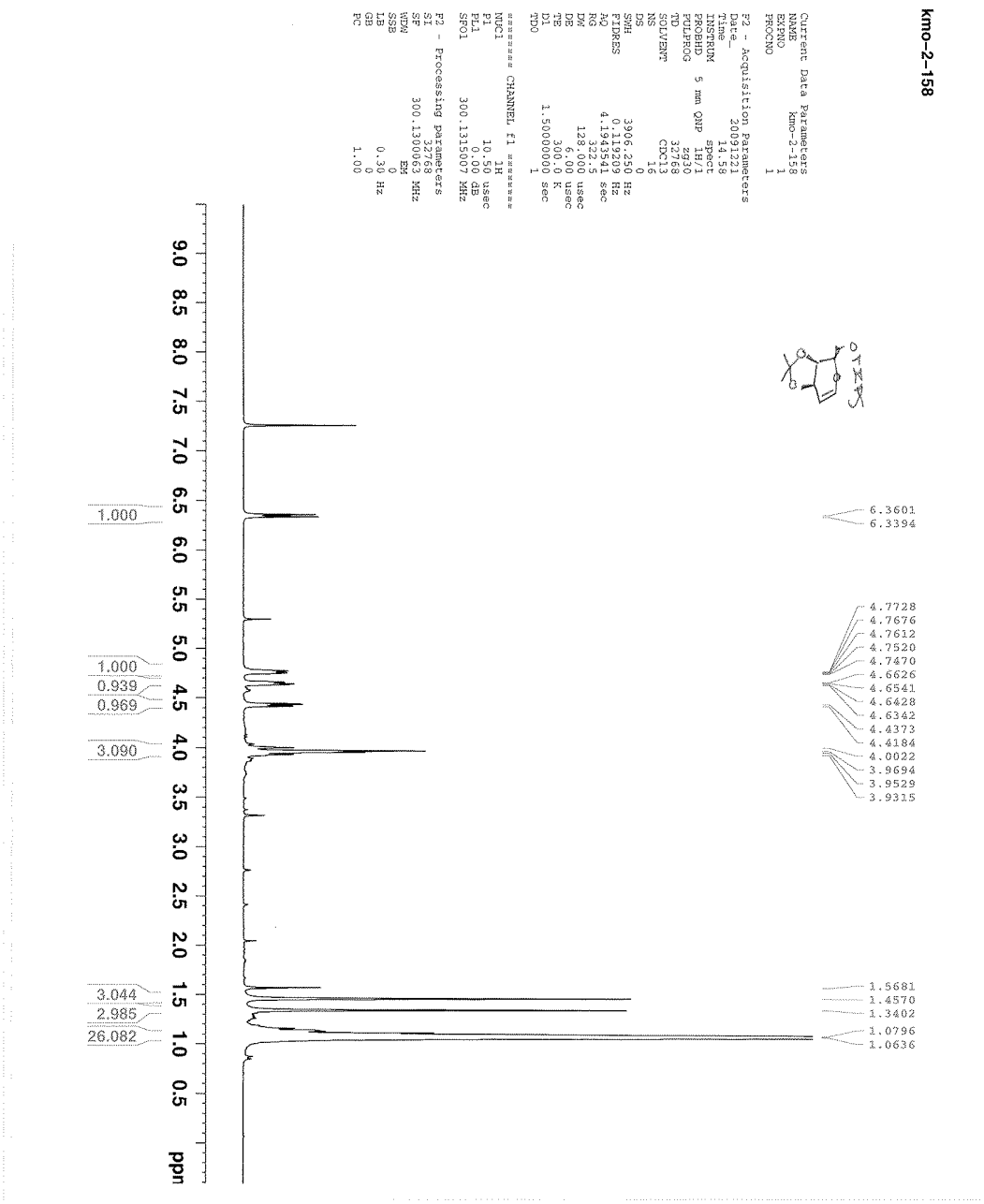


Figure A2 The 400MHz ^{1}H NMR of 1.4 in $CDCl_3$

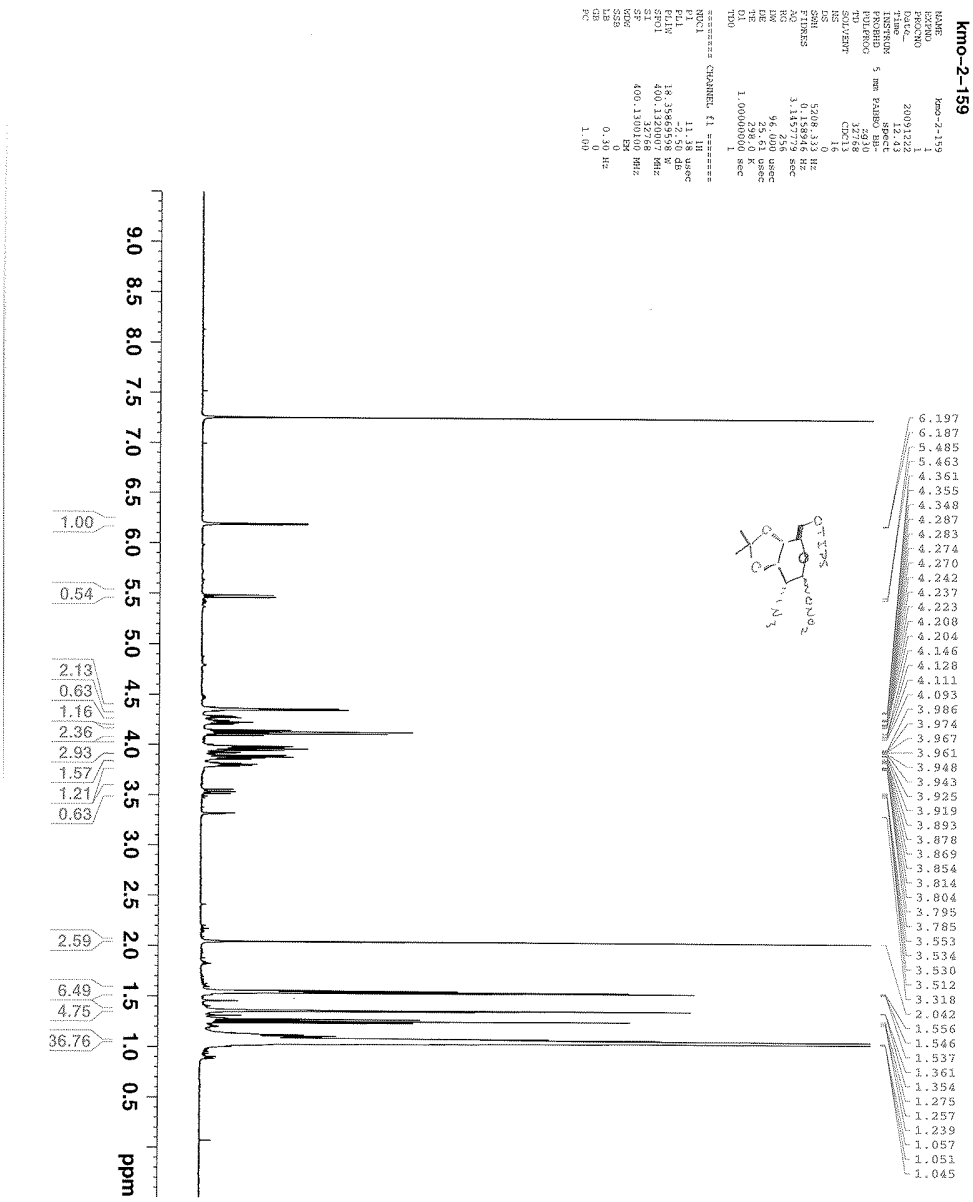


Figure A3 The 400MHz ^{1}H NMR of 1.5 in $CDCl_3$

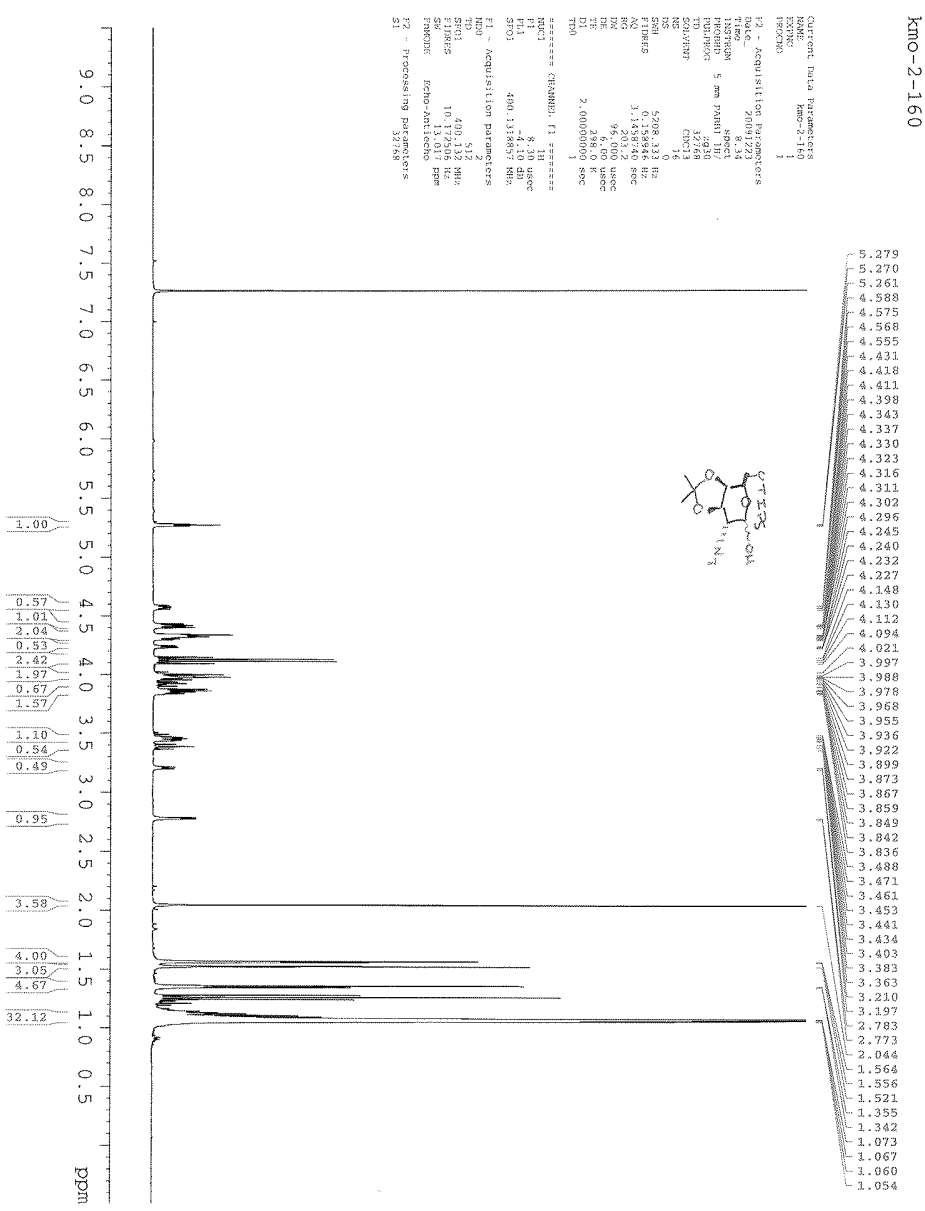


Figure A4 The 400MHz ^{1}H NMR of 1.6.1 in $CDCl_3$

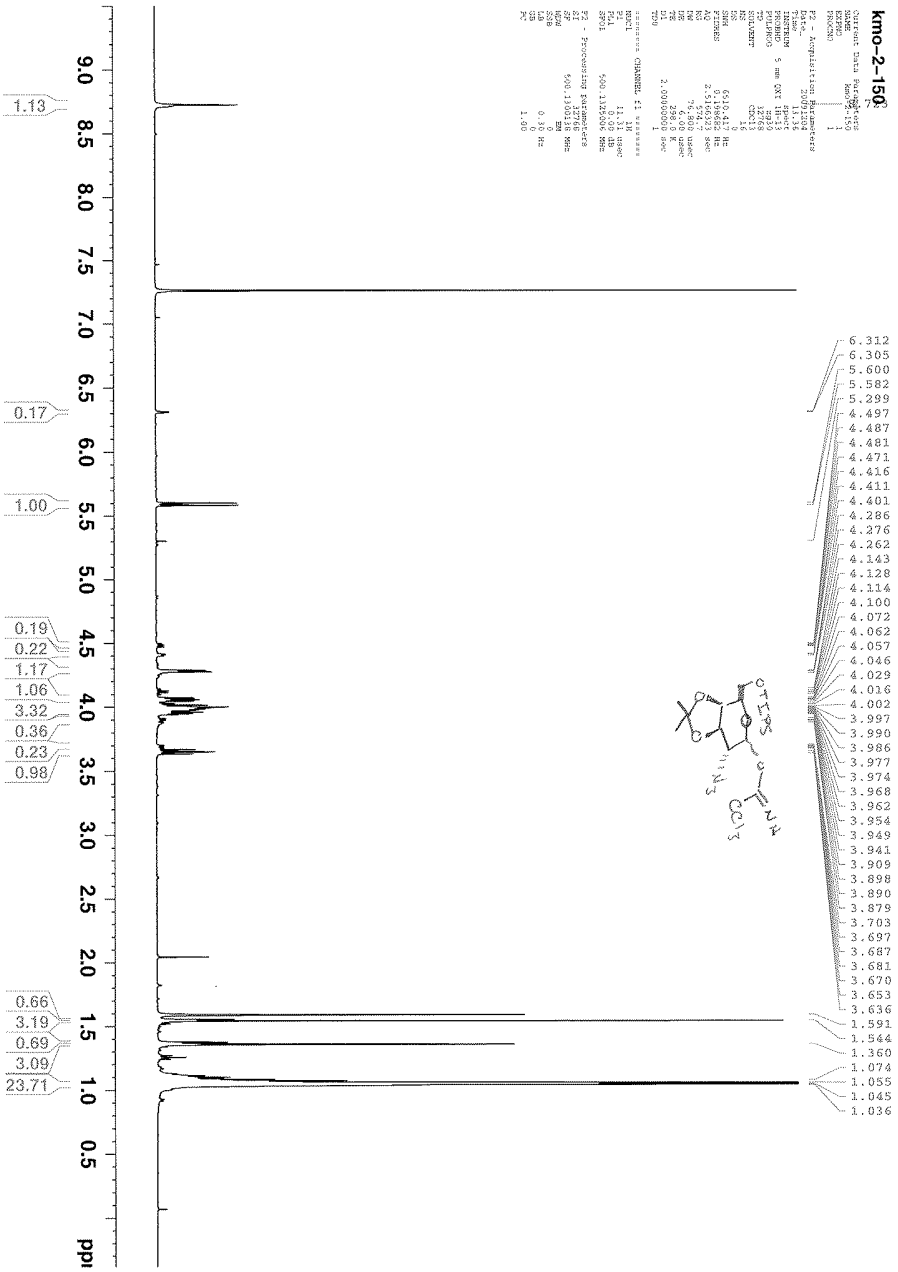


Figure A5 The 500MHz ^{1}H NMR of 1.6.2 in $CDCl_3$

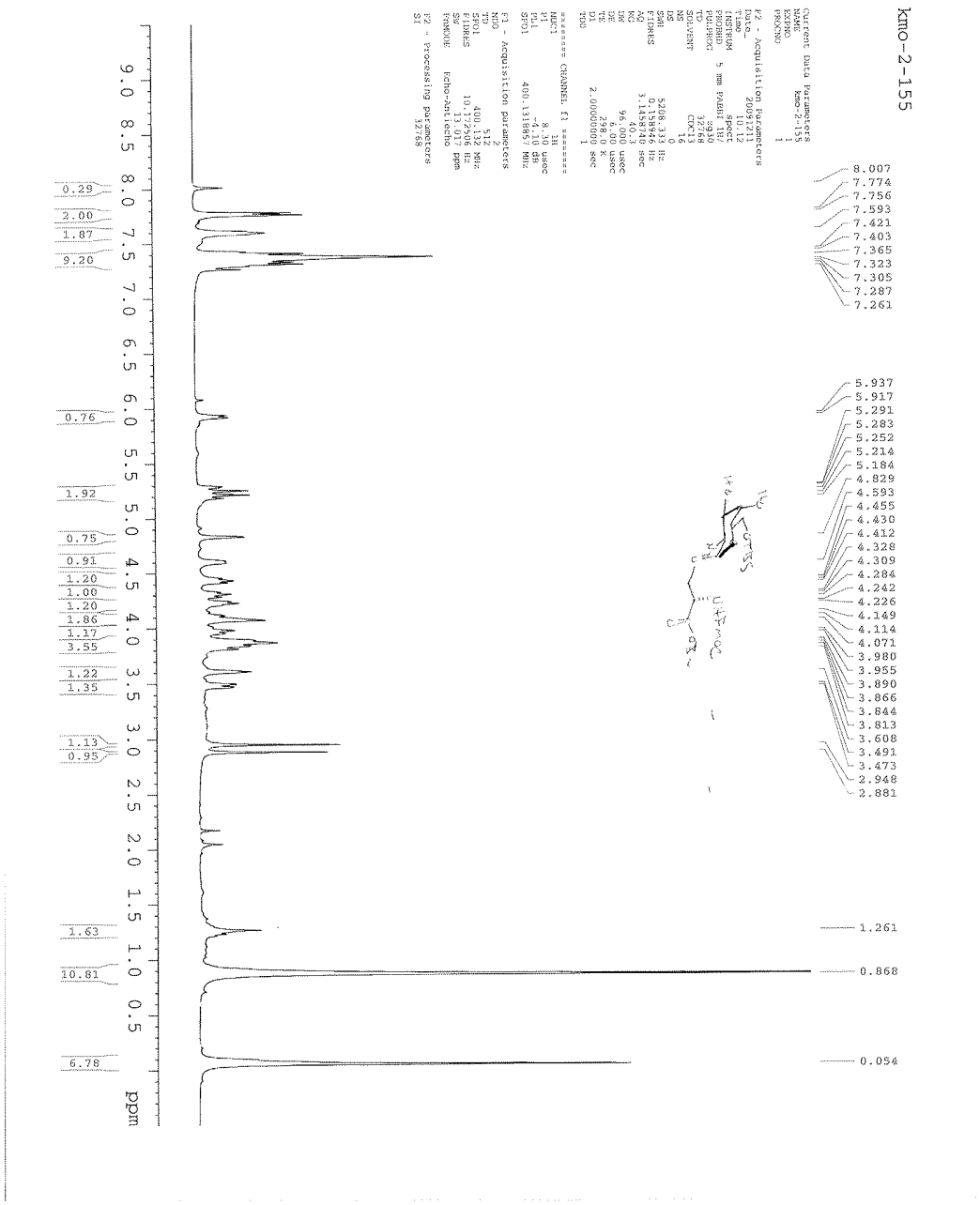


Figure A6 The 400MHz ^{1}H NMR of **A** in $CDCl_3$

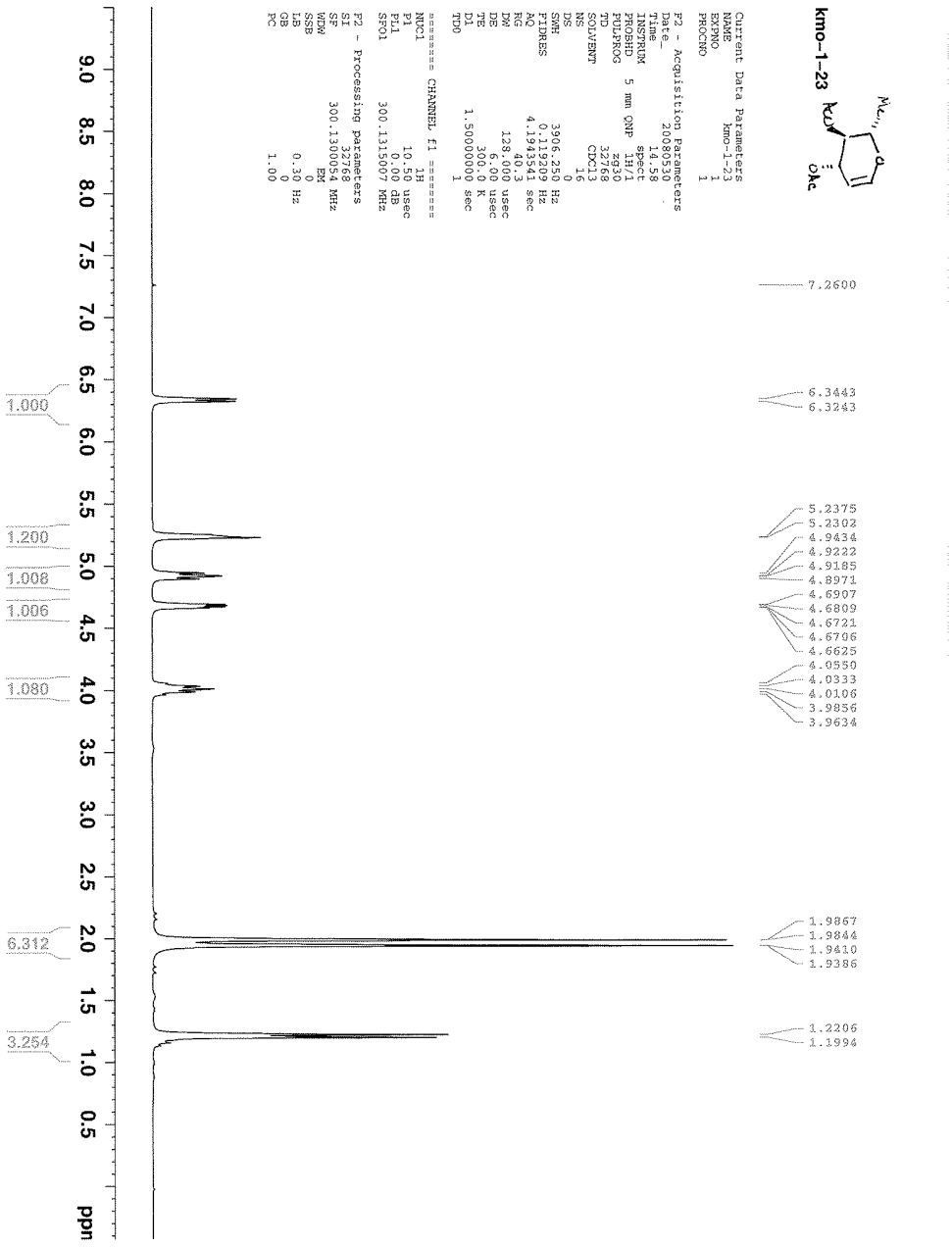


Figure A7 The 300MHz ^{1}H NMR of 2.2 in $CDCl_3$

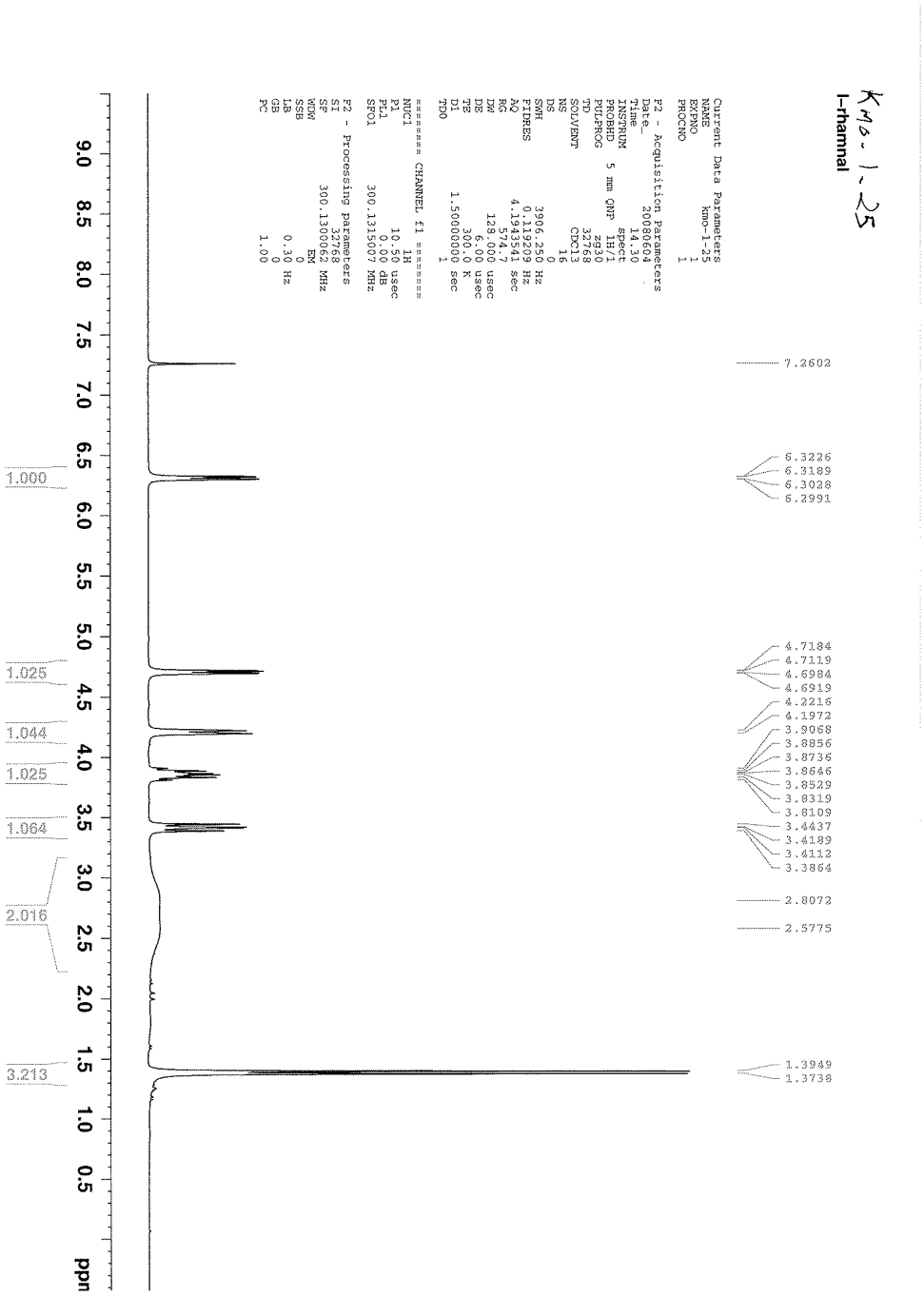


Figure A8 The 300MHz ^{1}H NMR of 2.3 in $CDCl_3$

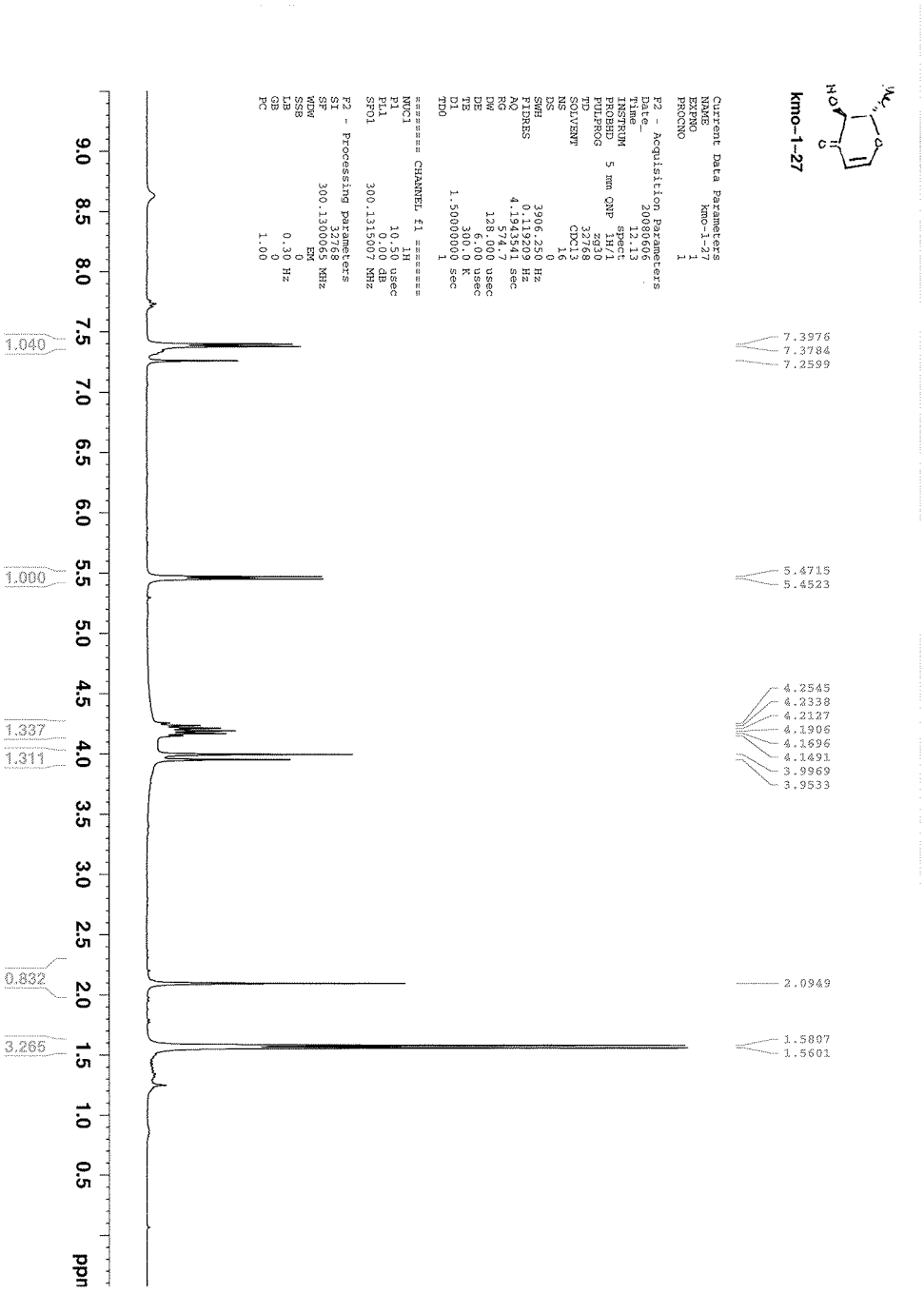


Figure A9 The 400MHz ^{1}H NMR of 2.4 in $CDCl_3$

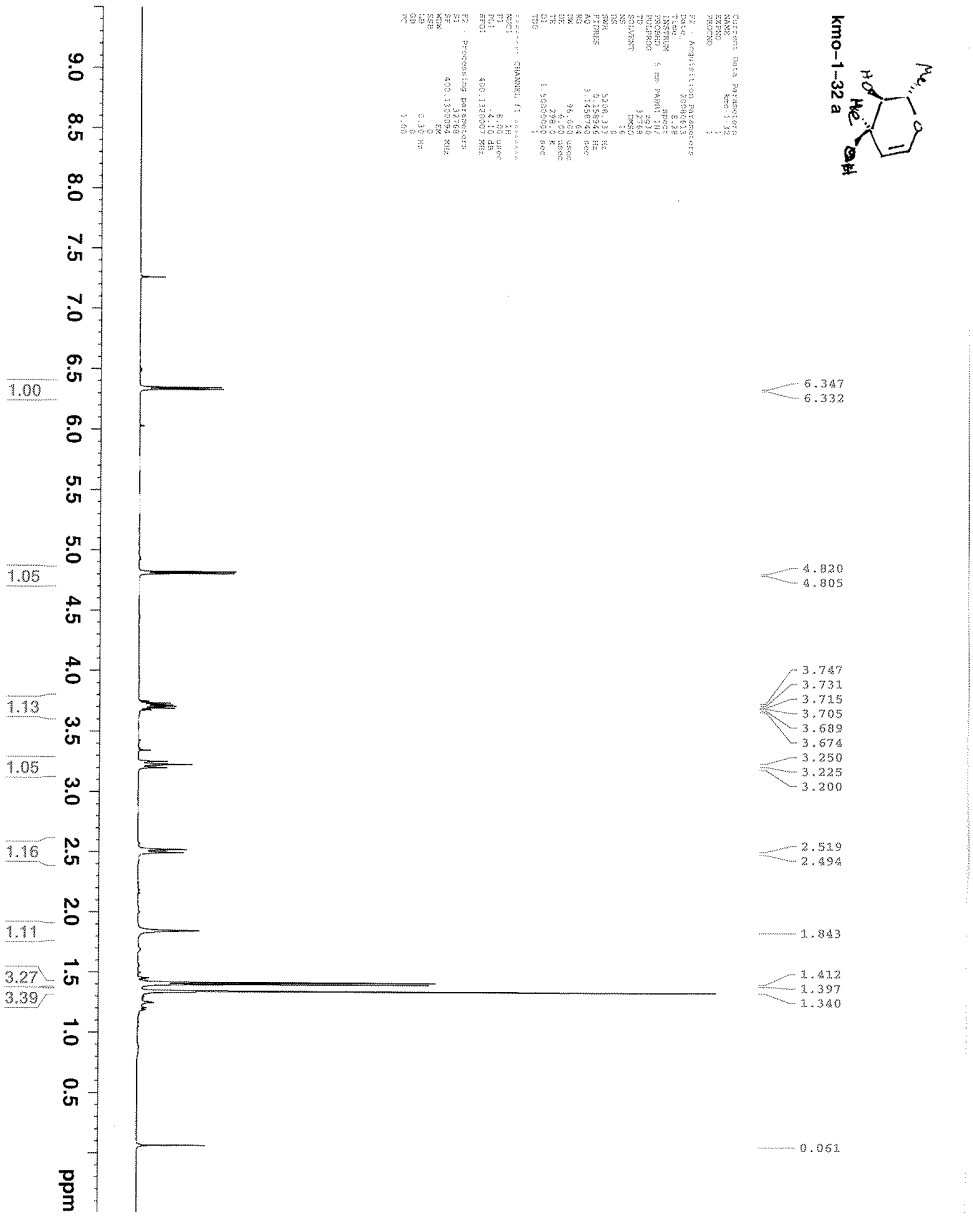


Figure A10 The 400MHz ^{1}H NMR of 2.5a in $CDCl_3$

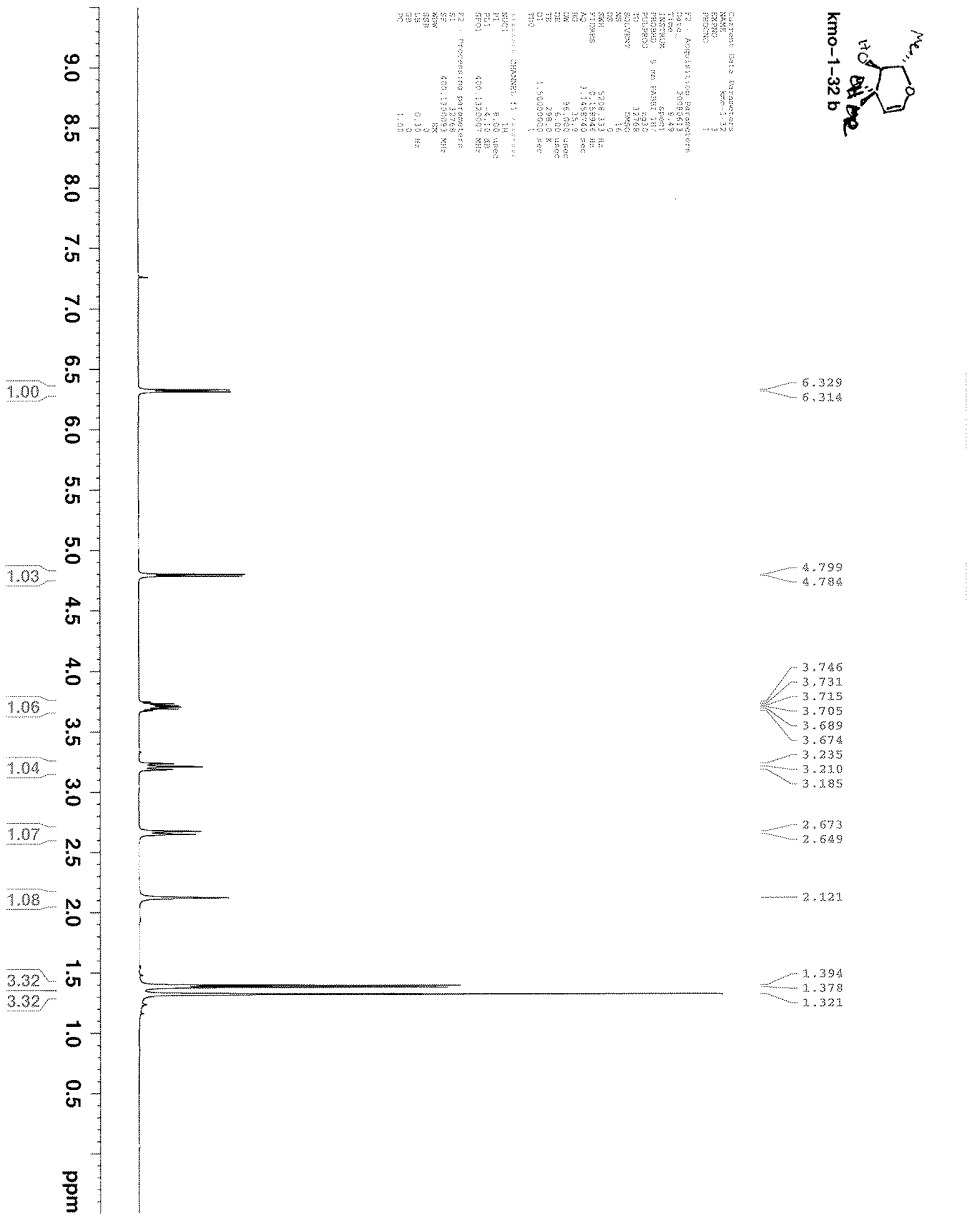


Figure A11 The 400MHz ^{1}H NMR of 2.5b in $CDCl_3$

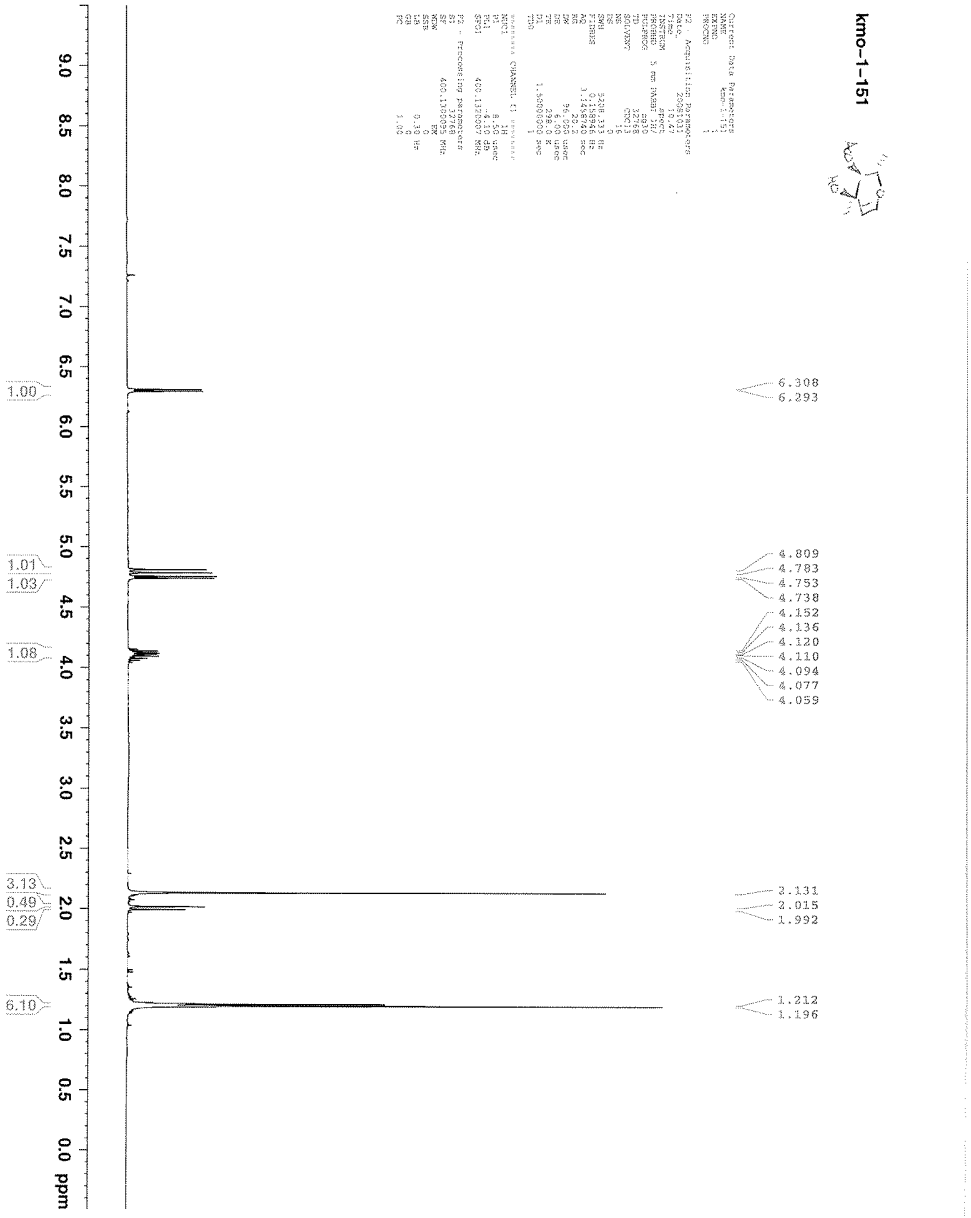


Figure A12 The 400MHz ^{1}H NMR of 2.6a in $CDCl_3$

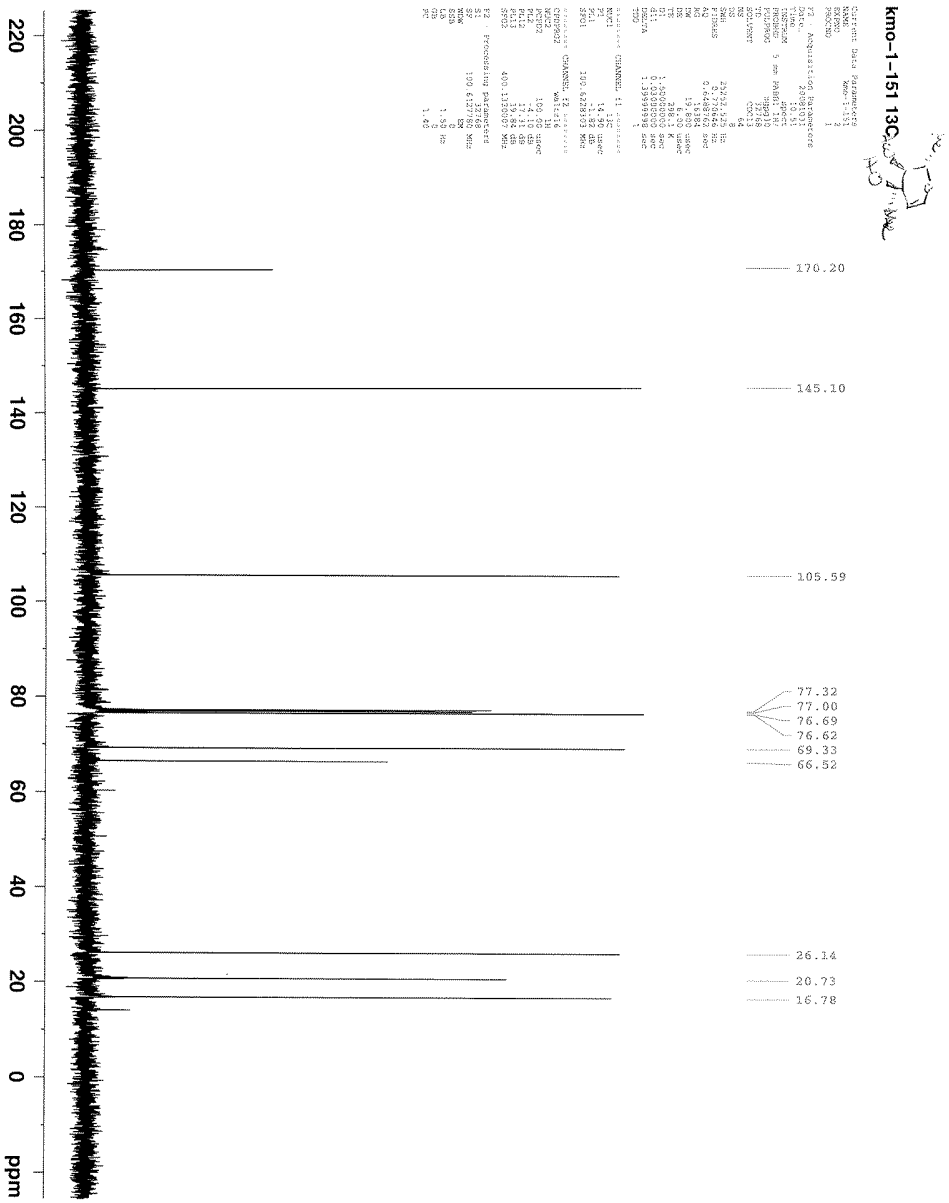


Figure A13 The 125MHz ^{13}C NMR of 2.6a in $CDCl_3$

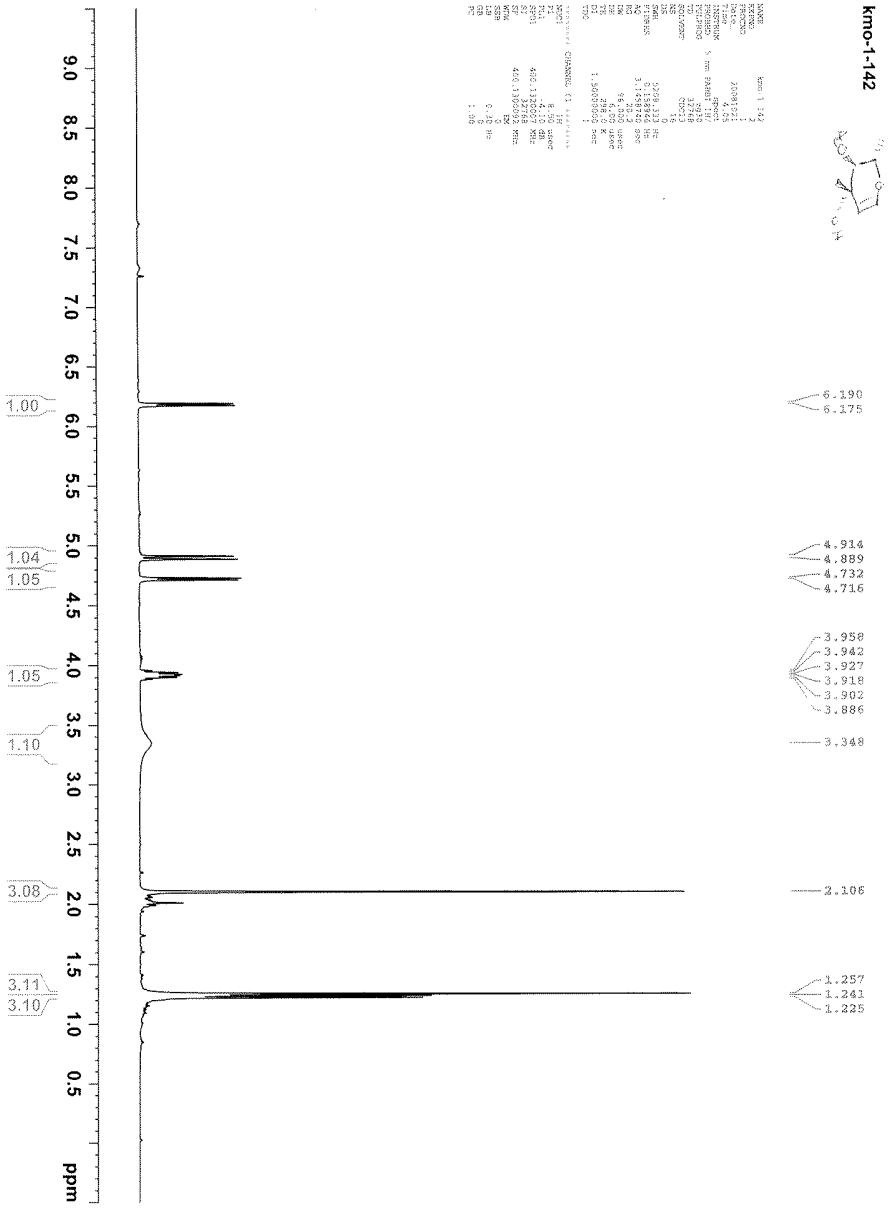


Figure A14 The 400MHz ^{1}H NMR of 2.6b in $CDCl_3$

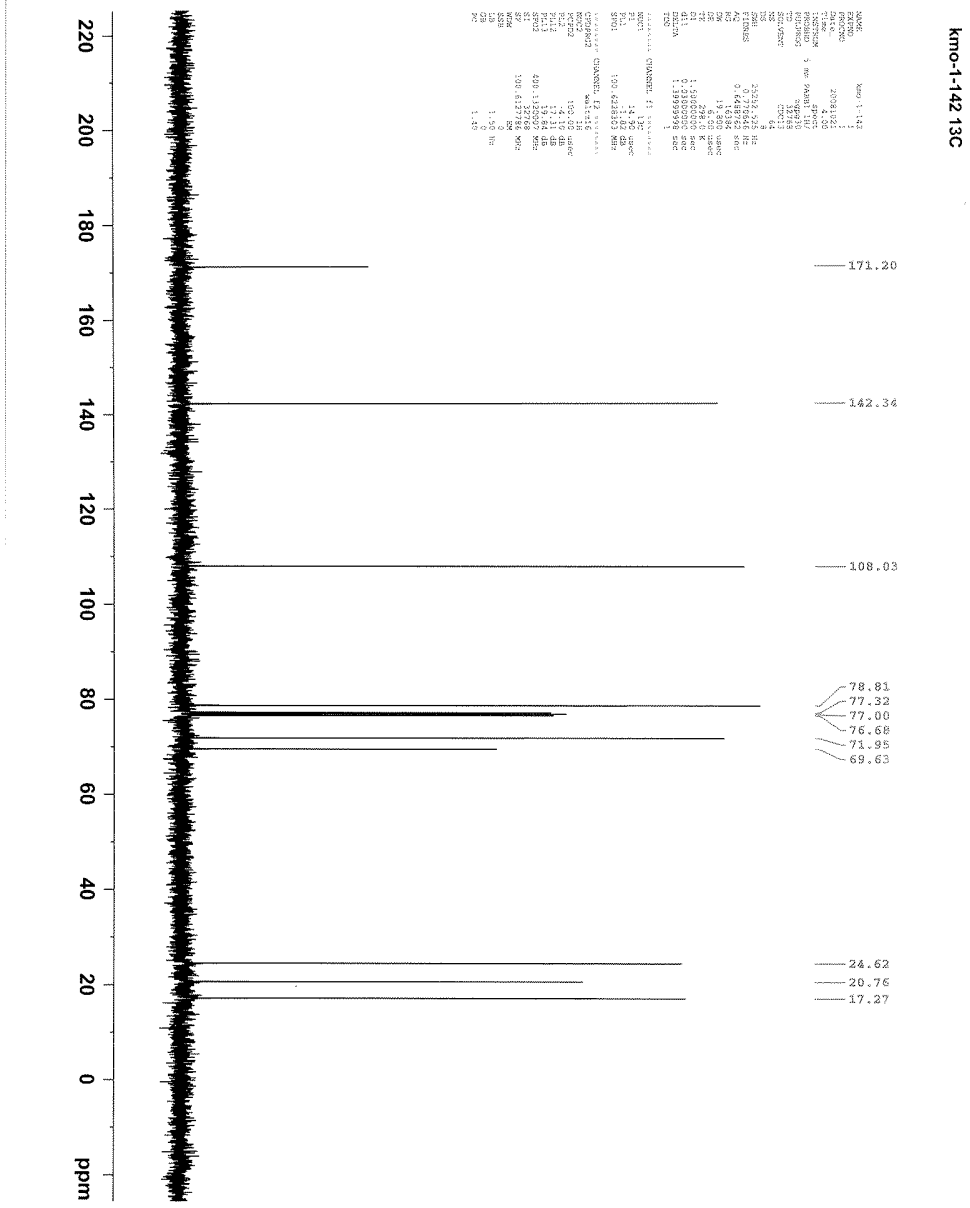


Figure A15 The 125MHz ^{13}C NMR of 2.6b in $CDCl_3$

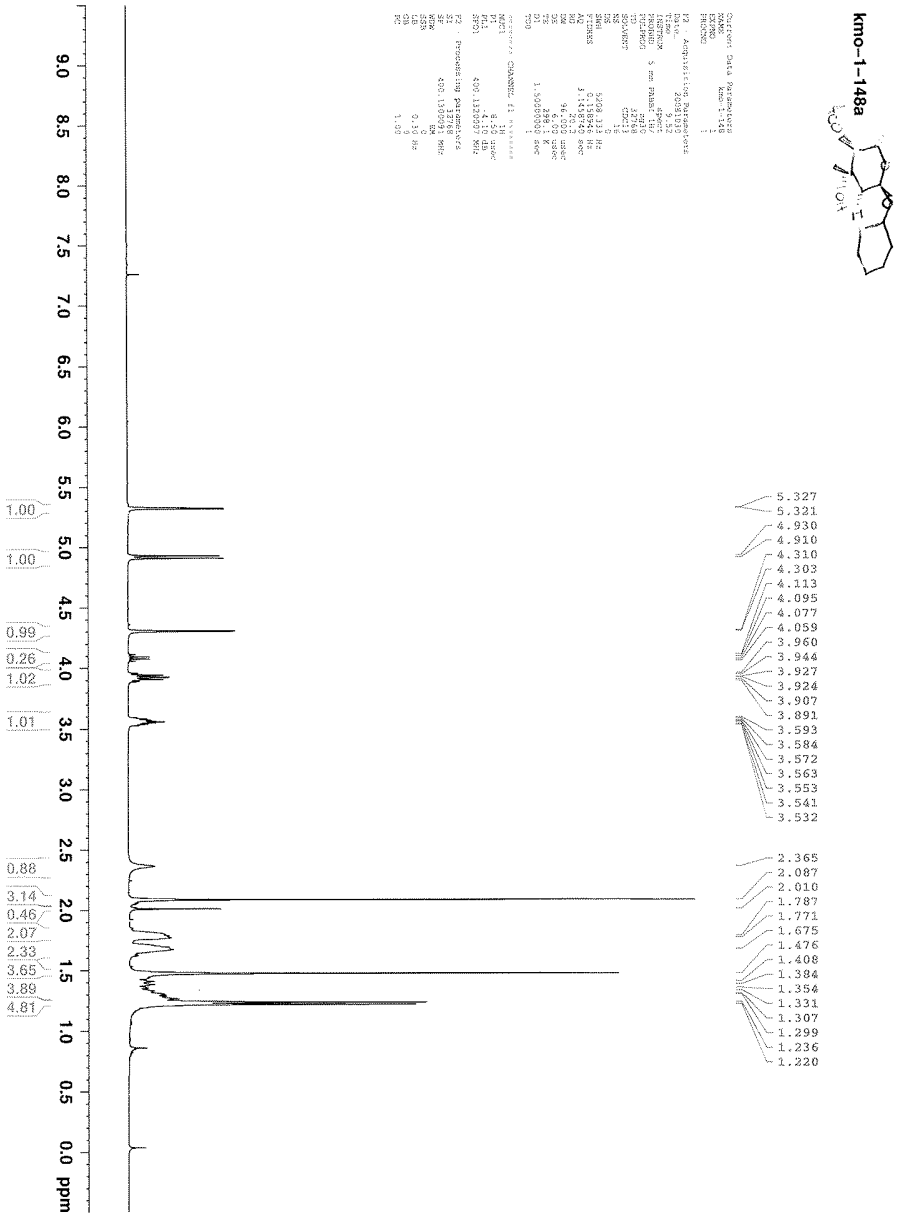


Figure A16 The 400MHz ^{1}H NMR of 2.7a in $CDCl_3$

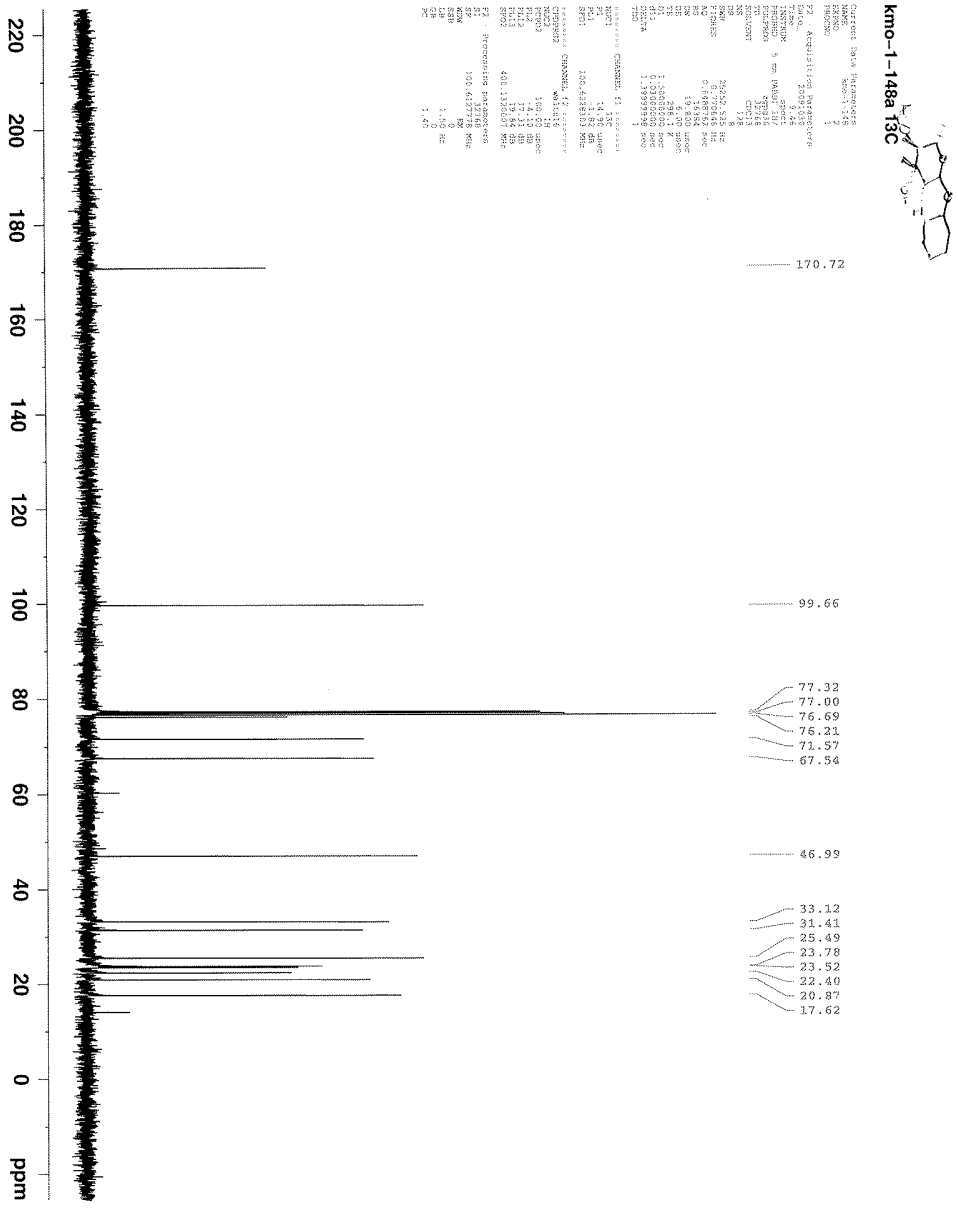


Figure A17 The 125MHz ^{13}C NMR of 2.7a in $CDCl_3$

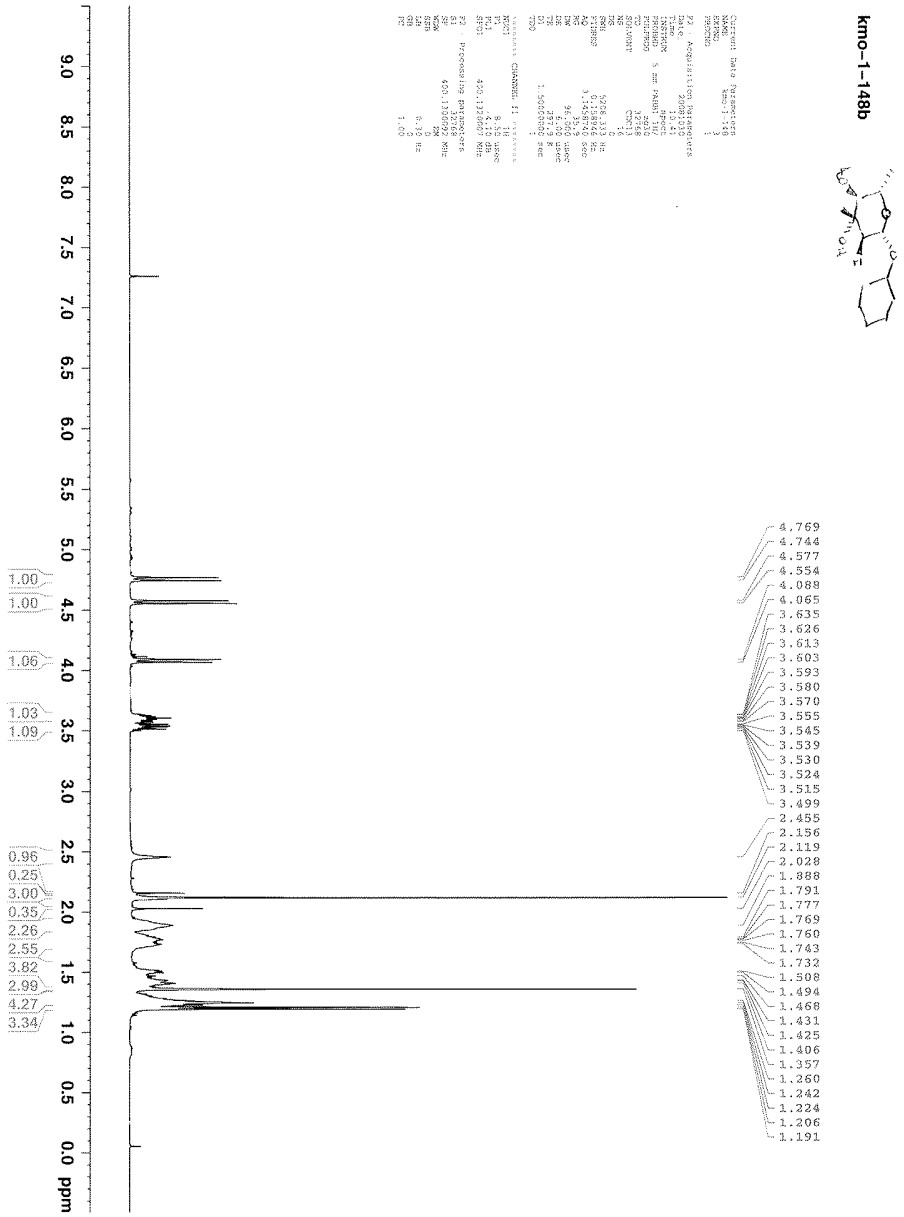


Figure A18 The 400MHz ^{1}H NMR of 2.7b in $CDCl_3$

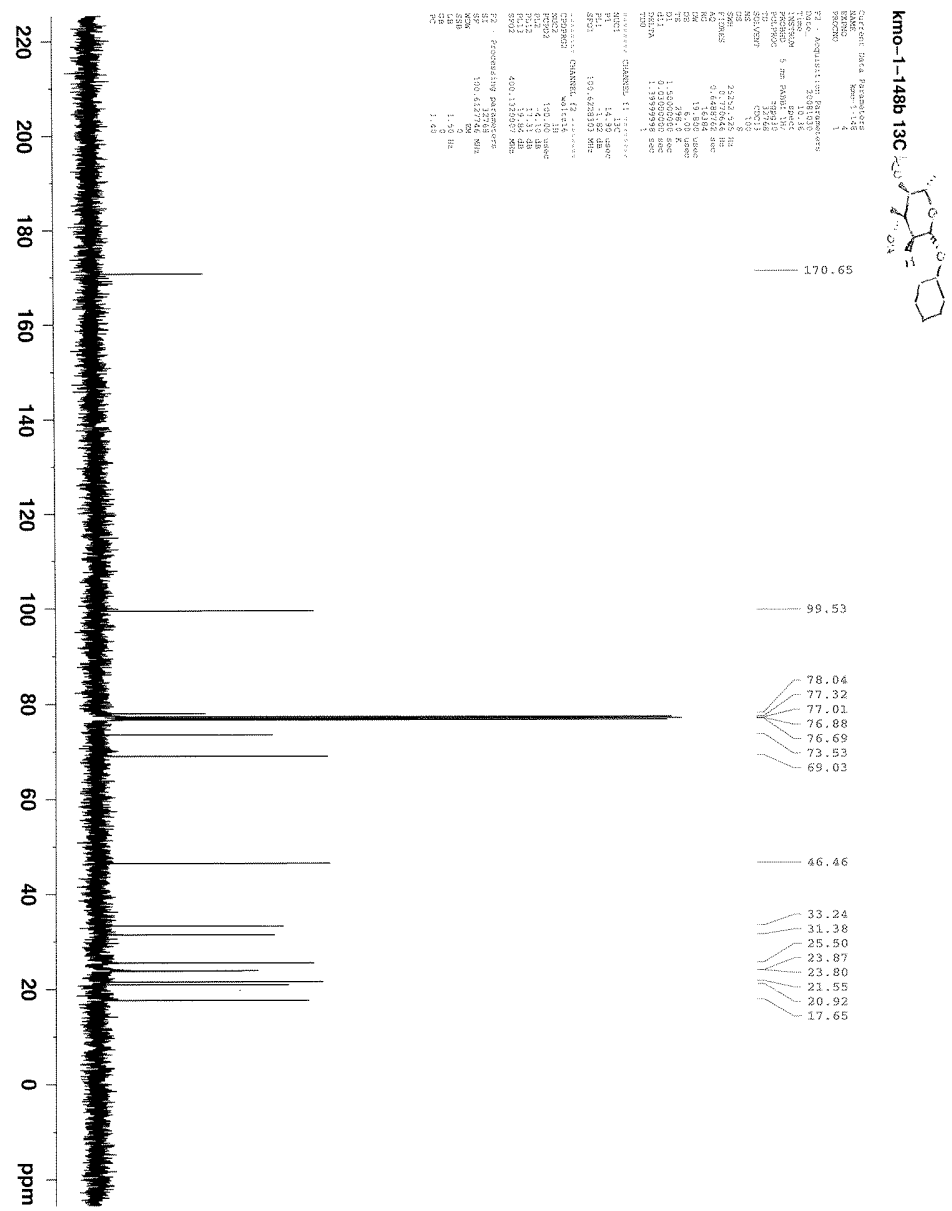


Figure A19 The 125MHz ^{13}C NMR of 2.7b in $CDCl_3$

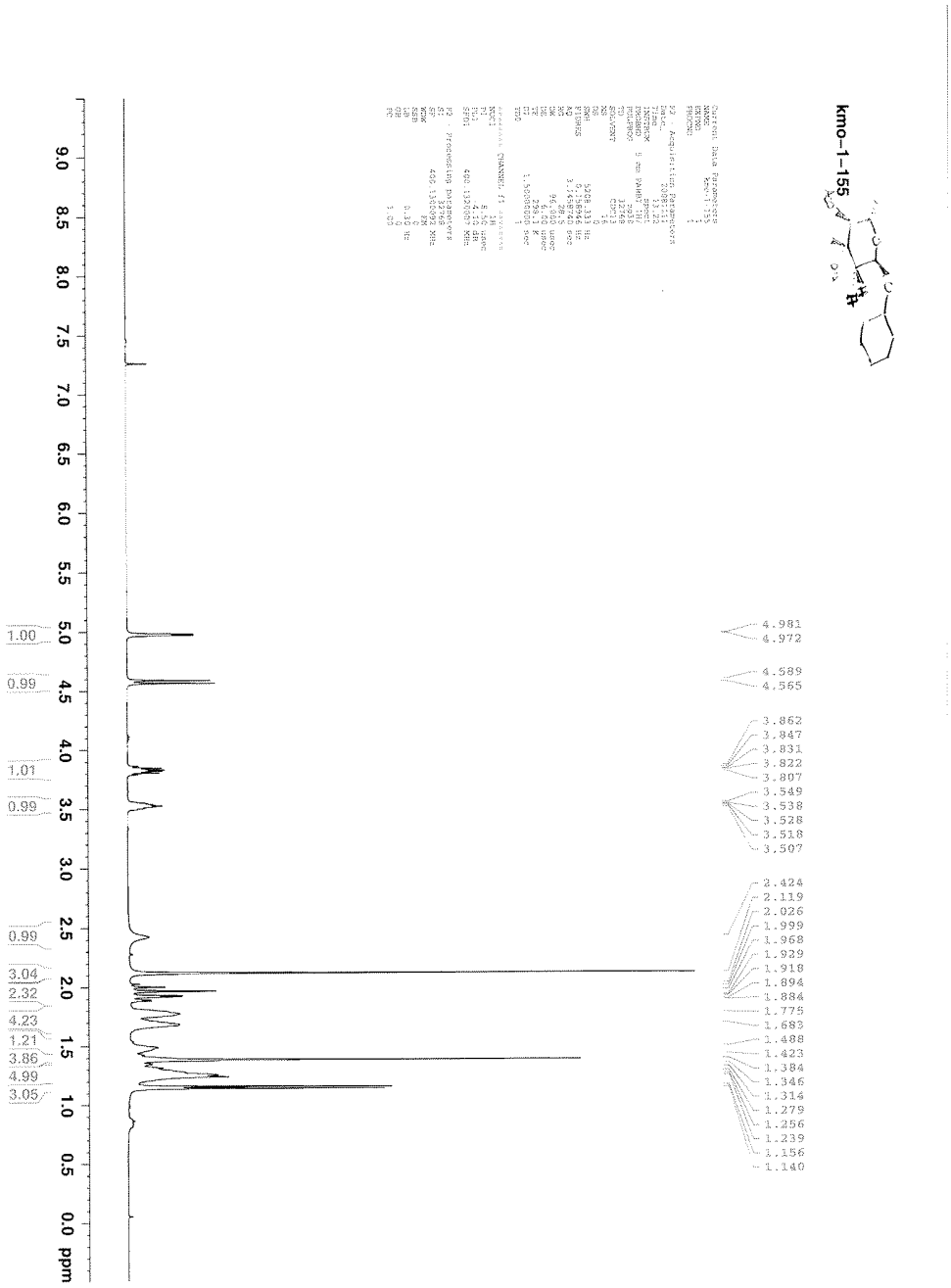


Figure A20 The 400MHz ^{1}H NMR of 2.8a in $CDCl_3$

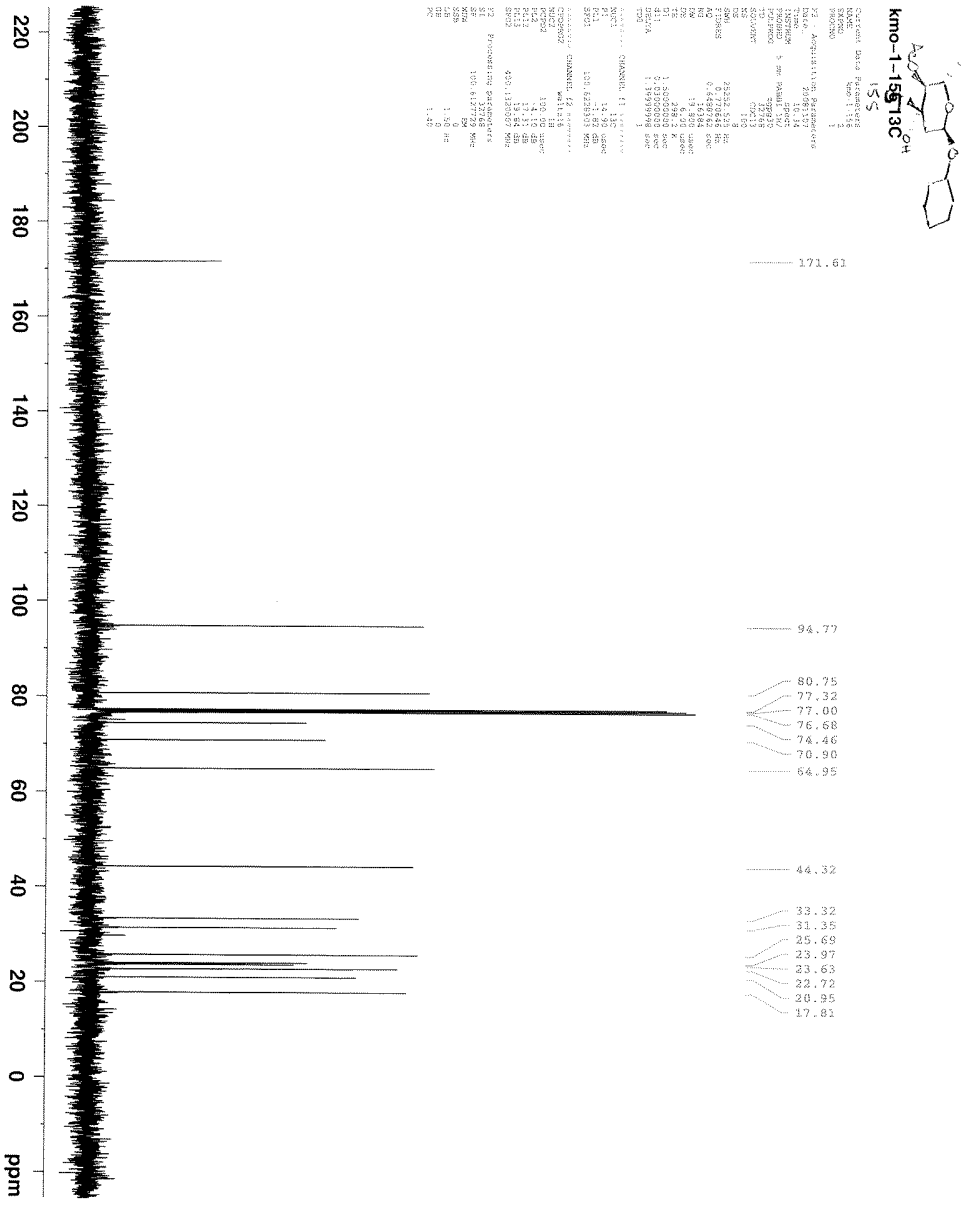


Figure A21 The 125MHz ^{13}C NMR of 2.8a in $CDCl_3$

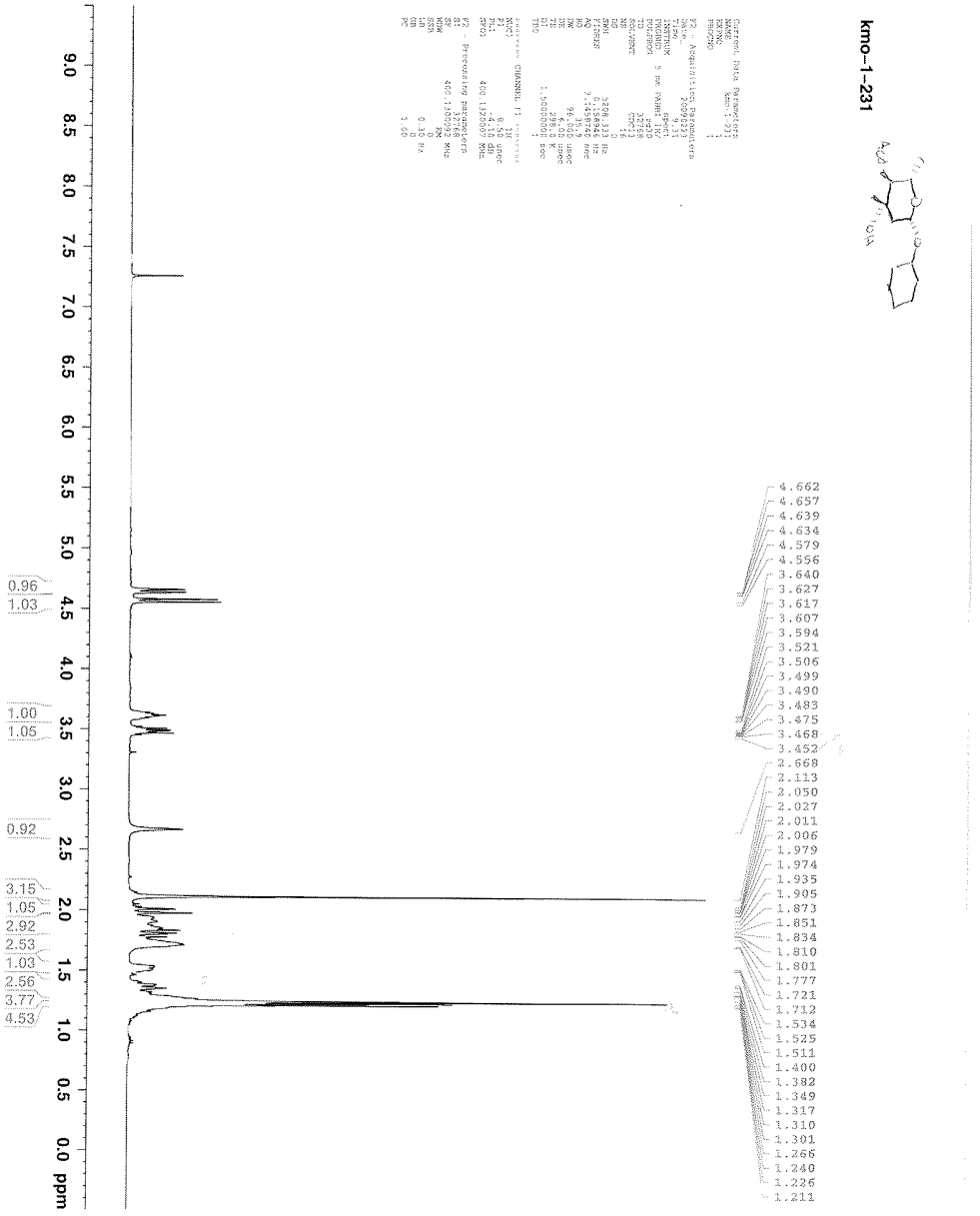


Figure A22 The 400MHz ^{1}H NMR of 2.8b in $CDCl_3$

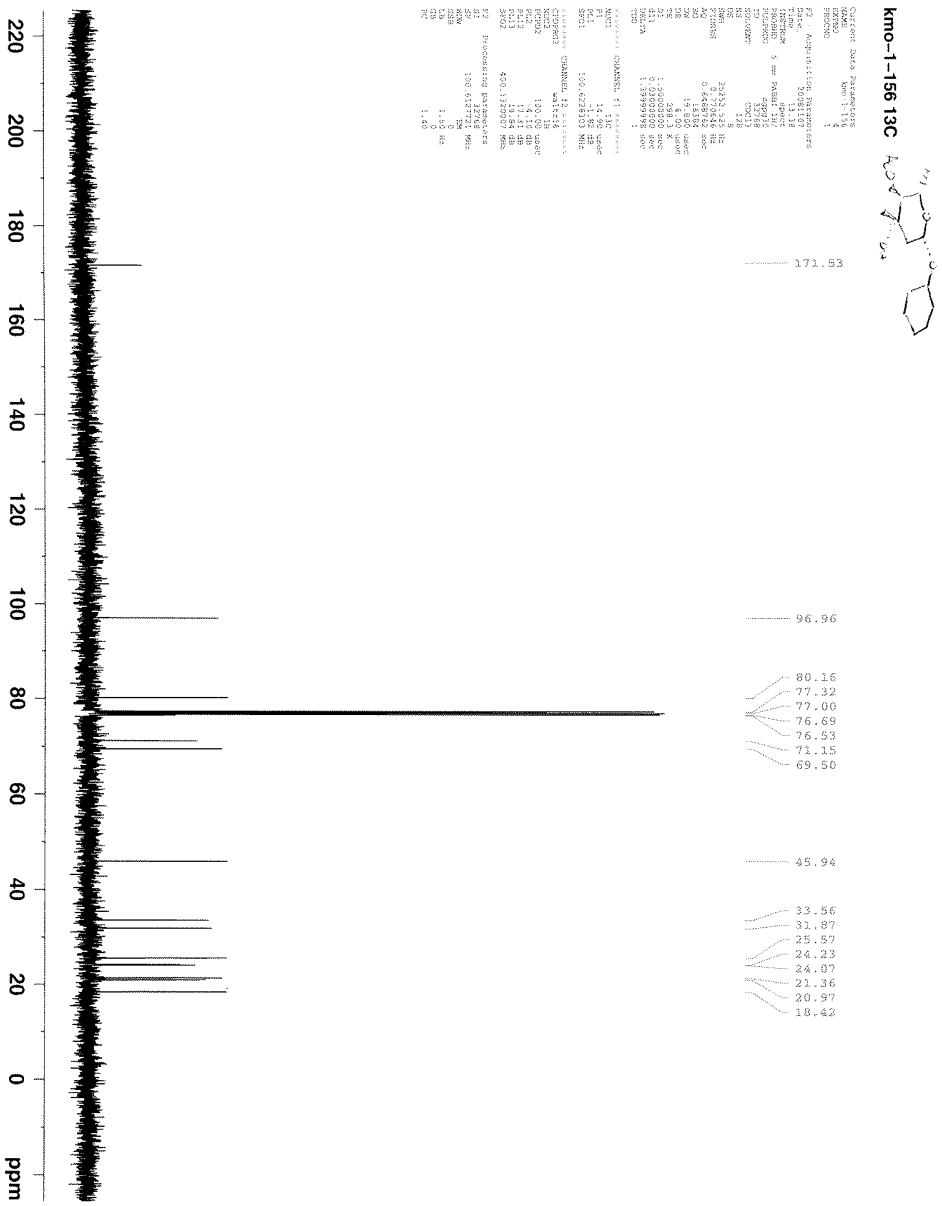


Figure A23 The 125MHz ^{13}C NMR of 2.8b in $CDCl_3$

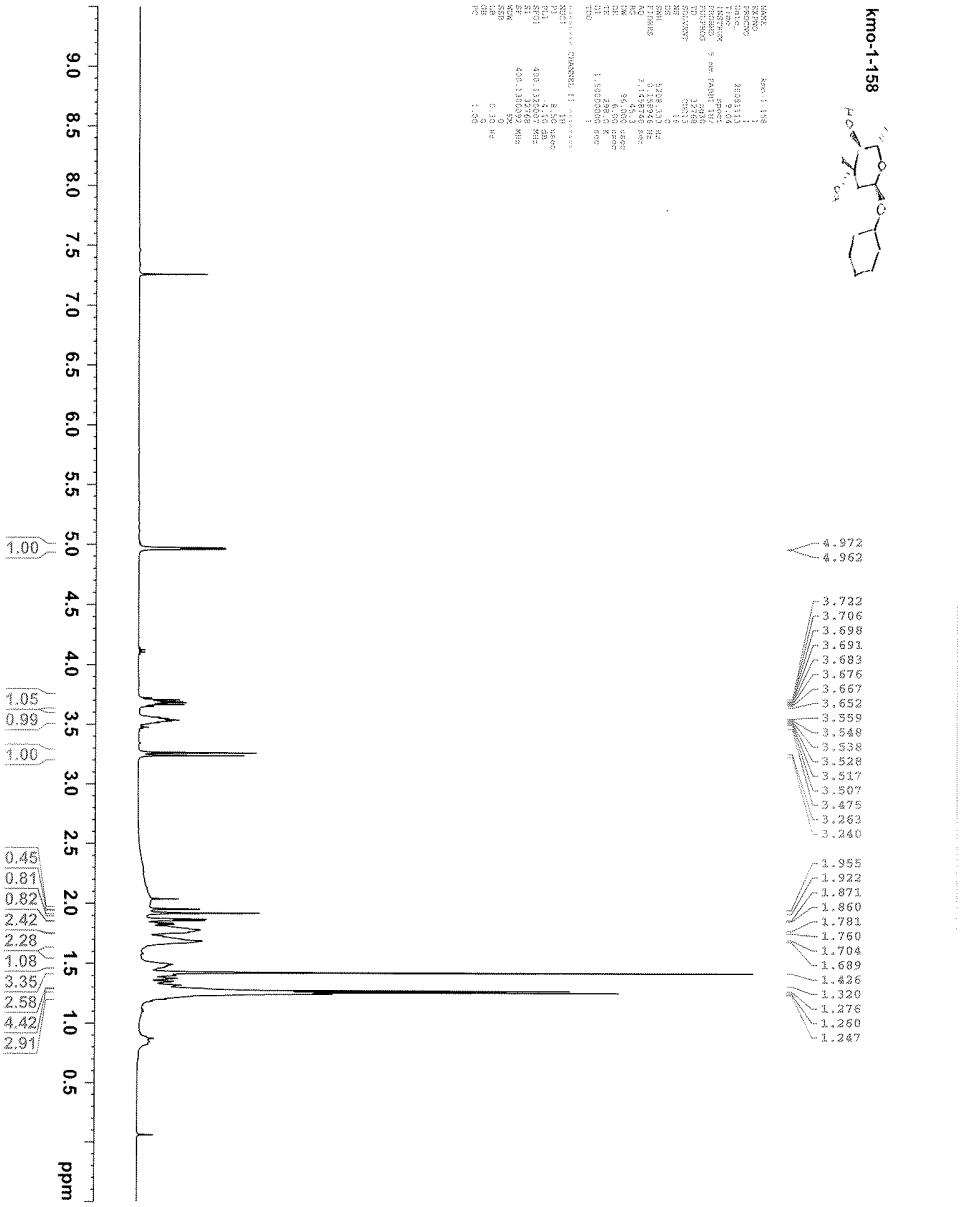


Figure A24 The 400MHz ^{1}H NMR of 2.9a in $CDCl_3$

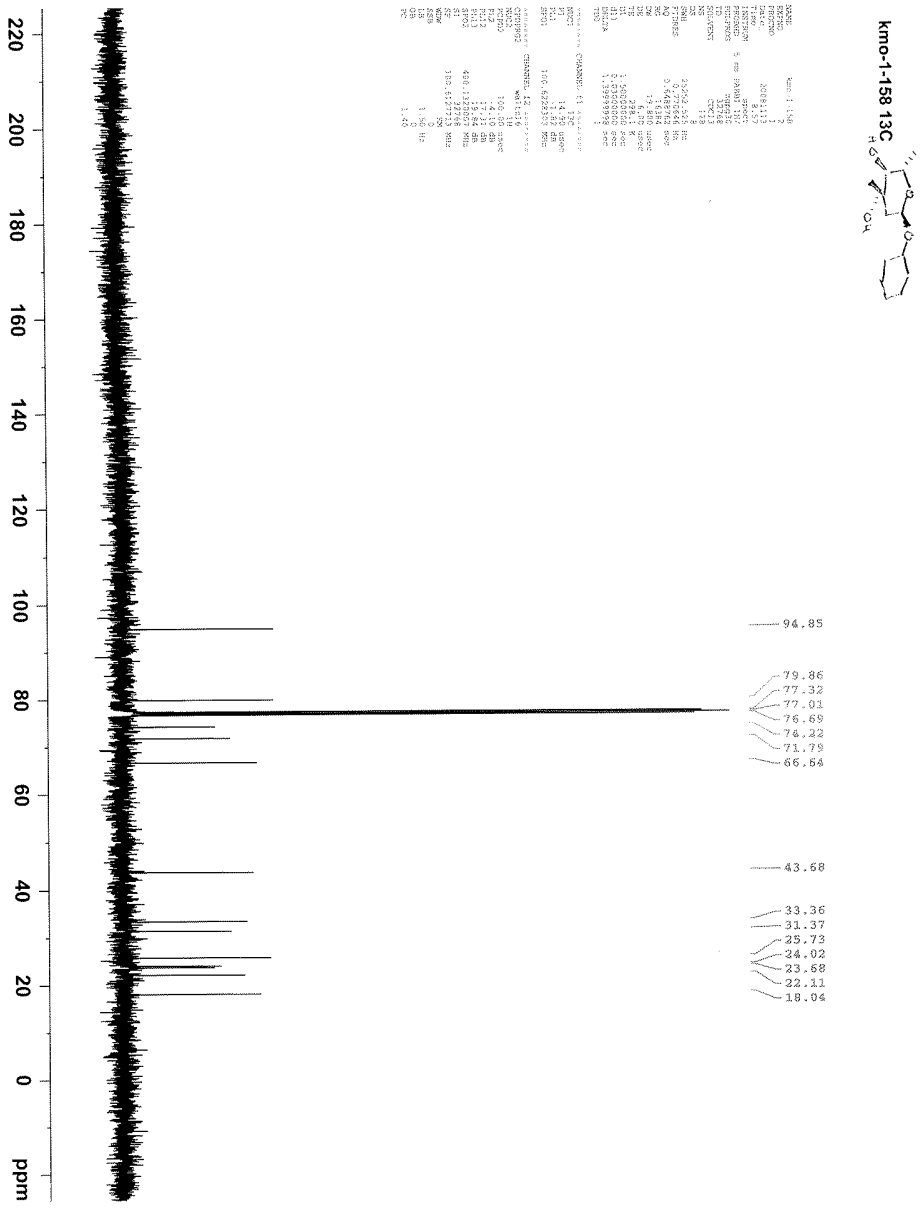


Figure A25 The 125MHz ^{13}C NMR of 2.9a in $CDCl_3$

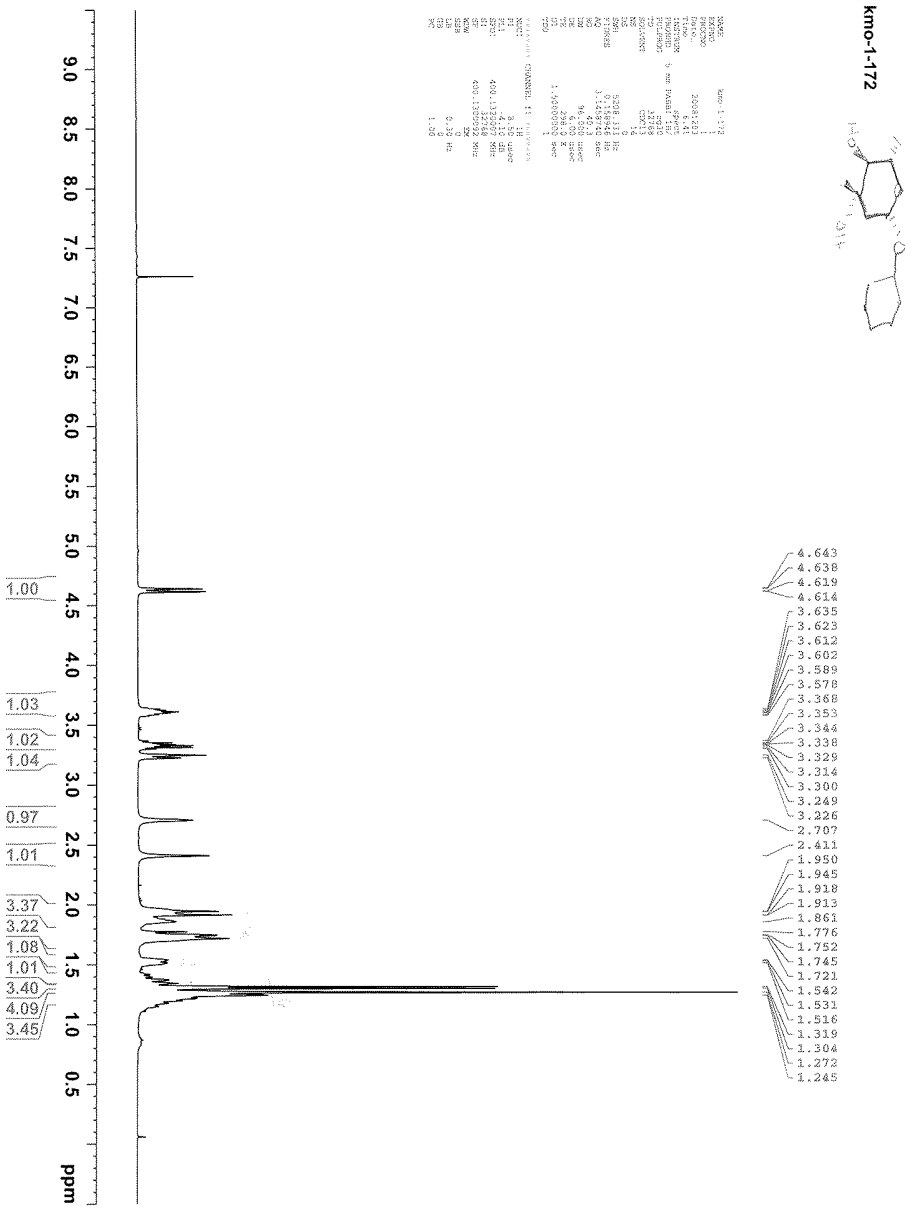


Figure A26 The 400MHz ^{1}H NMR of 2.9b in $CDCl_3$

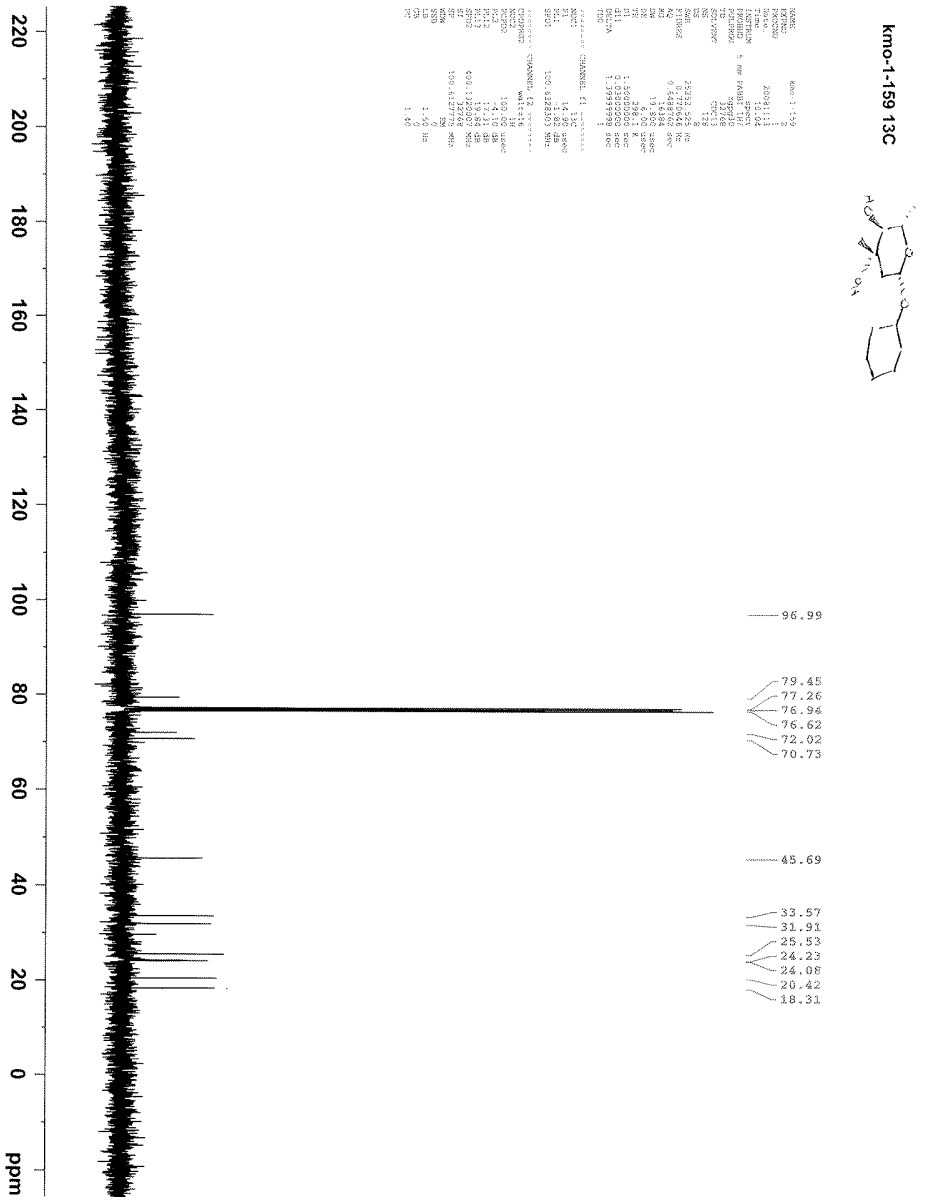


Figure A27 The 125MHz ^{13}C NMR of 2.9b in $CDCl_3$

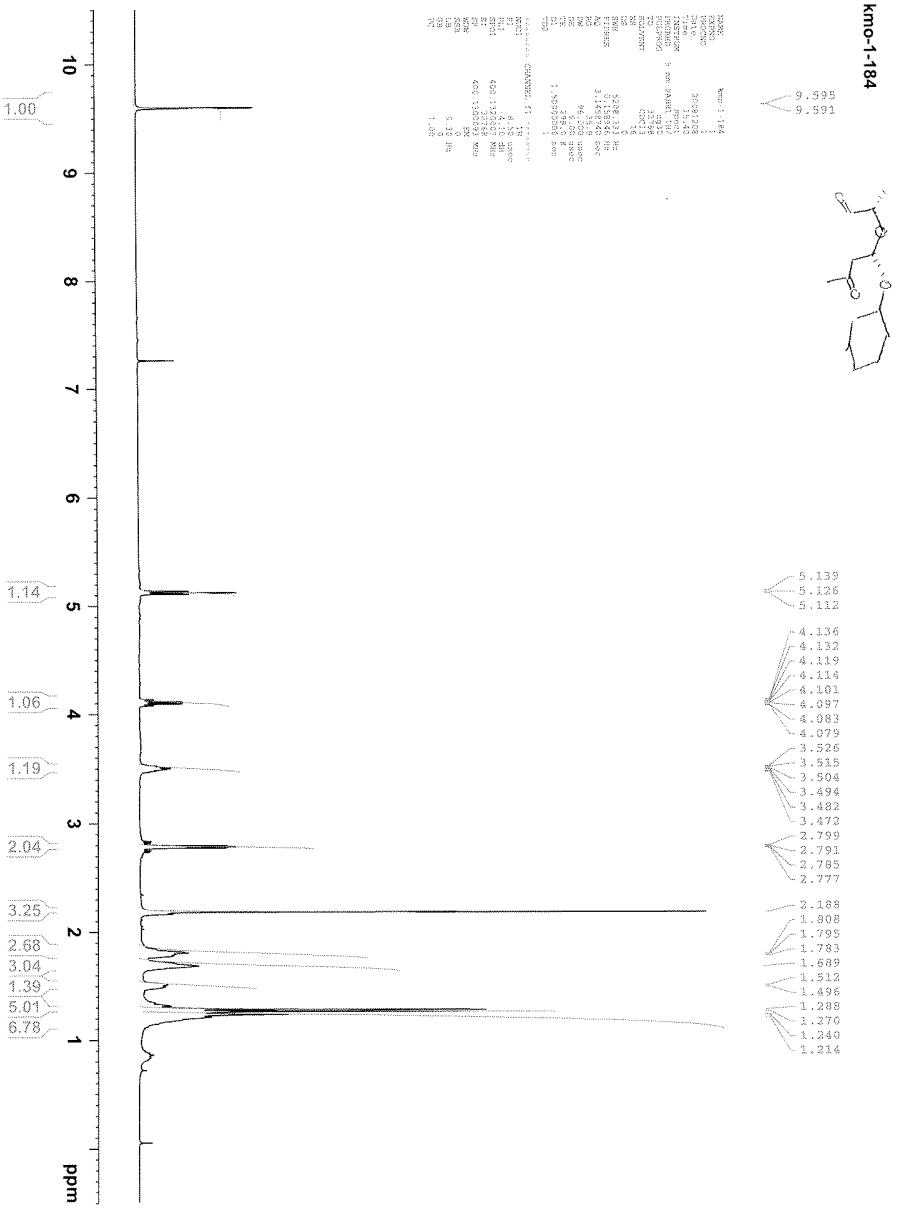


Figure A28 The 400MHz ^{1}H NMR of 2.10.1b in $CDCl_3$

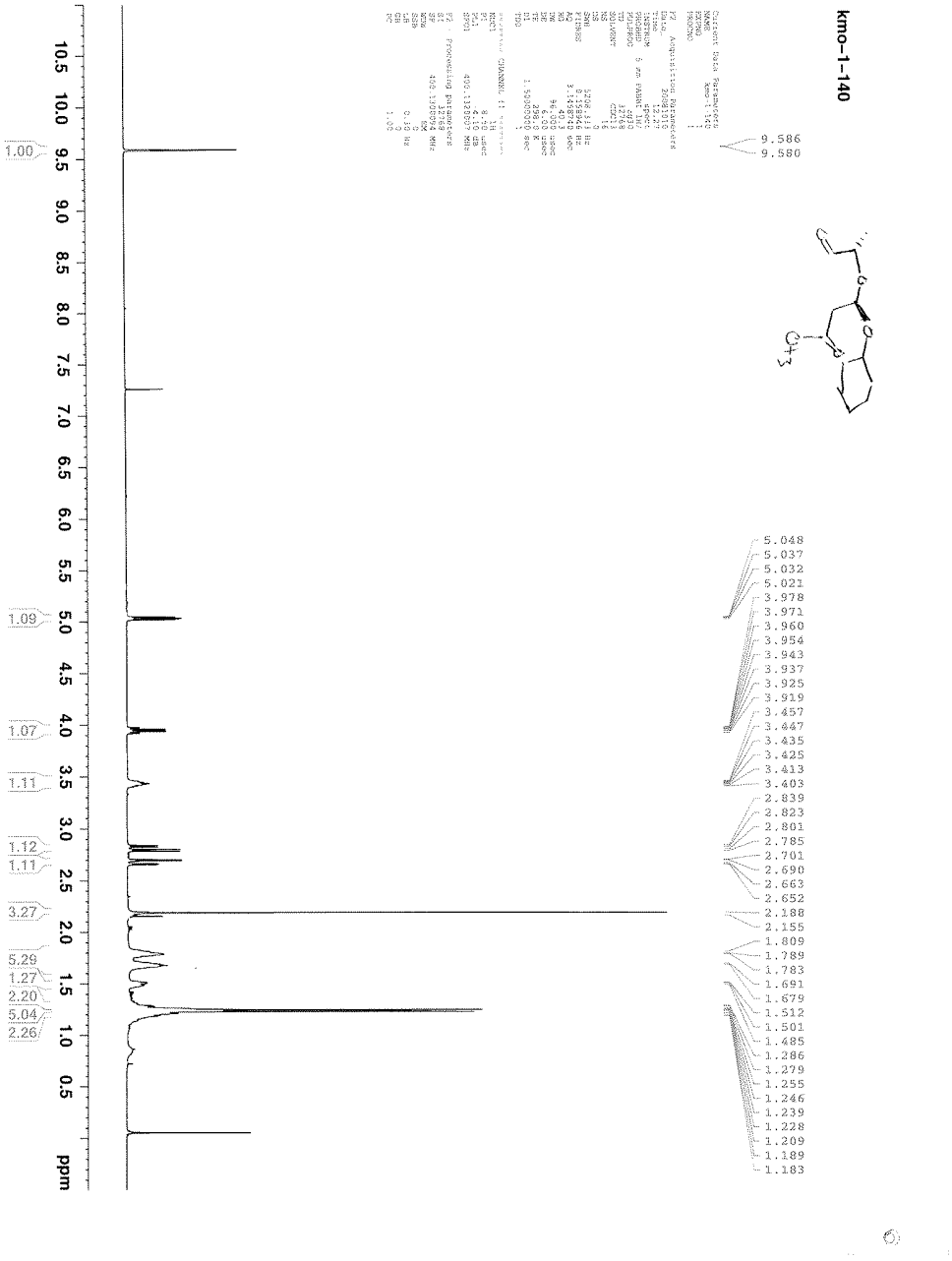


Figure A29 The 400MHz ^{1}H NMR of 2.10.1a in $CDCl_3$

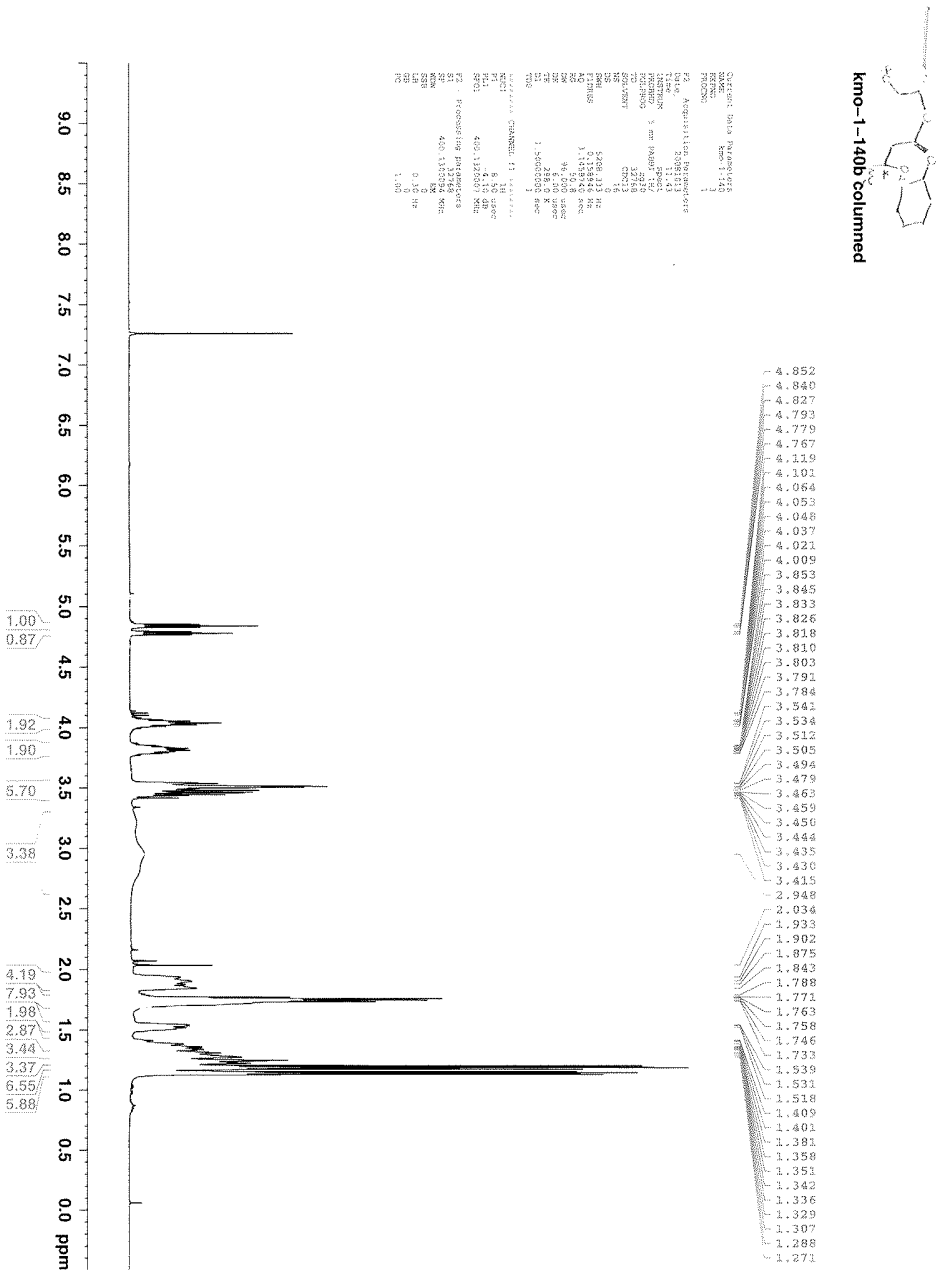


Figure A30 The 400MHz ^{1}H NMR of 2.10.2a in $CDCl_3$

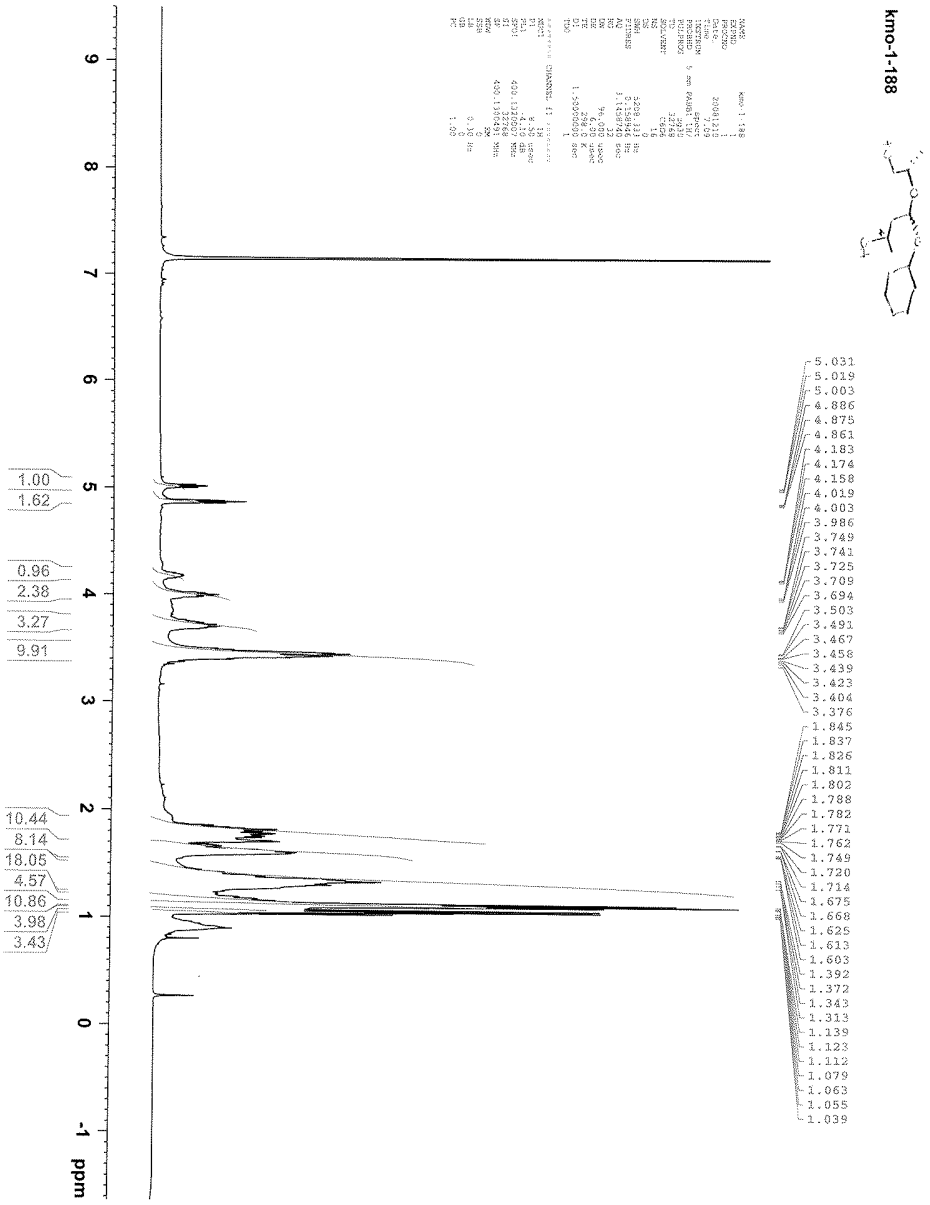


Figure A31 The 400MHz ^{1}H NMR of 2.10.2b in $CDCl_3$

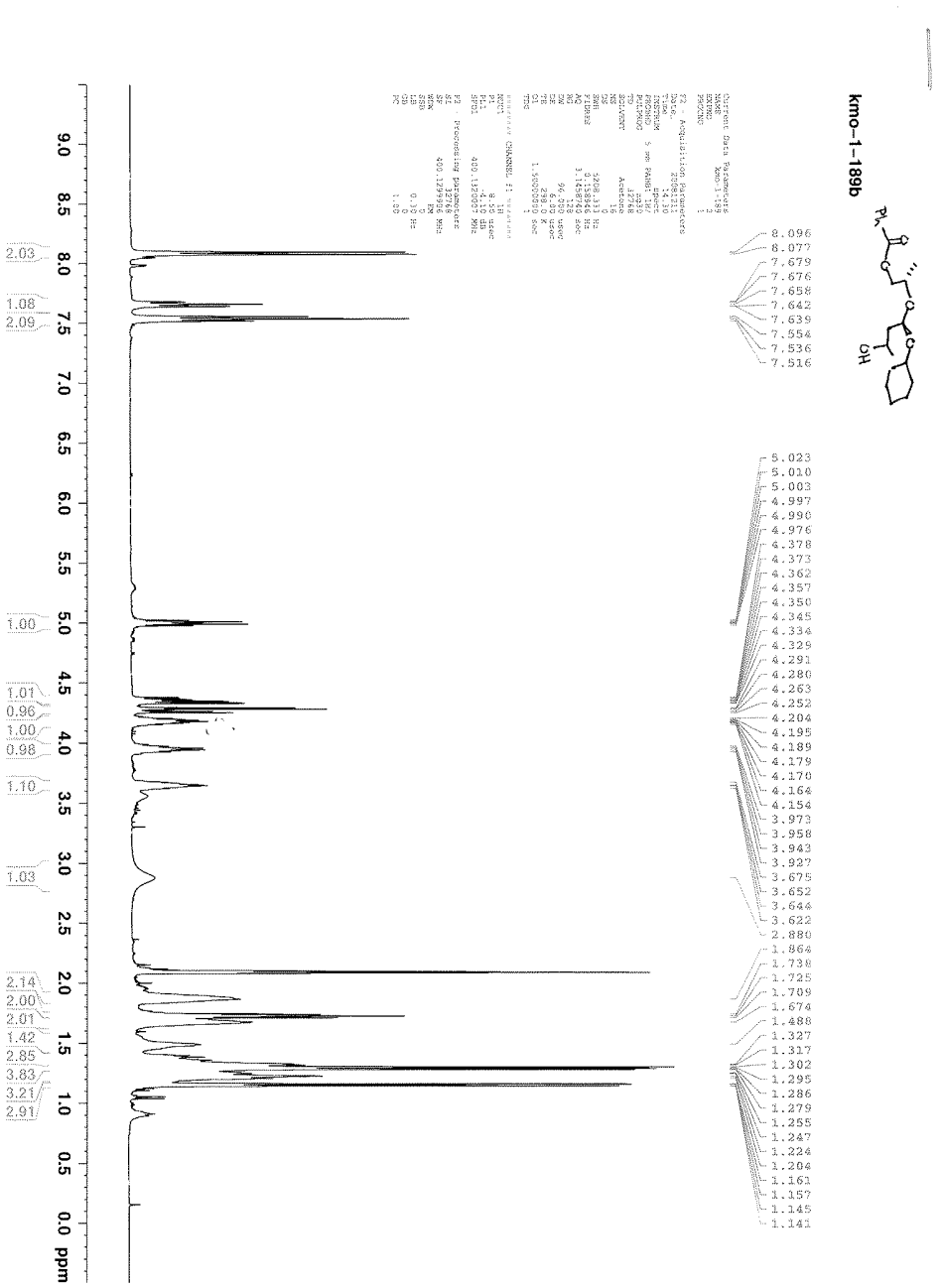


Figure A32 The 400MHz ^{1}H NMR of 2.11a in $CDCl_3$

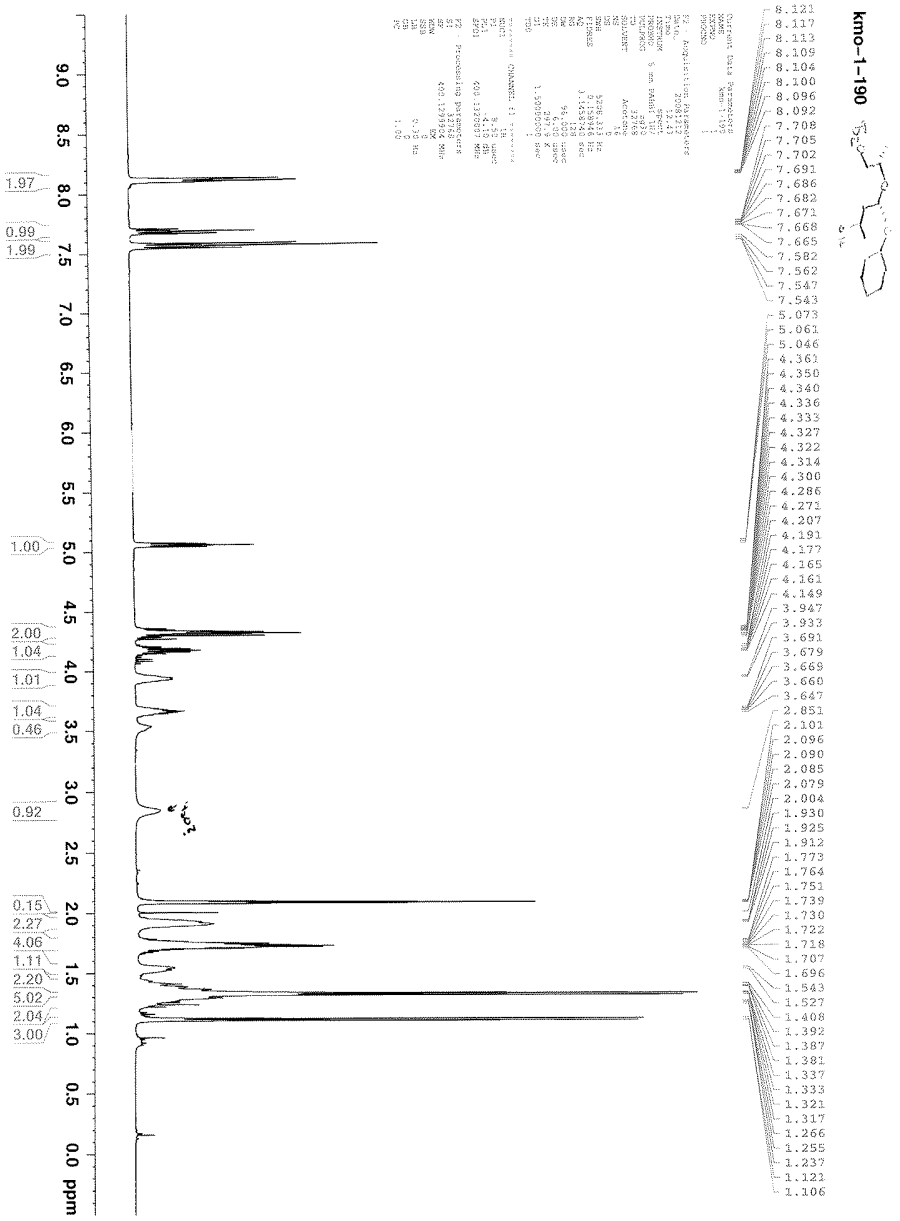


Figure A33 The 400MHz ^{1}H NMR of 2.11b in $CDCl_3$

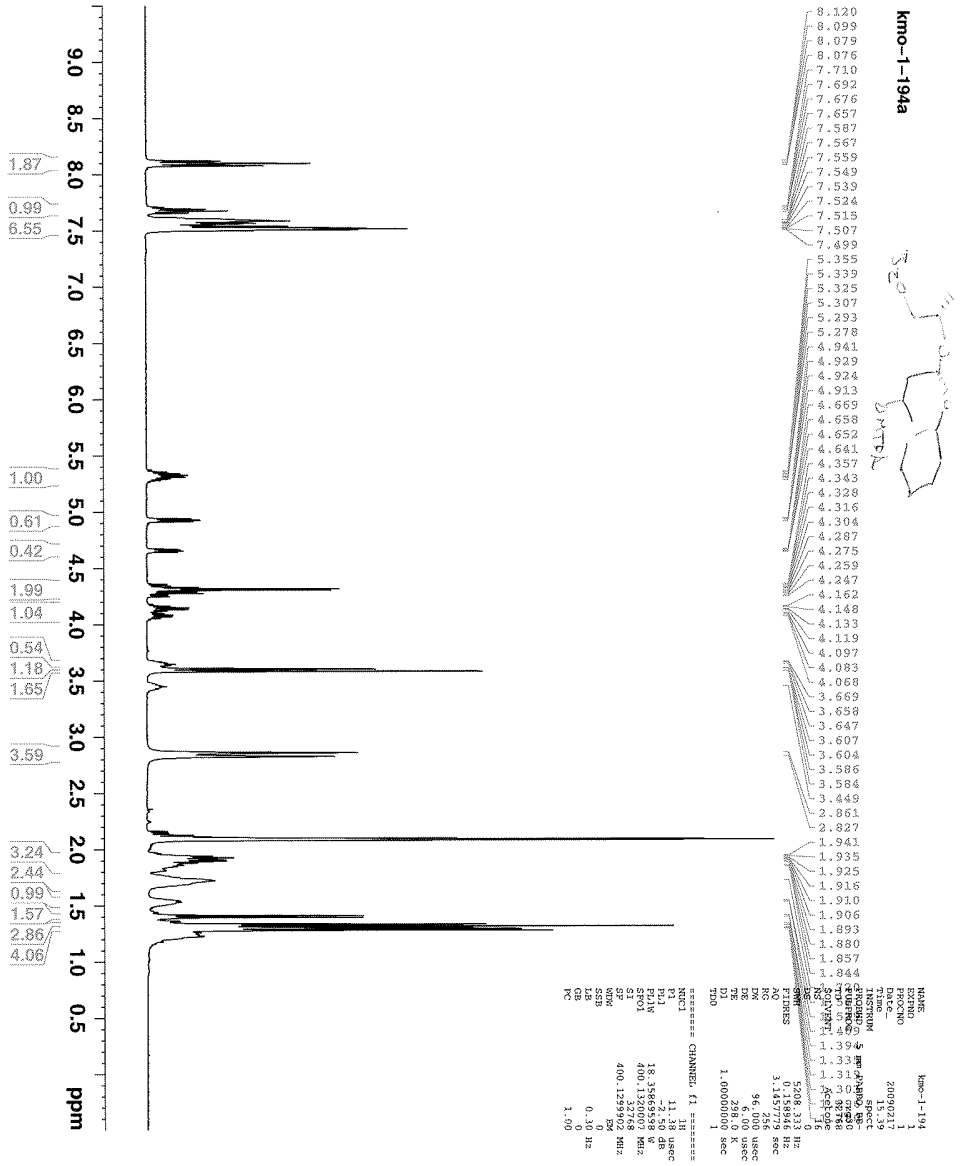


Figure A34 The 600MHz ^{1}H NMR of 2.12c-d in $CDCl_3$

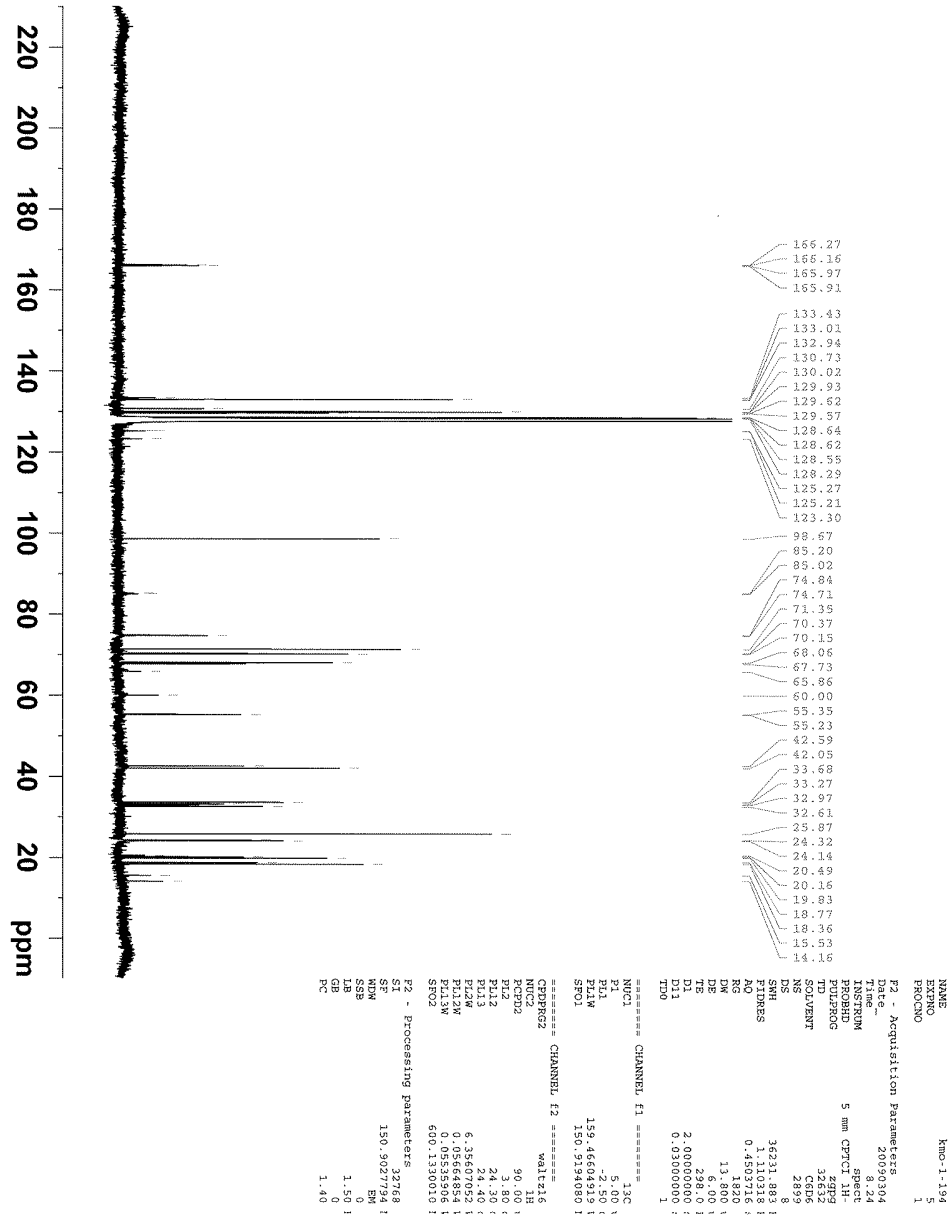


Figure A35 The 150MHz ^{13}C NMR of 2.12c-d in $CDCl_3$

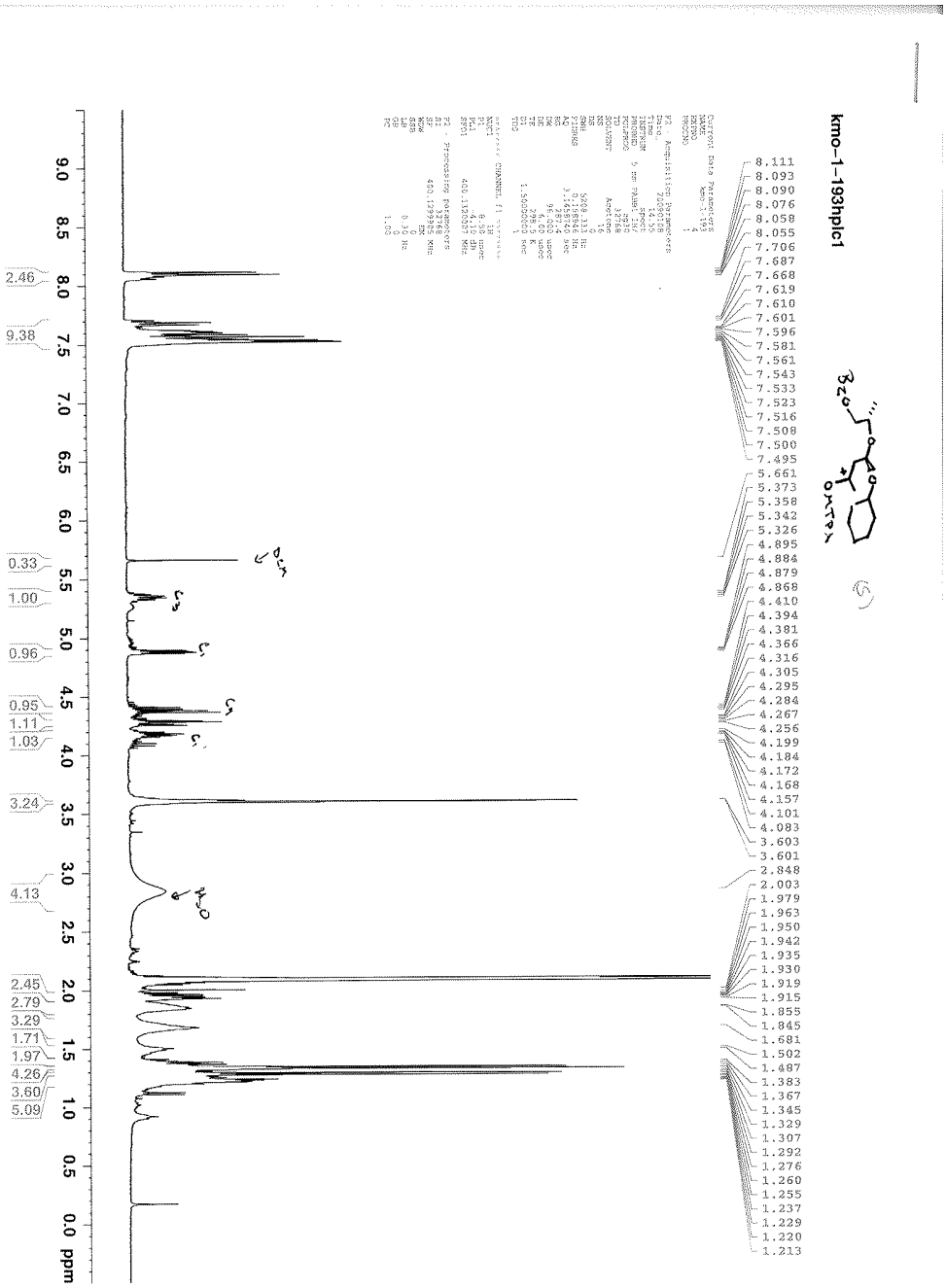


Figure A36 The 600MHz ^{1}H NMR of 2.12b in $CDCl_3$

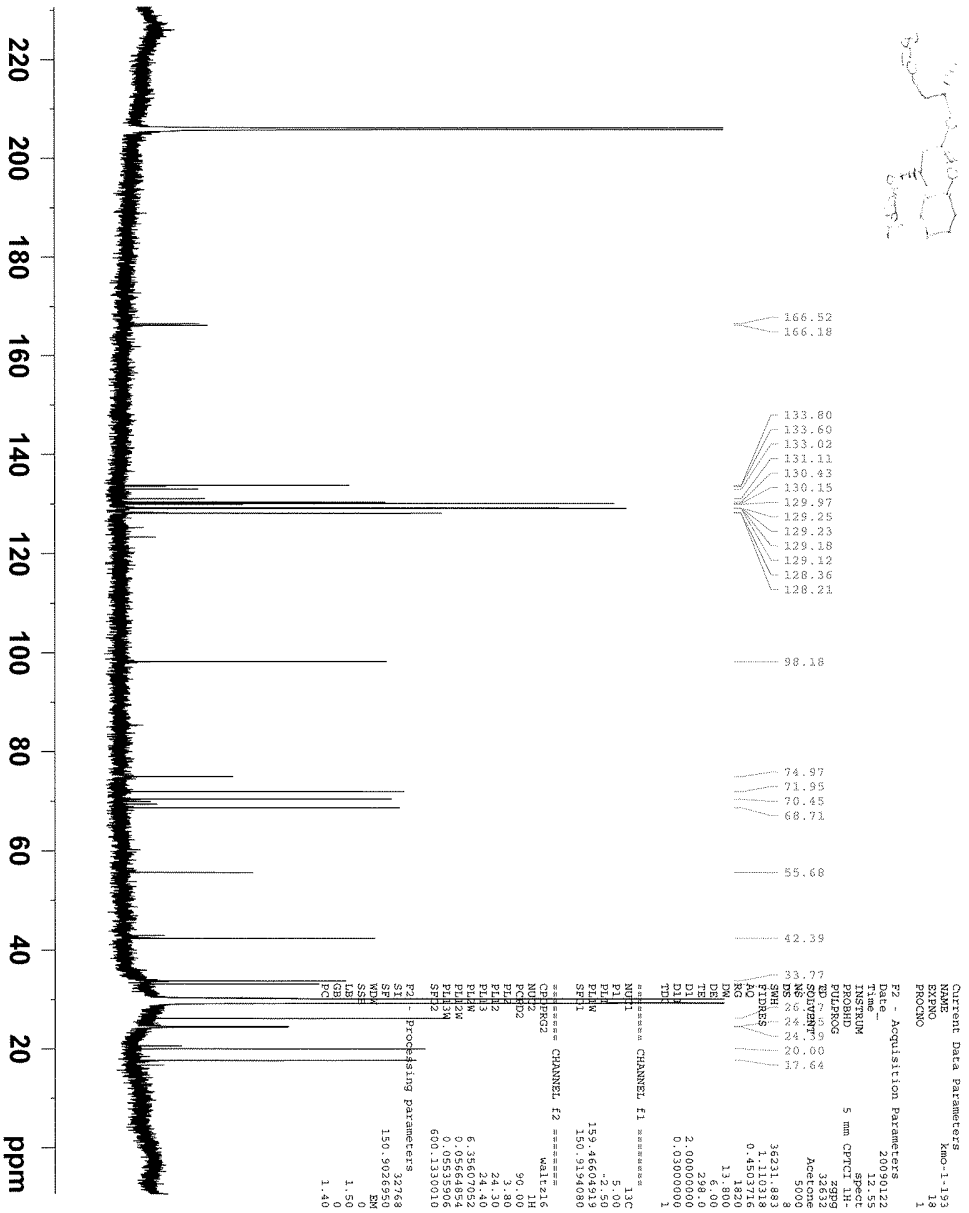


Figure A37 The 150MHz ^{13}C NMR of 2.12b in $CDCl_3$

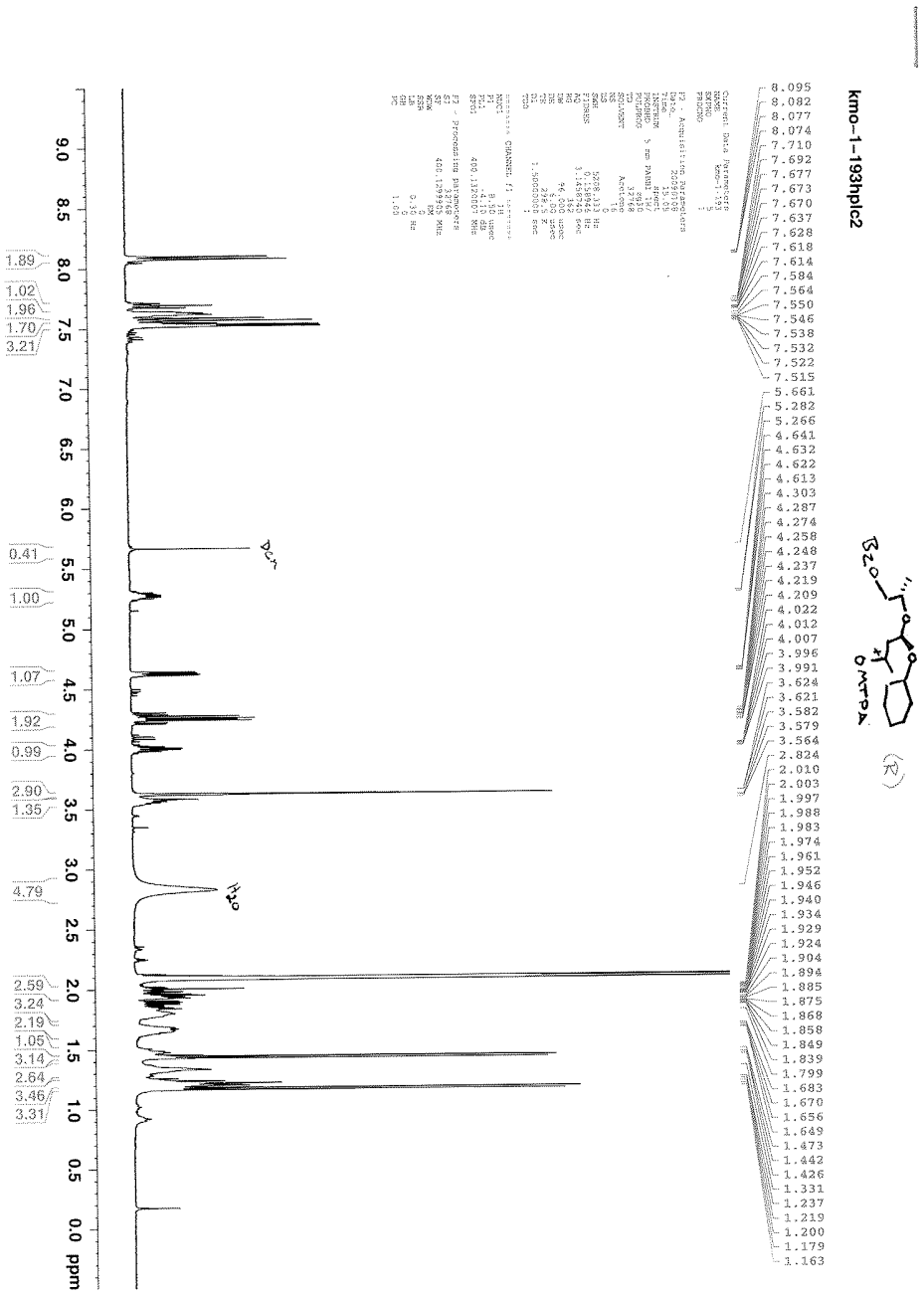


Figure A38 The 600MHz ^{1}H NMR of 2.12a in $CDCl_3$

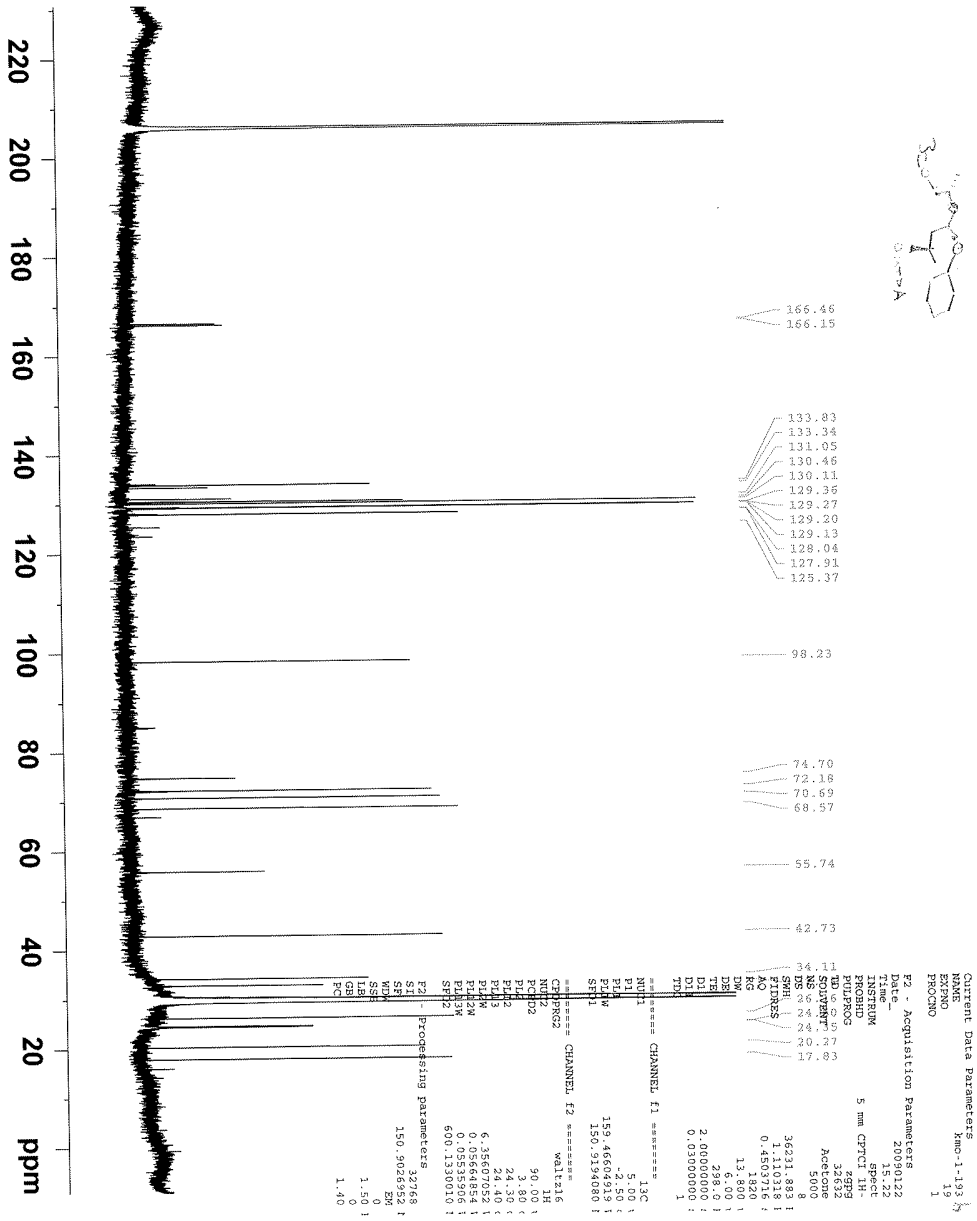


Figure A39 The 150MHz ^{13}C NMR of 2.12a in $CDCl_3$

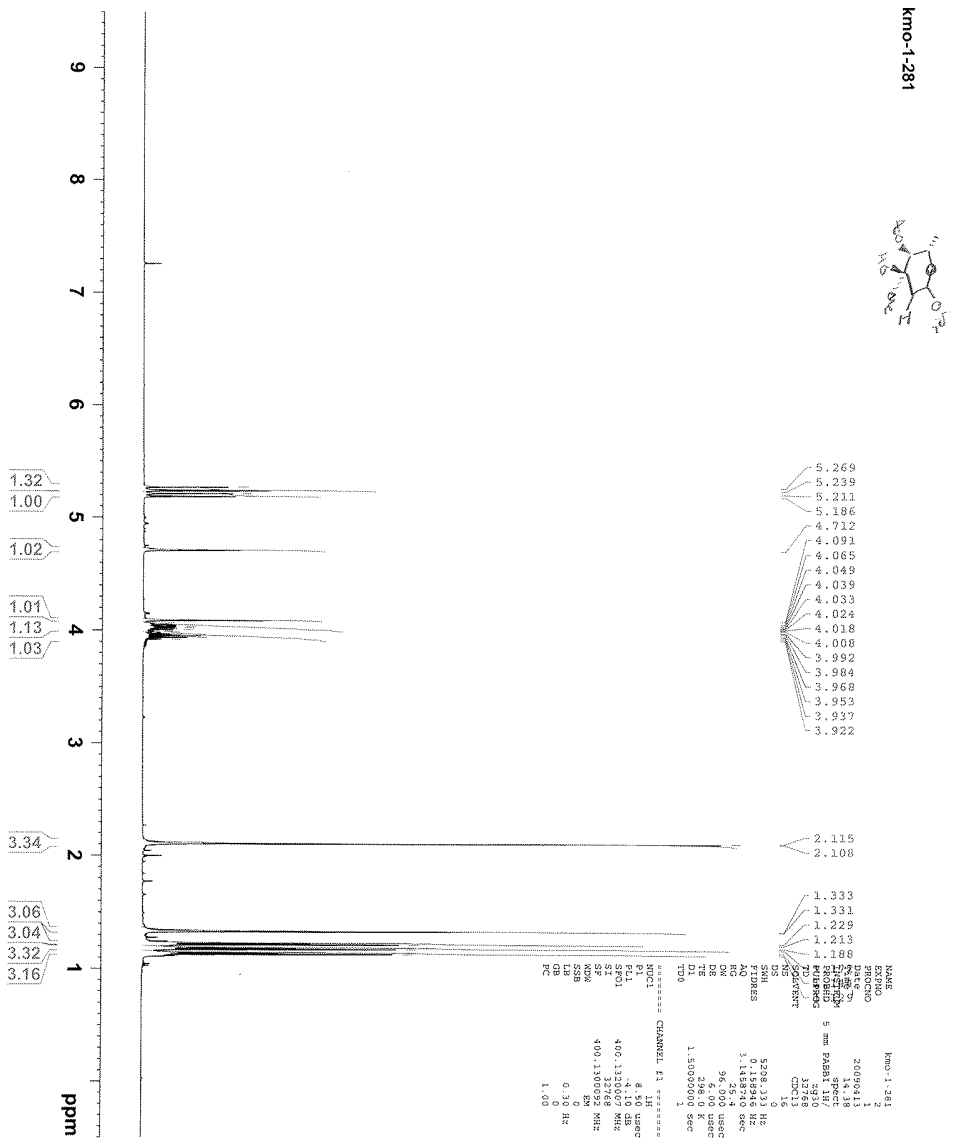


Figure A40 The 400MHz ^{1}H NMR of 2.15 in $CDCl_3$

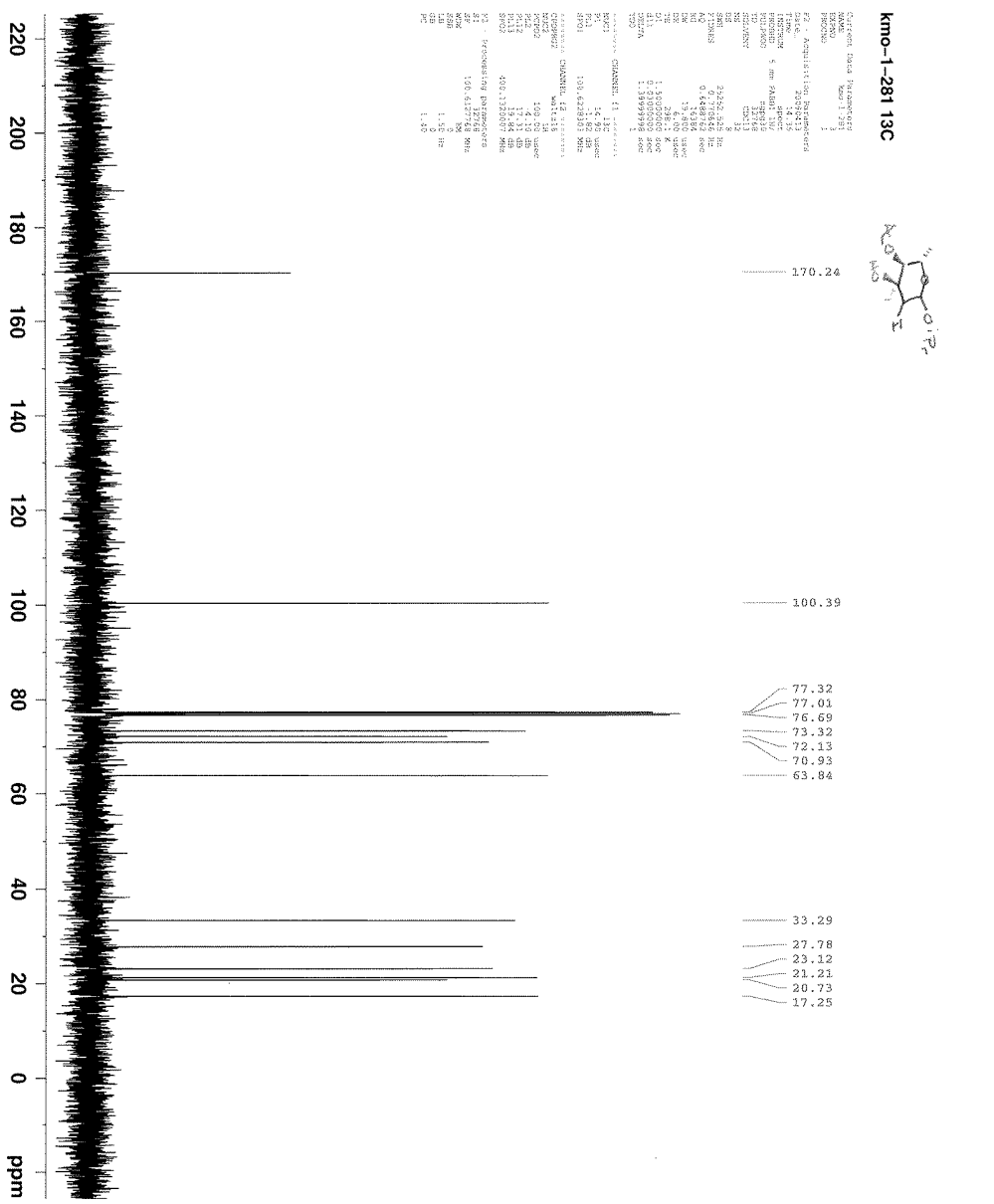


Figure A41 The 125MHz ^{13}C NMR of 2.15 in $CDCl_3$

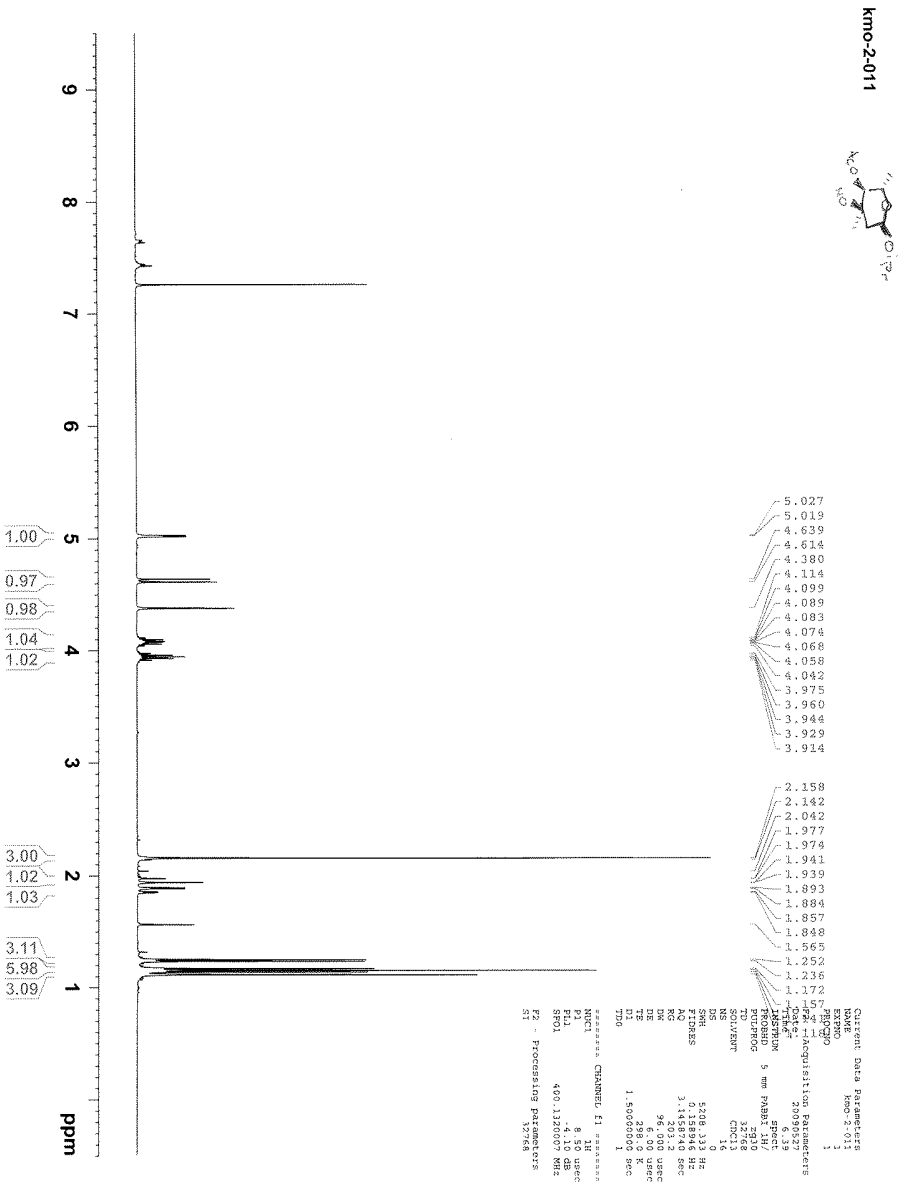


Figure A42 The 400MHz ^{1}H NMR of 2.16 in $CDCl_3$

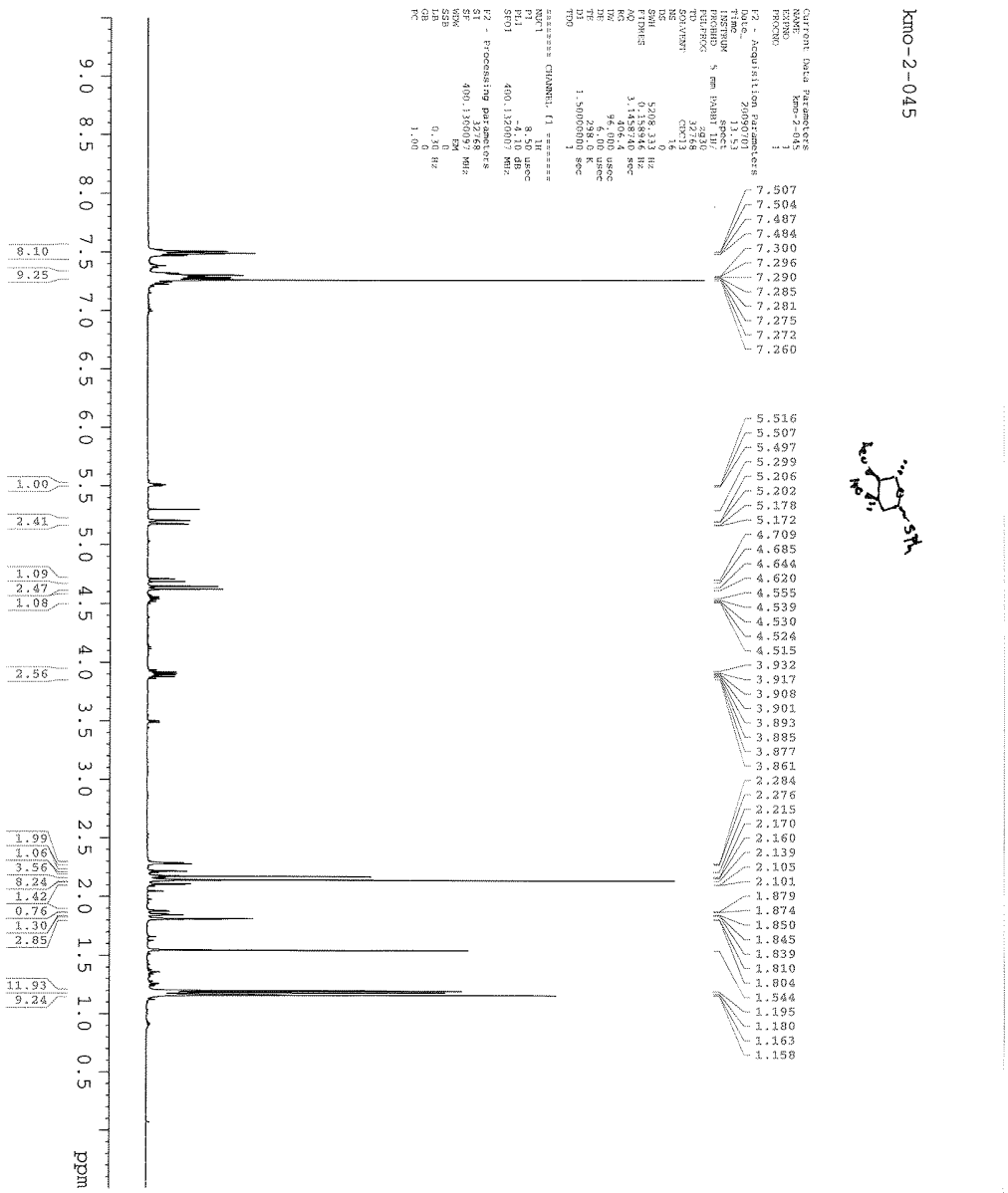


Figure A43 The 400MHz ^{1}H NMR of 2.17 in $CDCl_3$

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